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FORMULARIO UNICO DE SOLICITUD
PCT
ENTRADA EN FASE NACIONAL

(1) SOLICITUD DE :

[X] Patente de Invención [ ] Patente de Modelo de Utilidad

[ ] Capitulo I. [X] Capitulo II.

(2) SOLICITANTE (71)	Nombre: RECORDATI IRELAND LIMITED Dirección: 17, The Crescent, LIMERICK, IRLANDA Nacionalidad o Domicilio: IRLANDA Lugar de Constitución: IRLANDA Teléfono: Fax: E-mail:	IDENTIFICACIÓN C.C. [ ] NIT [ ] C.E. [ ] OTRO [ ] Cual: Número:
(3) REPRESENTANTE O APODERADO	Nombre: DORA MARIA GOMEZ DIAZ Dirección: CARRERA 7 No. 32 - 85 OFICINA 501 Teléfono: 2859058 - 2452462 Fax: 2859919 E-mail: gerencia@gomezydiaz.com	IDENTIFICACIÓN C.C. [X] NIT [ ] C.E. [ ] OTRO [ ] Cual: Número:52.145.354 Bogotá T.P.: 95.718 C.S. de la J.
(4) INVENTOR (ES) (72)	Nombre: Fausto BONIFACIO, Francesco CAMPANA, Gianluca DE IASI, Amadeo LEONARDI Dirección: Via G. D´Arezzo, 9, I-04100 LATINA-ITALIA, Via Mediana, I-00040 ROCCA PRIORA –ITALIA, Via del Bersaglieri, 27, I-04011 APRILIA – ITALIA, Via Poliziano, 16, I-20154 MILANO-ITALIA. (RESPECTIVAMENTE) Nacionalidad o Domicilio: ITALIA	

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NUEVAS FORMAS POLIMORFICAS CRISTALINAS DE CLORHIDRATO DE LERCANIDIPINA Y PROCESO PARA SU PREPARACION

(7) Clasificación Internacional (51): A61K 31/44 ; C07D 211/90

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(3) REPRESENTANTE APODERADO	**Nombre: DORA MARIA GOMEZ DIAZ** **Dirección: CARRERA 7 No. 32 - 85 OFICINA 501** **Teléfono: 2859058 - 2452462** **Fax: 2859919** **E-mail: gerencia@gomezydiaz.com**	**IDENTIFICACIÓN** **C.C.** [X] **NIT** [ ] **C.E.** [ ] **OTRO** [ ] **Cual:** **Número:52.145.354 Bogotá** **T.P.: 95.718 C.S. de la J.**
(4) INVENTOR (ES) (72)	**Nombre: Fausto BONIFACIO, Francesco CAMPANA, Gianluca DE IASI, Amadeo LEONARDI** **Dirección: Via G. D´Arezzo, 9, I-04100 LATINA-ITALIA, Via Mediana, I-00040 ROCCA PRIORA –ITALIA, Via dei Bersaglieri, 27, I-04011 APRILIA – ITALIA, Via Poliziano, 16, I-20154 MILANO-ITALIA. (RESPECTIVAMENTE)** **Nacionalidad o Domicilio: ITALIA**	

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ANEXOS

2

x	Comprobante de pago de la tasa de presentación de la solicitud $422.000.00
x	Comprobante de pago por reivindicación de prioridad $118.000.00
	Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica
x	Poderes, si fuere el caso
	Documento de cesión del inventor al solicitante o a su causante
	Declaraciones
x	Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
	Traducción de la solicitud internacional al castellano (Resumen, descripción, reivindicaciones, dibujos)
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	De ser el caso, modificaciones efectuadas a la solicitud internacional
	Otros

11

FIGURA CARACTERÍSTICA:

12

Solicito la concesión de la patente, NOMBRE: DORA MARIA GOMEZ DIAZ

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DORA MARIA CONEZ	CONSIGNACION	BANCO POPULAR	050 00110 6	15534512	02/02/2004	115,000.00
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CANT.	RETRITICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	422,000.00
		TOTAL : $	422,000.00

SON: CUATROCIENTOS VEINTIDOS MIL PESOS

RESPONSABLE : _____

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DORA MARIA GOMEZ	CONSIGNACION	BANCO POPULAR	050-00110-6	15534512	02/02/2004	110,000.00
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(54) Title: NOVEL CRYSTALLINE POLYMORPHIC FORMS OF LERCANIDIPINE HYDROCHLORIDE AND PROCESS FOR THEIR PREPARATION

(57) Abstract: The invention describes novel lercanidipine crude Forms (A) and (B), novel lercanidipine hydrochloride crystalline Forms (I) and (II) obtained form said crude Forms, pharmaceutical, antihypertensive compositions containing as active agent at least one of the lercanidipine hydrochloride crystalline Forms (I) and (II) and methods of use thereof

EXAMPLE 11 Preparation of lercanidipine hydrochloride crystalline Form (II) in aqueous N-methylpyrrolidone

A suspension of 50 g of lercanidipine hydrochloride crude Form (C) in 30 ml of N-methylpyrrolidone/water (1:1) was stirred at 20-25°C for 12 days. The solid thus formed was collected by filtration and dried, yielding 40 g of lercanidipine hydrochloride Form (II). Comparable results are obtained using crude Forms (A) or (B) or lercanidipine hydrochloride crystalline Form (I) as starting material.

EXAMPLE 12 DSC analysis of lercanidipine hydrochloride crystalline Forms (I) and (II)

DSC analysis measures changes that occur in a given sample with heating, wherein the changes identify transition phases. Enthalpy variations taking place in a transition phase are calculated on the basis of the area under the curve. The most common transition phases are melting and sublimation. The temperature at which transition starts, onset T, is given by the point in which the curve starts to deviate from the base line (flex point).

DSC of Form (I): 3.8 mg of Form (I) was placed in a golden pan of the apparatus Perkin Elmer DSC7. The heating speed during the test was 10°C/min.

DSC Form (II): 4.6 mg of Form (II) was placed in a golden pan of the apparatus Perkin Elmer DSC7. The heating speed during the test was 10°C/min.

The data are shown in Figures 1 and 2 and the characteristic points of the figures are briefly summarized in the following Table 1.

lercanidipine hydrochloride Form (II). Comparable results are obtained using crude Form (A) or (B).

EXAMPLE 9 Preparation of lercanidipine hydrochloride crystalline Form (II) in aqueous 1-propanol

60 g of lercanidipine HCl crude Form (C) was dissolved at 60°C in 100 ml of 1-propanol-H_2O (8:2). After filtering by gravity the possible insoluble portion the solution was cooled in two hours to 25°C and stirred for 120 hours at said temperature, with or without sonication for 6 seconds at 20 kHz and 100 Watts. The precipitate thus formed was collected, obtaining 90% yield with respect to stoichiometric lercanidipine hydrochloride Form (II) after a drying step. Comparable results are obtained using crude Forms (A) or (B) or lercanidipine hydrochloride crystalline Form (I) as starting material.

EXAMPLE 10 Preparation of lercanidipine hydrochloride crystalline Form (II) in aqueous 2-propanol

30 g of lercanidipine hydrochloride crude Form (C) was dissolved at 60°C in 100 ml of 2-propanol-H_2O (8:2). After filtering by gravity the possible insoluble portion the solution was cooled in two hours to 25°C and stirred for 72 hours at said temperature, with or without sonication for 6 seconds at 20 kHz and 100 Watts. The precipitate thus formed was collected, obtaining 85% yield with respect to stoichiometric lercanidipine hydrochloride Form (II) after a drying step. The same result is obtained by stirring for 168 hours at 10°C. Comparable results are obtained using crude Forms (A) or (B) or lercanidipine hydrochloride crystalline Form (I) as starting material.

25 g of lercanidipine HCl crude substance or Form (C) was dissolved at 60°C in 100 ml of a mixture ethanol-H_2O (8:2). The whole was filtered by gravity to eliminate the possible insoluble portion and diluted with 100 ml of H_2O. The solution thus obtained was stirred at 25°C as such, or it was added with 0.1 g of lercanidipine hydrochloride Form (II) or it was sonicated for 6 seconds at 20 kHz and 100 Watts, always at 25°C. Whatever the choice, after 48 hours under stirring the precipitate thus formed was collected and dried in an oven at 70°C for 24 hours, obtaining a 80-85% yield of Form (II). Comparable results are obtained using crude Forms (A) or (B) or lercanidipine hydrochloride crystalline Form (I) as starting material.

As an alternative, the initial clear solution is diluted with 100 ml of ethanol and seeded with lercanidipine hydrochloride Form (II) (0.1 g). After 48 hours with stirring at 25°C, 80% yield with respect to stoichiometric lercanidipine hydrochloride Form (II) is obtained.

EXAMPLE 8 . Preparation of lercanidipine hydrochloride crystalline Form (II) in aqueous methanol

In representative independent examples, 40 g of lercanidipine hydrochloride crude Form (C) or crystalline Form (I) was dissolved in 100 ml of methanol at 30°C. The whole was filtered by gravity to eliminate the possible insoluble portion and 25 ml of water was added. The solution thus obtained was stirred at 25°C as such, or was mixed with 0.1 g of lercanidipine hydrochloride Form (II), or was sonicated for 6 seconds at 20 kHz and 100 Watts, always at 25°C. Whichever the choice, after 48 hours under stirring the precipitate thus formed was collected and dried, with yields of 80-85% with respect to stoichiometric

for 24 hours. 20.2 kg of product was obtained, corresponding to a yield of 81%; HPLC purity in Form (I) > 99.5%. Comparable results are obtained with Form (C) as starting material.

EXAMPLE 6 Preparation of lercanidipine hydrochloride crystalline Form (II)

100 g of crude lercanidipine hydrochloride Form (C) and then 200 ml of acetonitrile was loaded into a reactor. The mixture was heated under strong reflux and under stirring, thus obtaining a complete dissolution. The mixture was brought to 20-30°C under slight stirring and kept at said temperature for 24 hours. The precipitate was filtered and dried in an oven at 70°C for 24 hours. 95 g of dry product was obtained, corresponding to a 95% yield; HPLC purity > 99.5% in lercanidipine hydrochloride Form (II). Comparable results are obtained when lercanidipine hydrochloride Form (A) or (B) is used as starting material.

EXAMPLE 7 Preparation of lercanidipine hydrochloride crystalline Form (II)

In separate representative experiments, 100 g of crude lercanidipine hydrochloride Form (A), (B), or (C) in 200 ml of 95% ethanol was loaded into a reactor, the mixture thus obtained was heated under stirring and under strong reflux and then cooled at 25°C always under stirring. The solution was kept at said temperature for 24 hours under stirring. The precipitate thus obtained was then filtered and dried in an oven at 70°C for 24 hours. 90 g of Form (II), HPLC purity > 99.5% was obtained.

EXAMPLE 7A Preparation of lercanidipine hydrochloride crystalline Form (II)

in an oven at 70°C under vacuum for 24 hours. Weight of dry product in each case was (lercanidipine HCl (I)) 90 g (HPLC purity of the product in Form (I) > 99.5%).

EXAMPLE 4A Preparation of lercanidipine hydrochloride crystalline Form (I)

In separate representative experiments, 100 g of crude lercanidipine hydrochloride Form (A), (B), or (C) was loaded into a reactor, followed by 400 ml of 2-propanol. The mixture was heated under strong reflux and under stirring, thus obtaining an almost complete dissolution of the crude substance. The mixture was hot filtered to eliminate a slight opalescence and the clear solution kept under stirring is slowly cooled to 40°C. Precipitation was then triggered with 100 mg of lercanidipine hydrochloride Form (I) and temperature was set at 35°C, keeping the mixture under stirring. The whole was kept for 24 hours under stirring at 35°C, then temperature was set at 30°C, keeping under stirring at said temperature for another 24 hours. The solid was filtered at 30°C and washed with 50 ml of 2-propanol, then dried in an oven at 70°C under vacuum for 24 hours. Weight of dry product (lercanidipine HCl (I)) was 90 g (HPLC purity of the product in Form (I) > 99.5%).

EXAMPLE 5 Preparation of lercanidipine hydrochloride crystalline Form (I)

In independent preparations, 25 kg of crude lercanidipine hydrochloride, Form (A) or (B), and then 100 mL of 95% ethanol were loaded and brought to strong reflux under stirring. The solution was cooled under stirring at 20°C and then seeded with crystalline Form (I). The whole was then cooled to a temperature between 10 and 15°C, keeping the reaction mixture under stirring for 4 days. The solid thus obtained was filtered and washed with 95% ethanol, the precipitate was filtered and dried in an oven under vacuum at 70°C

EXAMPLE 3A Crude lercanidipine hydrochloride Form (B)

The organic phase obtained at the end of Example 1 was heated under reflux (70-75°C) and the water contained in the solution was removed with a Dean Stark apparatus until a K.F. value of about 2% was obtained. The whole was then distilled at atmospheric pressure to reach ¾ of initial volume. The solution was brought to its initial volume by adding fresh ethyl acetate. The K.F. value at the end of this operation was 0.9-1.1%. The final solution was cooled to 20°C, seeded with 0.1% of crude lercanidipine hydrochloride Form (B) and cooled to 0-5°C. A solid slowly precipitated and was then filtered. The solid thus obtained was suspended in ethyl acetate (350 g) and stirred at 60-65°C for 1 hour. The whole was cooled at 5-10°C, then filtered and dried in an oven at 70°C for 24 hours, thus obtaining 133 g of crude lercanidipine hydrochloride Form (B), DSC peak 131-135°C; 75% yield.

EXAMPLE 4 Preparation of lercanidipine hydrochloride crystalline Form (I)

In separate representative experiments, 100 g of crude lercanidipine hydrochloride Form (A), (B), or (C) was loaded into a reactor, followed by 400 ml of 2-propanol. The mixture was heated under strong reflux and under stirring, thus obtaining an almost complete dissolution of the crude substance. The mixture was hot filtered to eliminate a slight opalescence and the clear solution kept under stirring was cooled to 40°C. Temperature was then set at 35°C. The whole was kept for 24 hours under stirring at 35°C, then temperature was set at 30°C, and stirring was continued at said temperature for another 24 hours. The solid was filtered at 30°C and washed with 50 ml of 2-propanol, then dried

EXAMPLE 2 Crude lercanidipine hydrochloride Form (A)

The organic phase obtained from Example 1 was then subjected to azeotropic distillation under vacuum at about 250 mmHg, without going above a temperature of 60°C. After removing about 50 ml of water, the solution was concentrated to about 1/3 of the initial volume in the same conditions of temperature and pressure and then brought to its initial volume with fresh ethyl acetate until the K.F. value (Karl Fisher value) was about 0.10-0.15%. The final suspension was cooled to 0-5°C. The solid was filtered, suspended in ethyl acetate (350 g) and stirred at 60-65°C for 1 hour. The whole was cooled to 5-10°C and then filtered. The solid was dried in an oven at 70°C. 133 g of dry raw lercanidipine hydrochloride Form (A) was obtained (75% yield), DSC peak 150-152°C.

EXAMPLE 3 Crude lercanidipine hydrochloride Form (B)

The organic phase obtained at the end of Example 1 was heated under reflux (70-75°C) and the water contained in the solution was removed with a Dean Stark apparatus (Spaziani Rolando, Nettuno, Rome, Italy) until a K.F. value of about 2% was obtained. The whole was then distilled at atmospheric pressure to reach ¾ of initial volume. The solution was brought to its initial volume by adding fresh ethyl acetate. The K.F. value at the end of this operation was 0.9-1.1%. The final solution was cooled to 0-5°C. A solid slowly precipitates which was filtered. The solid thus obtained was suspended in ethyl acetate (350 g) and stirred at 60-65°C for 1 hour. The whole was cooled to 5-10°C, then filtered and dried in an oven at 70°C, thus obtaining 133 g of crude lercanidipine hydrochloride Form (B), DSC peak 131-135°C; 75% yield.

dosage form containing from 0.1 to 400 mg lercanidipine hydrochloride. More preferably, the composition or dosage form comprises 1 to 200 mg lercanidipine hydrochloride. Even more preferably, the composition or dosage form comprises 5 to 40 mg lercanidipine hydrochloride.

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EXAMPLES

The following examples of preparation of lercanidipine hydrochloride crude Forms (A) and (B) and crystalline Forms (I) and (II) are now disclosed for illustrative non-limiting purposes, together with the results of DSC analysis and solubility, stability and 10 hygroscopicity tests; the bioavailability tests for the new crystalline forms are also disclosed.

EXAMPLE 1 Initial preparation

Thionyl chloride (36 g) diluted in ethyl acetate (25 g) was slowly added to a 15 solution of 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (90 g in dimethylformamide (115 g) and ethyl acetate (396 g), keeping temperature between -1 and +1°C. A solution of 2, N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol (84 g) in ethyl acetate (72 g) was slowly added to the mixture thus obtained. The whole was kept under stirring at the same temperature for 3 hours. The 20 mixture was then heated to 20-25°C and kept under stirring for 12 hours. Water (340 ml) was then added, the whole was stirred for 30 min and after settling the aqueous phase was discarded. The organic phase was washed again with water (340 ml).

achieved. Therefore, the patient may be titrated to the appropriate dosages for his/her particular hypertensive condition. After the appropriate dosage of each of the compounds is determined to achieve a decrease of the blood pressure without untoward side effects, the patient then may be switched to a single dosage form containing the appropriate dosages of each of the active agents, or may continue with a dual dosage form.

The exact dosage and administration regimen utilizing the combination therapy of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity and etiology of the hypertension to be treated; the route of administration; the renal and hepatic function of the patient; the treatment history of the patient; and the responsiveness of the patient. Optimal precision in achieving concentrations of compounds within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the absorption, distribution, metabolism, excretion of a drug, and responsiveness of the patient to the dosage regimen. However, such fine tuning of the therapeutic regimen is routine in light of the guidelines given herein.

A pharmaceutical composition for parenteral administration contains not below 0.1%, preferably from about 0.5% to about 30%, by weight of a polymorph or mixture of the present invention, based upon the total weight of the pharmaceutical composition. Individual isolated polymorphs are preferred for parenteral administration.

Generally, transdermal dosage forms contain from about 0.01% to about 100% by weight of the active agents, based upon 100% total weight of the dosage.

In a preferred embodiment of the present invention, the composition is administered daily to the patient. Preferably in said embodiment, the pharmaceutical composition has

A pharmaceutical composition comprising (1) lercanidipine hydrochloride, where the lercanidipine hydrochloride is selected from the group consisting of isolated lercanidipine hydrochloride crystalline Form (I), isolated lercanidipine hydrochloride crystalline Form (II), or combinations thereof of predetermined polymorph composition; and (2) at least one component selected from the group consisting of a pharmaceutically acceptable carrier or diluent, a flavorant, a sweetener, a preservative, a dye, a binder, a suspending agent, a dispersing agent, a colorant, a disintegrant, an excipient, a diluent, a lubricant, a plasticizer, and an edible oil. In a preferred embodiment, the pharmaceutical composition or dosage form 0.1 to 400 mg lercanidipine hydrochloride. Preferably, the composition or dosage form comprises 1 to 200 mg lercanidipine hydrochloride. More preferably, the composition or dosage form comprises 5 to 40 mg lercanidipine hydrochloride.

The pharmaceutical composition or unit dosage form may be administered in a single daily dose, or the total daily dosage may be administered in divided doses. In addition, co-administration or sequential administration of other active agents may be desirable. The polymorphs and mixtures thereof of the invention may be combined with any known drug therapy, preferably for treatment of hypertension. For example, bimodal therapy involving in addition a diuretic, a β-receptor blocker, an ACE inhibitor or an angiotensin II receptor antagonist is contemplated by the present invention (see, *e.g.*,U.S. Provisional Application No. 60/344,601, filed October 23, 2001 and Italian Application No. MI 2001 A 002136 filed October 16, 2001).

For combination therapy the compounds may initially be provided as separate dosage forms until an optimum dosage combination and administration regimen is

polyhydroxy-ethylaspartamidephenol, and polyethyl-eneoxideopolylysine substituted with palmitoyl residues.

Administration

The pharmaceutical composition or unit dosage forms of the present invention may be administered by a variety of routes such as intravenous, intratracheal, subcutaneous, oral, mucosal parenteral, buccal, sublingual, ophthalmic, pulmonary, transmucosal, transdermal, and intramuscular. Unit dosage forms also can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches known to those of ordinary skill in the art. Oral administration is preferred.

The pharmaceutical composition or unit dosage forms of the present invention may be administered to an animal, preferably a human being, in need of antihypertensive treatment. The pharmaceutical composition or unit dosage form of the present invention may be administered according to a dosage and administration regimen defined by routine testing in light of the guidelines given above in order to obtain optimal antihypertensive activity and a decreased in blood pressure while minimizing toxicity or side-effects for a particular patient. However, such fine turning of the therapeutic regimen is routine in light of the guidelines given herein.

The dosage of the composition containing polymorphs or mixtures of the present invention may vary according to a variety of factors such as underlying disease state, the individual's condition, weight, sex and age and the mode of administration. For oral administration, the pharmaceutical compositions can be provided in the form of scored or unscored solid unit dosage forms.

active combinations and the corresponding physiologically acceptable salts, can include water, physiological salt solutions or alcohols, e.g. ethanol, propane-diol or glycerol. Additionally, sugar solutions such as glucose or mannitol solutions may be used. A mixture of the various solvents mentioned may further be used in the present invention.

A transdermal dosage form also is contemplated by the present invention. Transdermal forms may be a diffusion-driven transdermal system (transdermal patch) using either a fluid reservoir or a drug-in-adhesive matrix system. Other transdermal dosage forms include, but are not limited to, topical gels, lotions, ointments, transmucosal systems and devices, and iontohoretic (electrical diffusion) delivery system. Transdermal dosage forms may be used for timed release and sustained release of the active agents of the present invention.

Pharmaceutical compositions and unit dosage forms of the present invention for administration parenterally, and in particular by injection, typically include a pharmaceutically acceptable carrier, as described above. A preferred liquid carrier is vegetable oil. Injection may be, for example, intravenous, intrathecal, intramuscular, intraruminal, intratracheal, or subcutaneous.

The active agent also can be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

The polymorphs of the present invention also may be coupled with soluble polymers as targetable drug carriers. Such polymers include, but are not limited to, polyvinyl-pyrrolidone, pyran copolymer, polyhydroxypropylmethacryl-amidephenol,

Dosage forms with predetermined amounts of lercanidipine hydrochloride may be formulated starting with compositions with known quantities of lercanidipine hydrochloride using methods well known in the art. In a preferred embodiment a dosage form is obtained by mixing compositions comprising known quantities of crystalline lercanidipine hydrochloride, e.g., Form (I) or (II), optionally including non-crystalline lercanidipine hydrochloride. Further preferred is where a dosage form with predetermined amounts of crystalline lercanidipine hydrochloride is formulated by mixing compositions comprising essentially pure crystalline lercanidipine hydrochloride are mixed to form dosage forms comprising a predetermined ratio of crystalline Forms (I) and (II).

Tablets or pills can be coated or otherwise compounded to form a unit dosage form which has delayed and/or prolonged action, such as time release and sustained release unit dosage forms. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of a layer or envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release.

Biodegradable polymers for controlling the release of the active agents, include, but are not limited to, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydro-pyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

For liquid dosage forms, the active substances or their physiologically acceptable salts are brought into solution, suspension or emulsion, optionally with the usually employed substances such as solubilizers, emulsifiers or other auxiliaries. Solvents for the

Suitable edible oils include, but are not limited to, cottonseed oil, sesame oil, coconut oil and peanut oil.

Examples of additional additives include, but are not limited to, sorbitol, talc, stearic acid, dicalcium phosphate and polydextrose.

Unit Dosage Forms

The pharmaceutical composition may be formulated as unit dosage forms, such as tablets, pills, capsules, caplets, boluses, powders, granules, sterile parenteral solutions, sterile parenteral suspensions, sterile parenteral emulsions, elixirs, tinctures, metered aerosol or liquid sprays, drops, ampoules, autoinjector devices or suppositories. Unit dosage forms may be used for oral, parenteral, intranasal, sublingual or rectal administration, or for administration by inhalation or insufflation, transdermal patches, and a lyophilized composition. In general, any delivery of active ingredients that results in systemic availability of them can be used. Preferably the unit dosage form is an oral dosage form, most preferably a solid oral dosage form, therefore the preferred dosage forms are tablets, pills, caplets and capsules. Parenteral preparations (e.g., injectable preparations and preparations for powder jet systems) also are preferred.

Solid unit dosage forms may be prepared by mixing an active agent of the present invention with a pharmaceutically acceptable carrier and any other desired additives as described above. The mixture is typically mixed until a homogeneous mixture of the active agents of the present invention and the carrier and any other desired additives is formed, *i.e.*, until the active agent is dispersed evenly throughout the composition. In this case, the compositions can be formed as dry or moist granules.

Suitable binders include, but are not limited to, starch; gelatin; natural sugars, such as glucose, sucrose and lactose; corn sweeteners; natural and synthetic gums, such as acacia, tragacanth, vegetable gum, and sodium alginate; carboxymethylcellulose; hydroxypropylmethylcellulose; polyethylene glycol; povidone; waxes; and the like.

Suitable disintegrants include, but are not limited to, starch, e.g., corn starch, methyl cellulose, agar, bentonite, xanthan gum, sodium starch glycolate, crosspovidone and the like.

Suitable lubricants include, but are not limited to, sodium oleate, sodium stearate, sodium stearyl fumarate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like.

A suitable suspending agent is, but is not limited to, bentonite, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, agar-agar and tragacanth, or mixtures of two or more of these substances, and the like.

Suitable dispersing and suspending agents include, but are not limited to, synthetic and natural gums, such as vegetable gum, tragacanth, acacia, alginate, dextran, sodium carboxymethylcellulose, methylcellulose, polyvinyl-pyrrolidone and gelatin.

Suitable film forming agents include, but are not limited to, hydroxypropylmethylcellulose, ethylcellulose and polymethacrylates.

Suitable plasticizers include, but are not limited to, polyethylene glycols of different molecular weights (e.g., 200-8000 Da) and propylene glycol.

Suitable colorants include, but are not limited to, ferric oxide(s), titanium dioxide and natural and synthetic lakes.

effective amount of isolated lercanidipine hydrochloride crystalline Form (I), isolated lercanidipine hydrochloride crystalline Form (II), or combinations thereof to a subject in need of such treatment.

Pharmaceutical Compositions

The compounds and polymorphs of the present invention may be formulated into a pharmaceutical composition. The pharmaceutical compositions according to the present invention may comprise lercanidipine hydrochloride (I), (II) or a mixture thereof. When said compositions contain a mixture of said crystalline forms the weight ratio of form (I) : (II) is preferably comprised between 1:9 and 9:1 more preferred embodiments of said pharmaceutical compositions are those wherein said weight ratio (I): (II) is selected from : 9:1, 7:3, 1:1, 3:7 and 1:9. The pharmaceutical composition also may include optional additives, such as a pharmaceutically acceptable carrier or diluent, a flavorant, a sweetener, a preservative, a dye, a binder, a suspending agent, a dispersing agent, a colorant, a disintegrant, an excipient, a film forming agent, a lubricant, a plasticizer, an edible oil or any combination of two or more of the foregoing.

Both crystalline forms can undergo micronization, using any method known in the art. The average size of particle produced by this method are preferably D(50%)2-8 μm, D(90%)<15 μm.

Suitable pharmaceutically acceptable carriers or diluents include, but are not limited to, ethanol; water; glycerol; propylene glycol, aloe vera gel; allantoin; glycerin; vitamin A and E oils; mineral oil; PPG2 myristyl propionate; magnesium carbonate; potassium phosphate; vegetable oil; animal oil; and solketal.

In addition to differences in melting point, the two crystalline forms exhibit differences in x-ray structure, solubility, and bioavailability. Solubility studies show that Form (I) is more soluble than Form (II) in water, ethanol, and mixtures thereof (See Tables 2 & 3). Bioavailability studies in dogs and humans indicate that Form (II) is more bioavailable than Form (I). The study in humans also indicates, however, that Form (I) has a shorter time to maximum concentration attainable and is thus suitable for use in immediate release formulations and dosage forms. Finally, x-ray diffraction studies show that these two forms have different diffraction patterns (see Figures 11 and 12 and Example 20). Form I has a smaller crystal and hence particle size before micronization and so is easier and faster to process than Form II, which presents with larger crystals.

The present application further discloses pharmaceutical formulations and unit dosage forms that comprise one of the isolated polymorphs of the present invention or a mixture thereof of predetermined polymorph content.

The present invention is also directed to a method of treating a subject with hypertension (e.g., essential hypertension, secondary hypertension or isolated systolic hypertension), coronary heart disease (e.g., chronic stable angina, myocardial infarction) or congestive heart failure the method comprising administering a therapeutically effective amount of isolated lercanidipine hydrochloride crystalline Form (I), lercanidipine hydrochloride crystalline Form (II), or combinations thereof of predetermined polymorph content (optionally with other form of lercanidipine, such as amorphous form) to a subject in need of such treatment.

The invention also contemplates a method of treating and preventing atherosclerotic lesions in arteries of a subject, the method comprising administering a therapeutically

d'''') dissolving crude lercanidipine hydrochloride or its crystalline Form (I) in a protic polar or an aprotic dipolar solvents preferably containing up to 50% by weight of water at a temperature preferably between 20 and 70°C to produce a solution;

e'''') stirring the solution of step d'''') at a temperature preferably between 20 and 25°C to produce a solid;

f'''') filtering the solid of step e'''') and drying (*e.g.*, in an oven) at preferably 70°C for preferably 12-18 hours.

The second alternative method may optionally comprise the step of adding up to 60% water to the solution of step d'''') prior to step e''''). The second alternative method may further comprise irradiating with ultrasound and/or adding preferably authentic crystalline seeds of Form (II) to step e''''). In step e''''), crystallization is considered complete when the content of the solution is $\leq$2% lercanidipine HCl. In a preferred embodiment, the protic polar solvent is an alcohol solvent such as, but not limited to, methanol, ethanol, n-propanol, isopropanol. In another preferred embodiment, the aprotic dipolar solvent is N-methylpyrrolidone.

The preferred process for preparing Form (I) is the γ process and the preferred process for preparing Form (II) is the δ process. Applicants have determined that Form (I) can be quantitatively obtained by use of C_1-C_5 anhydrous alcohol (preferably anhydrous ethanol or isopropanol) or C_1-C_5 alcohol containing up to 5% water under controlled conditions d' -f'). In fact, the foregoing processes, especially the γ and δ processes can be used to produce the desired polymorph reproducibly and consistently.

e") cooling of the mixture of step d") to room temperature and stirring preferably for 24 hours to form a solid,

f") filtering the solid obtained from step e") and drying it preferably in an oven.

In step e"), crystallization is considered complete when the content of the solution is ≤2% lercanidipine HCl.

The present application also contemplates two additional methods for producing Form (II).

First Alternative Process for Making Form (II)

The first alternative method comprises the steps of:

d"") adding isopropanol or ethanol, preferably ethanol, with a water content preferably between 5 to 10% by weight to lercanidipine hydrochloride, refluxing with stirring to produce a solution;

e"") cooling the mixture to a temperature preferably between 20 and 40°C and stirring for a period preferably between 24 and 96 hours to form a solid;

f"") filtering the solid and drying (e.g., in an oven) at preferably 70°C for 12-18 hours to produce lercanidipine hydrochloride Form (II).

In step e""), crystallization is considered complete when the content of the solution is ≤ 2% lercanidipine HCl.

Second Alternative Method for Making Form II

The second alternative method of obtaining the Form (II) polymorph comprises the steps of:

Alternative Process for Making Form (I)

The present application also contemplates an alternative method of producing lercanidipine hydrochloride having crystalline Form (I) which comprises the steps of:

d') adding ethanol to crude lercanidipine hydrochloride, preferably at a weight/volume ratio of lercanidipine hydrochloride solvent of 1:4 to 1:6, most preferably 1:4, refluxing under stirring in order to obtain a solution (if the solution is not clear it should preferably be filtered hot), cooling under stirring, preferably to 20°C, and adding crystalline seeds of Form (I);

e') cooling the seeded mixture of step d'), preferably to a temperature between 10 and 15°C, and stirring at this temperature for a period of time preferably between 24 and 96 hours to form a solid; and

f') filtering and drying the solid of step .e'), it preferably in an oven at preferably 70°C to obtain lercanidipine hydrochloride Form (I).

In step e'), crystallization is considered complete when the content of the solution is $\leq$2% lercanidipine hydrochloride. Crystalline seeds of Form (I) may also be added to steps e') to further promote crystal formation .

Process for Making Form (II)

The second purification process (δ process), which yields crystalline Form (II), comprises the steps of:

d") adding acetonitrile to crude lercanidipine hydrochloride (Form (A) or Form (B)) and heating the mixture under reflux and stirring.

specifically about 198.7°C. DSC analysis of crystalline Form (II) indicates that it has a melting peak of about 207°C to about 211°C, specifically about 209.3°C.

One purification process (γ process), that leads to formation of one of the novel crystalline forms (Form (I)) comprises the following steps:

Process for Making Form (I)

d) adding isopropanol to crude lercanidipine hydrochloride (Form (A) or Form (B)) and heating under reflux with stirring to produce a solution (if the solution is not clear, it should be filtered hot);

e) cooling the solution of step d) preferably to a temperature between 30 and 40°C and stirring for a period of time preferably between 12 and 48 hours to produce a solid; and

f) filtering the solid obtained from step e), washing the solid with isopropanol, re-filtering the solid, and drying the solid (*e.g.*, in an oven) at preferably 70°C for a period of time preferably between 12-48 hours.

Crude Form (C) may be also be used as starting material in step d). In such case, however, there is the risk of decreased yield of product because the solution should be filtered hot, resulting in the increased loss of lercanidipine hydrochloride in step d). In step e), crystallization is considered complete when the content of the solution is $\leq$2% lercanidipine HCl. Other alcohols may also be used as the solvent in step d). An alternatively preferred solvent is a C_1-C_5 alcohol containing a maximum of 5% water, e.g., anhydrous ethanol. Crystalline Form (I) may be added in step (e) as seeds to further promote crystal formation.

is achieved and (2) a water content, measured according to Karl Fischer, between 0.9 and 1.1% is obtained;

iv'). cooling the solution of step iii') preferably to 0-5°C to obtain a solid;

v') filtering the solid of step iv');

vi') re-suspending the solid of step v') preferably in ethyl acetate and stirring at preferably 60-65°C for about 1 hour; and

vii') cooling the suspension of step vi') preferably to 5-10°C , filtering and drying the solid obtained, preferably in an oven at about 70°C.

The temperature of step vii') should be carefully controlled at 5-10°C to maximize yield.

These novel crude forms of lercanidipine hydrochloride present the advantage of higher solubility and faster drying rate compared to Form (C) and make a simplified further crystallization process possible (which can advantageously be used to prepare Form (I) or Form (II)). Compared to the crude form produced by the method of U.S. Patent No. 5,912,351, these forms permit use of less solvent to recrystallize the compound. This also increases yield by reducing loss of compound. Additionally, the methods used to produce these crude forms are more adaptable to use in a large scale setting and commercial setting.

It has been surprisingly found that each of crude lercanidipine hydrochloride Form (A) and Form (B), when undergoing different purification treatments, result in two novel and different crystalline forms of lercanidipine hydrochloride. Studies indicate that these novel crystalline forms have different physical and chemical properties. DSC analysis of crystalline Form (I) indicates that it has a melting peak of about 197°C to about 201°C,

ii) removing water from the reaction mixture of step i), preferably by azeotropic distillation under vacuum at 200-300 mmHg at a temperature below about 60°C (preferably at 40-50°C);

iii) concentrating the mixture of step ii) preferably to about 1/3 of the initial volume at the same temperature and pressure as in step (ii), adding fresh solvent (e.g., ethyl acetate) preferably to obtain the initial volume, thus obtaining a suspension with a water content, as determined according to Karl Fischer (U.S. Pharmacopoeia 25, Method 921) preferably between 0.10 and 0.15%;

iv) cooling the suspension of step iii) preferably to 0-5°C ;

v) filtering the solid of step iv);

vi) re-suspending the solid of step v) preferably in ethyl acetate and stirring preferably at 60-65°C for about 1 hour; and

vii) cooling to 5-10°C, filtering and drying the obtained solid (e.g., in an oven at about 70°C).

The second process (β protocol; used to prepare Form (B)) is performed using the following steps:

i') washing the mixture of step (b) preferably with water,

ii') removing the water from step i') preferably by azeotropically refluxing the product of step i') with a Dean Stark apparatus until a water content of about 2%, measured according to Karl Fischer, is obtained;

iii') concentrating the mixture of step ii') to preferably ¾ of the initial volume and adding fresh solvent (ethyl acetate) to the mixture preferably until (1) the initial volume

has a melting point of about 131°C to about 135°C (DSC peak), and Form (C) has a melting point of about 186°C to about 192°C (DSC peak). Additionally, thermogravimetric studies show that Form (A) comprises 3 - 4 % residual ethyl acetate and Form (B) comprises 0.3-0.7 % residual ethyl acetate, by weight. Comparatively, the residual solvent present in Form (C) has been determined to be 0-0.1%.

Aspects of the invention are directed to processes for the preparation of lercanidipine hydrochloride, each resulting in a different crude form of the product. The first two steps in producing either crude form are identical and are:

(a) reacting 2, 6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (prepared as described in German patent DE 2847 237) with thionyl chloride or oxalyl chloride in a mixture of an aprotic dipolar solvent and of an aprotic polar solvent to yield a chloride compound, and

(b) in-situ reaction of the chloride obtained from the above step with 2, N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propyl alcohol, at a temperature preferably between –5 and +5°C, in a mixture of an aprotic dipolar solvent and of an aprotic polar solvent.

In a preferred embodiment, the mixture of an aprotic dipolar solvent and of an aprotic polar solvent is ethyl acetate and dimethylformamide used at a ratio of 4:1.

After the in-situ reaction, the lercanidipine hydrochloride is isolated and recovered from the mixture. The method of isolation used determines the crude form of lercanidipine hydrochloride obtained. Following the protocol below (α protocol) yields Form (A):

i) washing the mixture of step (b), preferably with water,

term crystalline forms refers to Forms (I) and (II) of lercanidipine hydrochloride. These crystalline forms have an HPLC purity $\geq$ 99.5 % and residual solvents content of < 3000 ppm.

As used herein, the term "polymorphism" refers to a property of a compound to crystallize in two or more forms with distinct structures. The different crystalline forms can be detected directly by crystallographic techniques or indirectly by assessment of differences in physical and/or chemical properties associated with each particular polymorph.

As used herein, a "subject in need of treatment" is a mammalian (e.g., human) subject suffering from or at risk of developing the particular condition to be treated, e.g., essential hypertension, secondary hypertension, isolated systolic hypertension , coronary heart disease (e.g., chronic stable angina, myocardial infarction), congestive heart failure. A subject in need of treatment for arterial hypertension may be identified using methods well known in the art such as, for example, by direct measurement of blood pressure using, for example, a manual sphygmomanometer, automatic/electronic devices or ambulatory blood pressure monitoring.

The present invention contemplates any method that may be used to produce the novel crude forms of lercanidipine hydrochloride described herein. These forms have different physico-chemical properties, e.g., melting points (which can be determined by DSC analysis), than the crude form of lercanidipine hydrochloride produced by other known methods, *e.g.*, by the method described in U.S. Patent No. 5,912,351; termed Form (C). Form (A) has a melting point of about 150°C to about 152°C (DSC peak), Form (B)

The purity and uniformity of Forms (I) and (II) allow for increased ease in production of lercanidipine dosage forms, due to, e.g., more precisely defined physico-chemical characteristics, such as, for example, increased uniformity of particle size following micronization and more reproducible solubility. Forms (I) and (II) also provide dosage forms with more precisely defined pharmacological characteristics, e.g., bioavailability, compared to previously achieved dosage forms that varied from batch-to-batch in their physico-chemical characteristics.

In a human study in man, where the plasma levels of lercanidipine were assessedafter administration of a single dose of either lercanidipine hydrochloride Form (I) or (II), Form (I) had a shorter time in obtaining the maximum concentration in plasma, relative to Form (II). Hence, Form (I) is more suited for immediate release formulations and dosage forms. From the same study, Form (II) showed a higher bioavailability, relative to Form (I), and is thus suited for use in controlled release formulations and dosage forms. Accordingly, the availability of pure Forms (I) and (II) provides for the ability to blend the two polymorphs into dosage forms with novel controlled characteristics, e.g., a dosage form with both a rapid onset and sustained biological action.

As used herein, the term "crude form" refers to precipitated solid forms comprising crystals of a compound that have not been washed and/or recrystallized to remove impurities (including but not limited to solvent) that may be present. In the present specification, the crude forms are referred to as Forms (A) and (B) of lercanidipine hydrochloride.

As used herein, the term "crystalline form" refers to crystals of a compound that have been washed and recrystallized to remove impurities. In the present invention, the

within the rage of about 197°C to about 201°C, more specifically, about 198.7°C, and the X-ray diffraction pattern set forth, supra.

Form (II) is characterized by a more pronounced yellow color, larger crystal size, slightly lower solubility in aqueous media (all compared to Form (I)), and a melting point (DSC peak) within the range of about 207-211°C, more specifically about 209.3°C.

Both Form (I) and Form (II) are stable. Form II exhibited higher bioavailability in the dog, and was also non equivalent to form I in man, showing a higher plasma concentration (AUCo-t) and a delayed time of maximum concentration (tmax), compared to Form (I).

Previously known methods for producing crystalline lercanidipine hydrochloride were inconsistent in producing lercanidipine hydrochloride with predictable physical and chemical characteristics. Hence, the previously known methods had the undesirable property of producing lercanidipine hydrochloride that varied, e.g., in physico-chemical properties, from batch to batch, even among batches produced by the same process and under the same conditions. The present inventors have discovered that the source of inconsistency exhibited by the previously known methods of producing lercanidipine hydrochloride is the presence of varying and unpredictable amounts of crystalline lercanidipine hydrochloride Form (II). In contrast to previously known methods of producing lercanidipine hydrochloride, the invention provides the novel crystalline Forms (I) and (II) that represent crystalline forms of lercanidipine hydrochloride of a purity and uniformity that has not been obtained with previously achieved solid forms of lercanidipine hydrochloride.

Additionally, the various novel forms have distinct chemical and physical properties and bioavailability profiles in animals, including man, as discussed herein.

The novel methods for preparation of crude of lercanidipine hydrochloride are suitable for highly reproducible commercial scale production of reproducible solid compositions of lercanidipine hydrochloride. The methods advantageously produce novel crude Forms (A) and (B) of lercanidipine hydrochloride which also exhibit characteristics desirable for industrial applications. Crude Forms (A) and (B), e.g., exhibit higher solubility and faster drying rates compared to other crude forms of lercanidipine hydrochloride that have previously been reported. Crude Forms (A) and (B) further allow simplified crystallization procedures to be used for production of novel isolated crystalline forms of lercanidipine hydrochloride.

The novel isolated crystalline forms of lercanidipine hydrochloride of the present invention can be obtained from lercanidipine hydrochloride crude Forms (A) and (B) and are termed lercanidipine hydrochloride crystalline Form (I) and Form (II). Either of isolated Form (I) or isolated Form (II) may be reproducibly obtained from the (A) and (B) intermediates by varying the crystallization conditions as described below. Forms (I) and (II) may also be obtained using other starting materials. Both of Forms (I) and (II) may be obtained using, for example, crude lercanidipine Form (C) as starting material, as described herein. Form (II) may also be obtained using Form (I) as starting material, as described herein.

Both lercanidipine hydrochloride crystalline Forms (I) and (II) exhibit good stability. Form (I) is characterized by a paler yellow color, smaller crystal size, higher solubility in aqueous media (all compared to Form (II)), and a melting point (DSC peak)

angles of the most significant peaks can be found in Tables 1 and 2 below. The ordinate indicates the number of counts/sec and the abscissa shows the values of 2θ angles.

Figures 13 and 14 are plots of percent mass change as a function of time in hygroscopicity tests carried out on Forms (I) and (II) of lercanidipine hydrochloride, respectively. The ordinate on the left indicates percent mass changes and the ordinate on the right percent relative humidity; the abscissa indicates time in minutes. The protocol for the hygroscopicity tests are described in Example 14.

Figures 15 and 16 show X-ray diffraction spectra at wavelength Kα of crude lercanidipine hydrochloride Form (A) and of crude lercanidipine hydrochloride Form (B), respectively.

Figures 17 and 18 show Raman spectra of crude lercanidipine hydrochloride Form (A) and of crude lercanidipine hydrochloride Form (B), respectively, where the ordinate represents Raman units and the abscissa represents wave number (cm^{-1}).

Figures 19 and 20 show the results of the thermogravimetric analysis carried out on crude lercanidipine hydrochloride Form (A) and on crude lercanidipine hydrochloride Form (B), respectively. In these figures, the abscissa indicates temperature (in °C) and the ordinate indicates percent mass variation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention discloses novel crude forms and crystalline forms of lercanidipine hydrochlorides and processes for making them. Applicants have determined that lercanidipine hydrochloride exhibits polymorphism and crystallizes in different forms depending on the process followed and on the solvents used, especially for crystallization.

Figure 4 is a graph of solubility at 25°C of Forms (I) and (II) in ethanol at increasing water concentrations. The experiments are described in Example 15. The ordinate indicates % solubility expressed as w/w and the abscissa % by weight of water in ethanol.

Figure 5 is a graph of solubility at 40°C of Forms (I) and (II) in ethanol at increasing water concentrations. The tests are described in Example 15. The ordinate indicates % solubility expressed as w/w and the abscissa % by weight of water in ethanol.

Figure 6 shows ^{13}C NMR spectra in solid phase of crystalline Form (I). The signals and attributes of the corresponding carbon atoms can be found in Table 4.

Figure 7 shows ^{13}C NMR spectra in solid phase of crystalline Form (II). The signals and attributes of the corresponding carbon atoms can be found in Table 5.

Figure 8 shows IR spectra of Form (I). The signal and corresponding attributes can be found in Table 6.

Figure 9 shows IR spectra of Form (II). The signal and corresponding attributes can be found in Table 7.

Figure 10 represents percent average concentration of lercanidipine hydrochloride in dog plasma after administration of crystalline Form (I) and of crystalline Form (II) in an amount of 3 mg/kg, in the form of a hard gelatin capsule. The ordinate indicates the mean value of concentration in plasma and the abscissa indicates time (in minutes).

Figures 11 and 12 show X-ray diffraction spectra at wavelength Kα of crystalline Forms (I) and (II), respectively. The distances (d) in X, the (I/Io) ratios and values of 2θ

hydrochloride crystalline Form (II), or combinations thereof comprising a predetermined content of each form to a subject in need of such treatment.

In other embodiments, a method of treating or preventing atherosclerotic lesions in arteries of a subject is provided, the method comprising administering a therapeutically effective amount of lercanidipine hydrochloride crystalline Form (I), lercanidipine hydrochloride crystalline Form (II), or combinations thereof comprising a predetermined amount of each form, to a subject in need of such treatment. In preferred aspect, a subject in need of treatment is a mammal. Most preferably the subject in need of treatment is a human.

These and other aspects of the present invention will be apparent to those of ordinary skill in the art in light of the present description, claims and figures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of DSC analysis carried out on crystalline Form (I), according to the working conditions described in Example 12. The ordinate indicates heat flow in mW and the abscissa temperature in °C.

Figure 2 is a graph of DSC analysis carried out on crystalline Form (II), according to the working conditions described in Example 12. The ordinate indicates heat flow in mW and the abscissa temperature in °C.

Figure 3 is a graph of the results of the thermogravimetric tests carried out on Form (I) and Form (II), respectively, as described in Example 13. The abscissa indicates temperature in °C and the ordinate indicates percent mass variation.

The present invention thus permits obtaining mixtures of Form I and Form II having a predetermined and reproducible content of each form, and optionally, also other forms of lercanidipine, such as amorphous.

Also provided are methods of syntheses in which each of isolated lercanidipine hydrochloride crystalline Form (I) and Form (II) may be obtained, independently, from the starting material of lercanidipine hydrochloride crude Form (A) or crude Form (B).

Also provided are pharmaceutical compositions comprising (1) crystalline lercanidipine hydrochloride and optionally other forms of lercanidipine, such as amorphous, wherein the crystalline lercanidipine hydrochloride is selected from the group consisting of lercanidipine hydrochloride crystalline Form (I), lercanidipine hydrochloride crystalline Form (II), and combinations thereof comprising a predetermined content of each crystalline form, and (2) at least one component selected from the group consisting of a pharmaceutically acceptable carrier or diluent, a flavorant, a sweetener, a preservative, a dye, a binder, a suspending agent, a dispersing agent, a colorant, a disintegrant, an excipient, a lubricant, a plasticizer, and an edible oil.

In certain embodiments the aforementioned pharmaceutical compositions are provided as a dosage form comprising lercanidipine hydrochloride crystalline Form (I) or Form (II) or a combination thereof having a predetermined formulation of each crystalline Form.

In further embodiments, the invention also provides for methods of treating a subject with arterial hypertension, the method comprising administering a therapeutically effective amount of lercanidipine hydrochloride crystalline Form (I), lercanidipine

D (X)	Relative intensity (I/Io)	2 θ angle
16.3	83	5.4
6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

The lercanidipine hydrochloride crystalline Form (I) has a melting point of about 197-201°C, when said melting point is determined as DSC peak.

In an alternative embodiment, isolated lercanidipine hydrochloride crystalline Form (II) is provided, which has the following X-ray diffraction pattern, at wavelength Kα, as shown wherein distances, (I/Io) ratios, and 2 θ angles of significant peaks are:

D (X)	Relative intensity (I/Io)	2 θ angle
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8
3.81	41	23.4
3.77	100	23.6
3.58	44	24.8
3.54	29	25.2

The lercanidipine hydrochloride crystalline Form (II) has a melting point of about 207-211°C, when said melting point is determined as DSC peak.

different composition having melting points within the same narrow range as the individual forms. As a result, more reproducible batches of lercanidipine more suitable for large scale manufacture and quality control were needed.

SUMMARY OF THE INVENTION

The present invention provides novel crude forms and crystalline forms of lercanidipine hydrochloride and processes for making them.

In one embodiment, the invention provides novel crude lercanidipine hydrochloride Form (A), which has a melting point of about 150-152°C (DSC peak) and comprises about 3-4% (w/w) ethyl acetate.

In another embodiment, the invention provides novel crude lercanidipine hydrochloride Form (B) which has a melting point of about 131-135°C (DSC peak) and comprises about 0.3-0.7% (w/w) ethyl acetate.

Methods are provided for the independent syntheses of crude lercanidipine hydrochloride Form (A) and crude lercanidipine hydrochloride Form (B), making possible to obtain each crude form in isolated form.

In a further embodiment, isolated lercanidipine hydrochloride crystalline Form (I) is provided which has the following X-ray diffraction pattern, at wavelength Kα wherein distances between peaks (D in Å), relative intensity ratios (I/Io) ratios, and angles of significant peaks (2θ) are:

Therefore, there is a need in the art for a process for the preparation of lercanidipine hydrochloride in crystalline form which avoids one more of the disadvantages of the currently used processes.

In addition, it was observed that lercanidipine, as produced by the second-described process above, displayed batch-to-batch variability despite careful process control and even observation of the melting point believed to be characteristic of the solid product obtained by the process of Example 3 of USP 5,767,136 of 186-188°C. This variability was manifest in seemingly unpredictably appearing (and disappearing) differences in one or more of product appearance (e.g., color), melting point and solubility. This raised issues as to whether assurances of purity and/or reproducibility can be made (e.g., to regulatory authorities) that the product is always the same.

Further research by the present inventors revealed batch-to-batch differences in bioavailability in animals, and differences in crystal size. In the course of researching the causes of the variability problem, the inventors surprisingly discovered novel lercanidipine hydrochloride polymorphs. They also discovered more suitable processes for the preparation and isolation of crystalline lercanidipine hydrochloride products from the reaction mixture. It was surprisingly determined that lercanidipine hydrochloride shows polymorphic features and crystallizes into different crystalline forms depending on the process followed and on the solvents used. Furthermore, the isolation of each of individual crystalline polymorphs has become possible, thus decreasing the possibility of batch to batch variability of lercanidipine, which the present inventors determined was due to mixtures of different solid forms being present by the same batch and to such mixtures of

hydrochloride salt is prepared by treatment with diluted hydrochloric acid in the presence of sodium chloride.

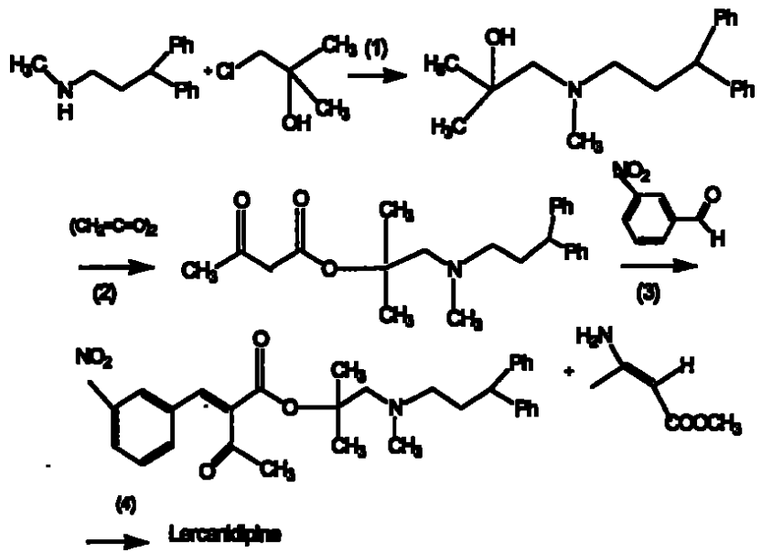
A major disadvantage of the process of preparing lercanidipine, as it is described in U.S. Patent No. 4,705,797, is that the disclosed cyclization reaction generates several by-products, which results in a lower yield for the desired product. Moreover, the purification and isolation of lercanidipine from the reaction mixture is quite complex, since it requires numerous treatments with different solvents. Finally, the purification and isolation steps are difficult to perform on an industrial scale because of the necessity of purifying the product by column chromatography.

U.S. Patent 5,912,351 describes a simpler process for the preparation of lercanidipine hydrochloride. It involves reaction of 1,4-dihydro-2,6-dimethyl-5-methoxycarbonyl-4- (3-nitrophenyl) pyridine-3-carboxylic acid with thionyl chloride in dichloromethane and dimethylformamide at a temperature between –4 and +1°C and subsequent esterification of the obtained acid chloride with 2, N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propyl alcohol at a temperature between –10 and 0°C. The process yields lercanidipine hydrochloride in an anhydrous non-hygroscopic crystalline form, and avoids the formation of unwanted by-products and the subsequent purification on chromatography columns.

However, the isolation of lercanidipine hydrochloride in crystalline form is again quite complex. After evaporating the solvent from the reaction mixture and dissolving the residue thus obtained in ethyl acetate, the solution is washed first with brine, then washed further five times with a 10% solution of sodium carbonate, five times with 1N hydrochloric acid, and eventually once again with brine.

comparison to other calcium antagonists, lercanidipine is characterized by gradual onset and long-lasting duration of action despite decreasing plasma levels. *In vitro* studies show that isolated rat aorta response to high K^+ may be attenuated by lercanidipine, even after the drug has been removed from the environment of the aortic tissue for 6 hours.

Lercanidipine is commercially available from Recordati S.p.A. (Milan, Italy) and has been described along with methods for making it and resolving it into individual enantiomers in U.S. Patents 4,705,797; 5,767,136; 4,968,832; 5,912,351; and 5,696,139. A process for preparing lercanidipine described in U.S. Patent No. 4,705,797 involves the following scheme:



(1): xylene at reflux; (2): toluene, 85°C; (3) HCl +$CHCl_3$; 0°C; (4) HO-$CH(CH_3)_2$ at reflux

Crude lercanidipine is an oily residue that must be purified by flash chromatography using chloroform, containing increasing amounts of acetone, as the eluant. The solvent is then evaporated to dryness and remaining residue is dissolved in methanol adding a small excess of hydrochloric acid in ethanol. After evaporation of the solvent, the hemi-hydrated

NOVEL CRYSTALLINE POLYMORPHIC FORMS OF LERCANIDIPINE HYDROCHLORIDE AND PROCESS FOR THEIR PREPARATION

FIELD OF THE INVENTION

The invention is directed to novel crude forms and crystalline forms of lercanidipine hydrochloride, and to processes for the preparation of these forms. Pharmaceutical compositions comprising the novel crystalline forms are also contemplated.

BACKGROUND OF THE INVENTION

Lercanidipine (methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate) is a highly lipophilic dihydropyridine calcium antagonist with long duration of action and high vascular selectivity. Its mechanism of antihypertensive activity is attributed to a direct relaxant effect on vascular smooth muscle, which lowers total peripheral resistance. The recommended starting dose of lercanidipine as monotherapy is 10 mg daily by oral route, with a drug titration as necessary to 20 mg daily. Lercanidipine is rapidly absorbed following oral administration with peak plasma levels occurring 2-3 hours following dosing. Elimination is essentially via the hepatic route.

By virtue of its high lipophilicity and high membrane coefficient, lercanidipine combines a short plasma half life with a long duration of action. In fact, the preferential distribution of the drug into membranes of smooth muscle cells results in membrane-controlled pharmacokinetics characterized by a prolonged pharmacological effect. In

Form (II) is less soluble than Form (I) in both solvents.

15.2 Solubility in mixtures of water-ethanol at 25°C and at 40°C, with increasing water concentrations

Figures 4 and 5 show solubility in water-ethanol at 25°C and at 40°C of Form (I) and of Form (II). The maximum solubility is reached for both forms, at both temperatures, when water concentration is of 20%. Also in this case the solubility of crystalline Form (I) is higher than that of crystalline Form (II).

EXAMPLE 16 Solid phase ^{13}C-NMR studies

The high resolution ^{13}C-NMR solid phase spectra were carried out with the Bruker, ASX300 Instrument equipped with a 7 mm Rotor accessory, using several combined techniques:

Magic angle spinning (MAS). About 300 mg of the sample was placed in the rotor spinning at 4.3 kHz around an axis oriented at the magic angle (54° 70') to the magnetic field to overcome the dipolar bradening caused by CSA (Chemical Shift Anisotropy). The experiments were conducted at room temerature.

Dipolar Coupling. Since much of line broadening in ^{13}C spectra of organic solids is due to coupling to protons, it was removed by heteronuclear decoupling (decoupling power level was almost 1 Kilowatt).

Cross polarization (CP). Cross polarization allowed carbon magnetization from larger proton magnetization via the dipolar coupling to increase signal intensity.

The exposure of Form (II) to humidity in DVS causes a negligible mass variation (< 0.05%) in the whole RH range tested.

EXAMPLE 15 Solubility of crystalline Forms (I) and (II)

15.1 Solubility in water and in ethanol at room temperature

The solubility at 23°C of both crystalline Forms (I) and (II) was evaluated by UV-Visible spectroscopy in bi-distilled water (at the pH value spontaneously reached by the system) and in absolute ethanol. The molar absorptivity had been previously determined in acetonitrile. The same molar absorptivity was considered for the determination in water and in ethanol. Solubility in water certainly depends on pH. The residual solid obtained by filtration of the suspension was immediately analyzed with Raman spectroscopy. The results are shown in the following Tables 2 and 3.

TABLE 2. Solubility in water (about 40 mg/ml as initial condition).

Starting material	Time [min]	Solubility [mg/ml]	Residual material
Form (I)	5/25/45/990	0.4/0.5/0.5/0.5	Form (I)
Form (II)	5/25/45/990	0.2/0.2/0.3/0.3	Form (II)

TABLE 3. Solubility in ethanol (100 mg/ml as initial condition)

Starting material	Time [min]	Solubility [mg/ml]	Residual material
Form (I)	15/45/120	28/27/27	Form (I)
Form (II)	15/45/120	11/12/12	Form (II)

acetate. During degradation (T > 170°C) a small amount of ethyl acetate in gas phase could be observed.

The results obtained with crude lercanidipine hydrochloride Form (B) are shown in Figure 20, where a weight loss of 0.5% in temperature range 25-153°C can be observed. The volatile compound identified with its corresponding IR spectrum is ethyl acetate (0.4%) and water (0.1%). During degradation (T > 170°C) a small amount of ethyl acetate in gas phase can be observed.

EXAMPLE 14 Hygroscopicity of crystalline Forms (I) and (II)

The hygroscopicity of both crystalline Forms (I) and (II) was measured with DVS analysis by means of a water absorption analyzer (SURFACE MEASUREMENT SYSTEM, Marion, Buckinghamshire, UK) according to the following working conditions:

10-15 mg of Form (I) and (II) respectively were placed in a quartz sample-holder, placed in its turn on a microbalance, and the sample underwent humidity cycles between 0 and 95%, starting from 50% of relative humidity (25°C, relative humidity (RH): 50-95-0-95-0-50% at RH/h:5%).

The results of the tests are shown in the diagrams of Figures 13 and 14.

14-1 Results obtained with crystalline Form (I)

The exposure of Form (I) to humidity in the DVS analyzer results in a mass change of +0.15% at 95% RH, and of -0.3% at 0% RH, with almost no hysteresis during mass increase and loss. These slight variations are probably due to a reversible surface absorption of water.

14-2 Results obtained with crystalline Form (II)

Table 1.

Compound	Melting T (Tpeak) [°C]	Onset T [°C]
Form (I)	198.7	179.8
Form (II)	209.3	169.0

Immediately after melting of Form (I) or (II) an exothermic event due to salt decomposition can be observed.

EXAMPLE 13 Thermogravimetry

A gravimetric analysis associated with an IR analysis was carried out on both crystalline Forms (I) and (II), and also on crude lercanidipine hydrochloride Form (A) and on crude lercanidipine hydrochloride Form (B), using a Netsch Thermomicrobalance 209 in combination with a spectrometer FTIR Bruker Vector 22.

The tests were carried out according to the following working conditions: 2-5 mg of sample was heated in a steel crucible in nitrogen atmosphere, with a heating speed of 10°C/min. The results obtained with crystalline Forms (I) and (II) are shown in Figure 3, from which it can be inferred that in both crystalline forms no weight loss can be observed up to their melting point (*i.e.*, until about 190-200°C).

During degradation, which takes places as indicated above after melting, a CO_2 loss can be observed.

The results obtained with crude lercanidipine hydrochloride Form (A) are shown in Figure 19, where a weight loss of 3.4% can be observed in the temperature range 25-153°C. The volatile compound has been identified by its corresponding IR spectrum and is ethyl

EXAMPLE 18: Raman Spectra

A Bruker FT-Raman RFS100 Spectrophotometer was utilized under the following typical conditions: about 10 mg sample (without any previous treatment), 64 scans 2 cm^{-1} resolution, 100 mW laser power, Ge-detector.

The following Tables 8 and 9 show the most significant peaks of Raman spectra of Form (I) and Form (II), respectively.

Table 8. Raman spectrum of crystalline Form (I)

Wave number (cm^{-1})	Peak intensity *
3054	M
3040	M
2981	M
2941	M
1675	S
1646	M
1583	M
1489	M
1349	Vs
1236	M
1005	S
821	M
174	M
98	S
73	Vs

* M= moderate; S= strong, Vs =very strong

EXAMPLE 17 IR Studies

The IR (infrared) spectra were recorded in KBr powder by Diffuse Reflectance Technique using a Perkin Elmer Spectrum-one instrument. IR spectra, whose wave lengths and corresponding attribution are shown in the following Tables 6 and 7, are clearly different for the new Forms (I) and (II).

Table 6. IR spectrum in KBr powder of lercanidipine hydrochloride Form (I)

Wavelength (cm^{-1})	Attribution
3186	NH stretching
3100-2800	Alkyl and phenyl stretching
2565	N^+H stretching
1673	C=O stretching
1525; 1348	Asymmetric and symmetric stretching of NO_2 group
1405; 1386	Bending of geminal methyl groups
785-685	Out-of-plane bending of 5 and 3 adjacent hydrogens on aromatic rings

Table 7. IR spectrum in KBr powder of lercanidipine hydrochloride Form (II)

Wavelength (cm^{-1})	Attribution
3183	NH stretching
3100-2800	Alkyl and phenyl stretching
2684	N^+H stretching
1705;1675	C=O stretching
1526; 1350	Asymmetric and symmetric stretching of NO_2 group
1402; 1380	Bending of geminal methyl groups
800-680	Out-of-plane bending of 5 and 3 adjacent hydrogens on aromatic rings

Table 4. Lercanidipine hydrochloride crystalline Form (I)

Chemical shift (, ppm)	Attribution of carbon atoms
168.7; 167.7	9; 11 or 11; 9
150.1 to 120.4	2; 6 and 20 to 37
104.3; 100.9	3; 5 o 5; 3
79.7	12
63.0; 60.1 (weak)	15; 17 o r 17;15
48.6	10
47.7	16
45.4	19
41.1	4
31.6	18
27.7; 26.4	13; 14 or 14; 13
19.6; 18.0	7; 8 or 8; 7

Table 5. Lercanidipine hydrochloride crystalline Form (II)

Chemical shift (, ppm)	Attribution of carbon atoms
168.1; 166.6	9; 11 or 11; 9
151.9 to 121.9	2; 6 and from 20 to 37
104.0; 102.8	3; 5 or 5; 3
79.0	12
66.0; 58.0 (weak)	15; 17 or 17;15
49.7	10
48.8	16
44.3	19
40.5	4
29.8	18
27.6; 23.5	13; 14 or 14; 13
19.6; 18.3	7; 8 or 8; 7

Total suppression of sidebands (TOSS). TOSS was performed using spin-echoes synchronized with the rotation of the sample to cause phase alteration of the spinning sidebands, resulting in cancellation when successive spectra were added together.

Crystalline Forms (I) and (II) show different ^{13}C-NMR spectra in solid phase. The signals (chemical shift) and attribution of the corresponding carbon atoms (as numbered in the formula of lercanidipine hydrochloride shown below) are represented in the following Tables 4 and 5, respectively.

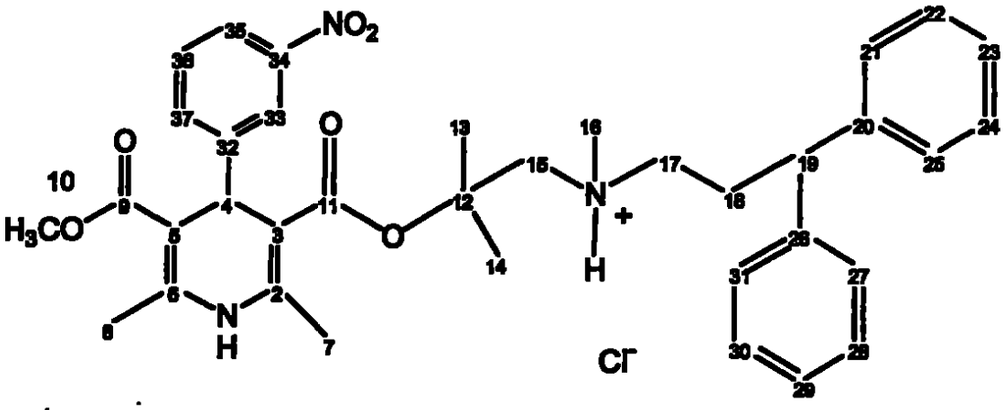


Table 9. Raman spectrum of crystalline Form (II)

Wave number (cm^{-1})	Peak intensity *
3074	M
3064	M
3055	M
3048	M
3030	M
2973	M
2940	M
1675	S
1647	S
1630	M
1584	M
1489	M
1351	Vs
1005	M
995	M
103	Vs
85	S

* M= moderate; S= strong, Vs =very strong

EXAMPLE 19 Bioavailability of crystalline Forms (I) and (II)

Example 19a-Dog

A study was carried out on six Beagle dogs to evaluate the bioavailability of crystalline Forms (I) and (II).

The products, in micronized form, were administered orally by hard gelatin capsules filled up with the active agent, Form (I) and (II), at a dosage of 3 mg/kg, administered once in the morning of the day of the experiment.

Blood samples were taken at given times and plasma concentrations of lercanidipine were determined with a stereoselective analytical method HPLC-MS/MS, according to the following working conditions;

Lercanidipine was extracted from dog plasma by means of a liquid-liquid extraction with a mixture of n-hexane and ethyl ether. The dry residue of the organic phase was taken up with a mixture of methanol and water and a liquid-phase chromatographic separation (LC) was carried out; the two enantiomers of lercanidipine were separated on a CHIROBIOTIC V column (Vancomycin) (particle size 5 m, column size 150 x 4.6 mm (ASTEC, NJ, USA)) and were detected with a mass spectrometer (MS/MS) by using an electrospray technique.

The analytical method was validated in a concentration range between 0.1 and 20 ng/ml of plasma for both enantiomers. The method has shown to be specific with an accuracy of 15%. The average concentrations of lercanidipine in the tables represent the sum of both enantiomers.

The profiles referring to the average concentrations of lercanidipine for both forms are shown in Figure 10. The following Tables 10 and 11 show single values referring to AUC, Tmax, C_{max} and to plasma concentrations.

TABLE 10. Mean values (n=5) of AUC_{0-t}, C_{max} and T_{max} of lercanidipine hydrochloride (S+R) crystalline Form (I) and crystalline Form (II), in dogs, after oral administration at a dosage of 3 mg/kg.

Form (I)

Parameter	Dog 1	Dog 2*	Dog 3	Dog 4	Dog 5	Dog 6	Mean	SD
AUC_{0-t} ng/h/ml	15.41	263.83	27.544	46.57	70.39	28.72	37.73	19.12
T_{max} (h)	2.00	4.00	6.00	3.00	3.00	6.00	4.00	1.67
C_{max} (ng/ml)	8.29	128.87	11.62	27.17	22.58	17.83	17.50	6.91

Form (II)

Parameter	Dog 1	Dog 2*	Dog 3	Dog 4	Dog 5	Dog 6	Mean	SD
AUC_{0-t} ng/h/ml	54.59	119.77	75.62	173.82	142.34	61.91	104.68	43.99
T_{max} (h)	3.00	1.50	1.50	4.00	2.00	6.00	3.00	1.61
C_{max} (ng/ml)	18.46	52.19	19.78	52.64	55.38	18.56	36.17	17.27

* not included in the calculation of mean value

Table 11. Average concentration in plasma of lercanidipine hydrochloride (S+R) crystalline Form (I) and crystalline Form (II), in dogs, after oral administration at a dosage of 3 mg/kg

Form (I)

Time (h)	Dog 1	Dog 2*	Dog 3	Dog 4	Dog 5	Dog 6	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.1	0.20	0.00	0.00	0.00	0.00	0.00	0.02
1	0.59	0.29	0.00	0.00	0.00	0.00	0.12	0.22
1.5	1.83	1.06	0.32	0.00	1.33	0.00	0.70	0.73
2	8.29	8.94	0.94	0.35	17.11	0.28	5.39	6.34
3	4.44	36.39	0.92	27.17	22.58	1.29	11.28	11.11
4	1.81	128.87	9.42	11.07	16.39	6.26	8.99	5.56
6	0.80	26.65	11.62	2.53	9.73	17.83	8.50	6.50

Form (II)

Time (h)	Dog 1	Dog 2*	Dog 3	Dog 4	Dog 5	Dog 6	Mean	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.00	22.67	6.99	0.00	0.00	0.00	1.40	2.61
1	0.00	52.13	16.61	5.50	3.28	0.00	5.08	5.91
1.5	0.23	52.19	19.78	35.43	32.69	3.49	18.32	14.88
2	7.63	35.45	17.81	38.10	55.38	10.19	25.82	19.23
3	18.46	17.43	15.80	28.36	40.57	14.10	23.46	12.56
4	14.83	5.17	14.10	52.64	23.66	13.24	23.69	16.26
6	8.05	4.50	3.62	17.46	6.76	18.56	10.89	6.82

* not included in the calculation of mean value

The formulation containing Form (II) is more bioavailable than the one containing crystalline Form (I) in 5 animals out of 6.

To simplify the comparison, dog 2 was excluded from the evaluation, since after the administration of Form (I) dog 2 shows a plasma AUC of 264 ng/h/ml versus a mean value of 38 ± 19 (SD) of the other 5 dogs. On the other hand, its AUC after administration of Form (I) is similar to that of the other animals, the value being 120 versus 105 ± 44 ng/h/ml.

The bioavailability of lercanidipine hydrochloride (Form (II)), expressed as increase in the AUC of lercanidipine (R+S) obtained after administration of Form (II), is about 3 times higher than that obtained with Form (I). The average profile of plasma concentrations for both crystalline forms is shown in Figure 10.

The analysis of these results shows that the amount of lercanidipine (S+R) absorbed after administration of crystalline Form (II) is 3 times higher that of Form (I), whereas the absorption speed, expressed as T_{max}, is practically unchanged.

Plasma concentrations 6 hours after administration (last sampling time) are similar, the concentrations being of 8.5 ± 6.5 ng/ml after administration of Form (I) and of 10.9 ± 6.8 ng/ml after administration of Form (II).

EXAMPLE 19b-Man

A study was carried out on 16 healty volunteers to assess the relative bioavailability of lercanidipine hydrochloride Form (I) and Form (II). Form (I) was represented by a tablet of Zanedip® corresponding to10 mg of lercanidipine hydrochloride (Reference –R). Form (II) was administered in form of a 10 mg tablet prepared exactly in the same way and with the

same composition of Zanedip[R] 10mg, starting from micronized Form (II) having the same particle size of Form I (Test-T). Blood samples were taken at 15 points from time 0 to 24 h post-dosing and plasma concentrations of lercanidipine were determined with a stereoselective analytical method HPLC-MS/MS. The pharmacokinetic parameters obtained are given in the following table

	Form (I) geom. least square mean	Form (II) geom. least square mean	Point Estimate (T/R)	90%C.I.
AUC $_{0-t}$ (ng·h/mL)	8.82	10.36	1.17	0.93 – 1.48
C_{max} (ng/mL)	3.18	3.22	1.01	0.73 – 1.42
t_{max} (h)	1.50*	2.50*	0.75**	0.00 – 1.25
C_{max}/AUC	0.386^	0.329^	0.85	0.69 – 1.02

* median
** median difference
^ least square mean

The obtained results indicated that lercanidipine hdyrochloride Form (II) was not bioequivalent to Form I, with Form (II) obtaining higher plasma levels, that lercanidipine hydrochloride Form (I) has a t_{max} that is shorter than that of Form (II), suggesting s its use in immediate release formulations.

EXAMPLE 20 X-ray diffraction studies

Philips PW 1710 and Philips X pert PW 3040 powder diffractometer (Copper Kα radiation) were used, under the following typical conditions: about 5-70 mg sample

(without any previous treatment) with application of a slight pressure to obtain a flat surface. Ambient air atmosphere. 0.02° 2θ stepsize, 2 sec step-1, 2-50 2θ.

--The obtained spectra are given in Figures 11 and 12 and the corresponding main peaks are described in Tables 12 and 13. The data are clearly different for new isolated Forms (I) and (II).

Table 12. X RD spectrum of lercanidipine hydrochloride Form (I).

D (Δ)	Relative intensity (I/Io)	2 θ angle
16.3	83	5.4
6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

Table 13. X RD spectrum of lercanidipine hydrochloride Form (II).

D (Δ)	Relative intensity (I/Io)	2 θ angle
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8
3.81	41	23.4
3.77	100	23.6
3.58	44	24.8
3.54	29	25.2

EXAMPLE 21 Melting point determination of various mixtures of lercanidipine hydrochloride crystalline Forms (I) and (II)

The melting points of compositions consisting of known ratios of lercanidipine hydrochloride crystalline Forms (I) and (II) were determined manually. Conditions consisted of using a set point of 177°C and introducing the capillary into the instrument (Melting Point Apparatus model 535, Büchi Labortechnik AG, Flawil, Switzerland) at approximately 5°C below the melting point. Results are shown in Table 14.

Table 14. Melting points of compositions consisting of known ratios of lercanidipine hydrochloride crystalline Forms (I) and (II). Samples in Series A and Series B were heated at a gradient of 1°C/min and 0.5°C/min, respectively. Results are given in °C.

Sample	Pure Form (I)	Ratio lercanidipine hydrochloride crystalline Form (I): Form (II)					Pure Form (II)
		9:1	7:3	1:1	3:7	1:9	
Series A	186.8	188.0	189.5	190.0	192.2	194.2	194.3
Series B	185.9-186.8	184.4-186.1	184.5-187.0	186.7-187.4	186.5-189.4	188.7-190.5	190.6-192.9

U.S. Patent No. 5,767,136 discloses crystalline lercanidipine hydrochloride as having a melting point of 186-188°C. Table 14 shows that this melting point is exhibited by mixtures of Form (I) and Form(II) in which the ratio of Form (I):Form (II) varies between 9:1 to 3:7. Bianchi et al. (Drugs of the Future, 1987, 12:1113-1115) report a

melting point of 186-188°C (non DSC) for a lercanidipine product they characterize as "crystals". Hence, the melting point of a preparation of lercanidipine hydrochloride is not sufficient by itself to distinguish the particular form or forms present therein, and many mixtures of different compositions have the same melting point range.

EXAMPLE 22. Micronization of lercanidipine hydrochloride.

Micronization is carried out by a jet-mill process using a MICRONETTE M300 from the firm NUOVA GUSEO (Villanova sull'Arda -PC- Italy). Parameters are as follows: Injection pressure, 5 Kg/cmq; micronization pressure, 9 Kg/cmq; and cyclone pressure, 2.5 Kg/cmq. Capacity of micronization is 16 Kg/h. Particle size is determined by laser light scattering using a GALAI CIS 1 laser instrument (GALAI, Haifa, Israel). Micronization is performed to obtain an average particle size of D (50%) 2-8 μm and D (90%) < 15 μm.

* * *

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Patents, patent applications, publications, procedures, and the like are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties.

CLAIMS

1. Crude lercanidipine hydrochloride solid Form (A), having a melting point of about 150-152°C (DSC peak) and comprising about 3-4% (w/w) ethyl acetate.

2. Crude lercanidipine hydrochloride solid Form (B), having a melting point of about 131-135°C (DSC peak) and comprising about 0.3-0.7% (w/w) ethyl acetate.

3. A method of producing the crude lercanidipine hydrochloride Form of claim 1, comprising the steps of:

a) reacting 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid with a chloride selected from the group consisting of thionyl chloride and oxalyl chloride in an aprotic dipolar solvent and an aprotic polar solvent to produce the corresponding carbonyl chloride;

b) reacting, *in-situ*, the chloride of step a) with 2, N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propyl alcohol to form crude lercanidipine hydrochloride; and

c) isolating the crude lercanidipine hydrochloride of step b and recovering crude lercanidipine hydrochloride Form (A).

4. The method of claim 3 wherein the reacting step b) is conducted at a temperature between -5 and +5°C.

5. The method of claim 3 wherein step c) comprises the steps of:

i) washing the crude lercanidipine hydrochloride of step b) with water;

ii) removing the water from step i) to produce a mixture;

iii) concentrating the mixture of step ii) and adding solvent to produce a suspension having about the same volume as the initial volume of the mixture of step ii) and a water content, according to Karl Fischer, of between 0.10 and 0.15%;

iv) cooling the suspension obtained in step iii) to obtain a solid;

v) filtering the solid from step iv);

vi) re-suspending the solid of step v) in ethyl acetate;

vii) cooling the suspension of step vi) ; and

viii) filtering and drying the precipitate of step vii) to produce the crude lercanidipine hydrochloride Form (A).

6. The method of claim 3 wherein the chloride in step a) is thionyl chloride.

7. The method of claim 5 wherein step c) ii) comprises removing the water from step c) i) by azeotropic distillation under vacuum within the range 200-300 mm Hg, at a temperature not higher than 60°C, to produce a mixture.

8. The method of claim 5 wherein the resuspending step vi) comprises stirring at 60-65°C for about 1 hour.

9. The method of claim 5 wherein the drying in step viii) is in an oven at 70°C.

10. The method of claim 5, wherein the washing step i) is with water; the mixture in step iii) is concentrated to 1/3 of its initial volume and solvent is added to produce a suspension having about the same volume as the initial volume of said mixture;

and the water content of said suspension according to Karl Fischer , is between 0.1 and 0.15%.

~~11.~~ ~~The method of claim 5, wherein cooling in step iv) is to a~~ temperature within the range of 0-5°C.

12. The method of claim 5 wherein cooling in step vii) is to a temperature within range of 5-10°C.

13. A method of producing the crude lercanidipine hydrochloride Form of claim 2, comprising the steps of:

a) reacting 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid with a chloride selected from the group consisting of thionyl chloride and oxalyl chloride in an aprotic dipolar solvent and an aprotic polar solvent to produce the corresponding carbonyl chloride;

b) reacting, *in-situ*, the chloride of step a) with 2, N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propyl alcohol to yield crude lercanidipine hydrochloride; and

c) isolating the crude lercanidipine hydrochloride of step b) and recovering crude lercanidipine hydrochloride Form (B).

14. The method of claim 13 wherein the reacting step b) is conducted at a temperature between -5 and +5°C.

15. The method of claim 13 wherein step c) comprises the further steps of:

i') washing the crude lercanidipine hydrochloride of step b) with water,

ii') removing the water from step i') to produce a mixture having a water content of about 2%, measured according to Karl Fischer;

iii') concentrating the mixture of step ii') and adding solvent to produce a solution having about the same volume as the initial volume of the mixture of step ii') and a water content, according to Karl Fischer, of between 0.9 and 1.1%;

iv') cooling the solution of step iii') to obtain a solid;

v') filtering the solid of step iv');

vi') re-suspending the solid of step v') in a solvent;

vii') cooling the suspension of step vi'); and

viii') filtering and drying the solid obtained to produce the crude lercanidipine hydrochloride Form (B).

16. The method of claim 13 wherein the chloride is thionyl chloride.

17. The method of claim 15 wherein step c) ii') comprises removing the water from step i') by azeotropic reflux to produce said mixture.

18. The method of claim 15 wherein step c) iii') comprises concentrating said mixture to 3/4 of its initial volume.

19. The method of claim 15 wherein said solvent in steps c) iii') and vi') is ethyl acetate.

20. The method of claim 15 wherein the step c) iv') comprises cooling the solution to a temperature within the range of 0-5°C.

21. The method of claim 15 wherein said step c) vi') further comprises stirring the suspension at 60-65°C for about one hour.

22. The method of claim 21 wherein said step c) vii') further comprises cooling the solid to a temperature between 5 and 10°C.

23. The method of claim 15 wherein said drying in step viii') is in an oven at approximately 70°C.

24. The method of any one of claims 1-7, wherein said aprotic dipolar solvent is dimethylformamide and said aprotic polar solvent is ethyl acetate.

25. Isolated lercanidipine hydrochloride crystalline Form (I), which has the X-ray diffraction pattern, at wavelength Kα , as shown in Figure 11.

26. The lercanidipine crystalline Form of claim 10, wherein distances, (I/Io) ratios, and 2 θ angles of significant peaks in Figure 11 are:

D (X)	Relative intensity (I/Io)	2 θ angle
16.3	83	5.4
6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

27. Isolated lercanidipine hydrochloride crystalline Form (II), which has an X-ray diffraction pattern, at wavelength Kα , as shown in Figure 12.

28. The lercanidipine crystalline Form of claim 27, wherein distances, (I/Io) ratios, and 2 θ angles of significant peaks in Figure 12 are:

D (Å)	Relative intensity (I/Io)	2 θ angle
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8
3.81	41	23.4
3.77	100	23.6
3.58	44	24.8
3.54	29	25.2

29. A method of producing lercanidipine hydrochloride crystalline Form (I), which has an X-ray diffraction pattern, at wavelength Kα, as shown in Figure 11, which comprises:

d) adding a C1-C5 alcohol solvent containing a maximum of 5% water (v/v) to a crude lercanidipine hydrochloride Form and heating under reflux and with stirring to produce a clear solution;

e) cooling the solution of step d) and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is $\leq$2%; and

f) recovering the solid obtained from step e), and drying said solid to produce the lercanidipine hydrochloride crystalline Form (I).

30. The method of claim 29, wherein step f) comprises filtering the solid obtained from step e), washing the solid with isopropanol and re-filtering the solid before drying.

31. The method of claim 29 wherein the alcohol of step d) is selected from the group consisting of isopropanol, ethanol and anhydrous ethanol.

32. The method of claim 29, wherein the crude Form is lercanidipine hydrochloride crude Form (A), lercanidipine hydrochloride crude Form (B) or lercanidipine crude Form (C)

33. The method of claim 29 wherein said step d) further comprises filtering the heated solution.

34. The method of claim 29 wherein said step e) comprises cooling the solution to a temperature between 30 and 40°C.

35. The method of claim 34 wherein said step e) further comprises stirring for a period of time of 12-48 hours.

36. The method of claim 29 wherein said drying in step f) takes place in an oven.

37. A method of producing lercanidipine hydrochloride crystalline Form (II), which has an x-ray diffraction pattern, at wavelength Kα, as shown in Figure 12, the method comprising the steps of:

d") adding acetonitrile to lercanidipine hydrochloride and heating the mixture thus obtained to form a solution;

e") cooling of the solution of step d") and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is $\leq$2%; and

f") recovering the solid of step e") and drying said solid to produce the lercanidipine hydrochloride Form (II).

38. The method of claim 37 wherein said step d") comprises heating said mixture under reflux with stirring.

39. The method of claim 37 wherein said step e") comprises cooling the solution to room temperature.

40. The method of claim 39 wherein said step e") comprises stirring the solution at room temperature for 24 hours.

41. The method of claim 37 wherein drying step f") takes place in an oven.

42. The method of claim 37, wherein the crude Form is lercanidipine hydrochloride crude Form (A), lercanidipine hydrochloride crude Form (B) or lercanidipine crude Form (C).

43. A method of producing lercanidipine hydrochloride crystalline Form (I), which has an x-ray diffraction pattern, at wavelength Kα, as shown in Figure 12, which comprises:

d') providing a mixture of ethanol and lercanidipine hydrochloride, refluxing under stirring and cooling and adding crystalline seeds of Form (I);

e') further cooling the seeded mixture of step d') and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is $\leq$2%; and

f') recovering the solid of step e') to form lercanidipine hydrochloride Form (I).

44. The method of claim 43 wherein the ratio of lercanidipine hydrochloride to volume of solvent in step d') on a weight volume ratio is within the range of about 1:4 to 1:6.

45. The method of claim 44 wherein said ratio is 1:4.

46. The method of claim 43 wherein said step d') further comprises filtering the heated solution.

47. The method of claim 43 wherein cooling in said step d') is to a temperature of 20°C while stirring.

48. The method of claim 43 wherein cooling in said step e') is to a temperature between 10 and 15°C.

49. The method of claim 43 wherein the drying in said step f') takes place in an oven at 70°C.

50. The method of claim 47 wherein authentic seeds of lercanidipine Form (I) are added at the end of cooling in steps e') and d').

51. A method of producing lercanidipine hydrochloride crystalline Form (II), which has an X-ray diffraction pattern, at wavelength K , as shown in Figure 12, which comprises:

d''') adding ethanol or isopropanol with a water content below 10% by weight to lercanidipine hydrochloride and refluxing to produce a solution;

e''') cooling the solution and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is $\leq$2%; and

f''') recovering the solid produced in step e''') to produce lercanidipine hydrochloride Form (II).

52. The method of claim 51 wherein ethanol is added in said step d''').

53. The method of claims 51 wherein the water content of the solvent in step d''') is between 5 and 10%.

54. The method of claim 51 wherein cooling in said step e''') is to a temperature between 20 and 40°C.

55. The method of claim 51 wherein step f''') comprises filtering said solid and drying in an oven.

56. A method of producing the lercanidipine hydrochloride crystalline Form (II), which has an x-ray diffraction pattern, at wavelength Kα, as shown in Figure 12, which comprises:

d'''') dissolving crude lercanidipine hydrochloride or lercanidipine hydrochloride crystalline Form (I) in a protic polar or an aprotic dipolar solvent containing up to 50% by weight of water to produce a solution;

e'''') stirring the solution of step d'''') until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is $\leq$2%; and

f'''') recovering the solid of step e'''') to produce lercanidipine Form (II).

57. The method of claim 56, further comprising irradiating with ultrasound and/or adding crystalline seeds of Form (II) to step e'''').

58. The method of claim 56, further comprising adding up to 60% water to the solution of step d'''').

59. The method of claim 56, wherein the protic polar solvent is an alcohol solvent.

60. The method of claim 56, wherein the alcohol solvent is selected from the group consisting of methanol, ethanol, n-propanol, isopropanol.

61. The method of claim 56, wherein the aprotic dipolar solvent is N-methyl-pyrrolidone.

62. The method of claim 56, wherein the temperature of said step d"") is between 20 and 70°C.

63. The method of claim 56, wherein stirring in said step e"") takes place at a temperature between 20 and 25°C.

64. The method of claim 56, wherein drying in said step f"") takes place at 70°C.

65. An antihypertensive pharmaceutical composition comprising (1) crystalline lercanidipine hydrochloride and optionally other forms of lercanidipine, wherein the crystalline lercanidipine hydrochloride is selected from the group consisting of lercanidipine hydrochloride crystalline Form (I), lercanidipine hydrochloride crystalline Form (II), and combinations thereof comprising a predetermined content of each crystalline form, and (2) at least one component selected from the group consisting of a pharmaceutically acceptable carrier or diluent, a flavorant, a sweetener, a preservative, a dye, a binder, a suspending agent, a dispersing agent, a colorant, a disintegrant, an excipient, a lubricant, a plasticizer, and an edible oil..

66. A unit dosage form comprising the antihypertensive pharmaceutical composition of claim 65.

67. The unit dosage form of claim 66 wherein the dosage form is a lercanidipine immediate release dosage form.

68. The unit dosage form of claim 66 wherein the dosage form is a lercanidipine sustained release dosage form.

69. The unit dosage form of claim 66 wherein the dosage form comprises a lercanidipine immediate release phase and a lercanidipine sustained release phase.

70. The unit dosage form of claim 66, wherein the composition comprises 0.1 to 400 mg lercanidipine hydrochloride.

71. The unit dosage form of claim 70, wherein the composition comprises 1 to 200 mg lercanidipine hydrochloride.

72. The unit dosage form of claim 71, wherein the composition comprises 5 to 40 mg lercanidipine hydrochloride.

73. An antihypertensive composition comprising predetermined amounts of lercanidipine hydrochloride crystalline Form (I) and lercanidipine hydrochloride crystalline Form (II).

74. The antihypertensive composition of claim 73 wherein the lercanidipine hydrochloride crystalline Form (I) has a melting point of about 197-201°C and the lercanidipine hydrochloride crystalline Form (II) has a melting point of about 207-211°C, when said melting points are determined as DSC peaks.

75. The antihypertensive composition of claim 73 or claim 74 wherein the ratio of Form (I) : Form (II) is between 1:9 to 9:1.

76. The antihypertensive composition of claim 75 wherein the ratio of Form (I) : Form (II) is selected from the group consisting of 9:1, 7:3, 1:1, 3:7 and 1:9.

77. The isolated lercanidipine crystal Form of any one of claims 25, 26, 27 or 28 comprising an average particle size of D (50%) 2-8 μm and D (90%) < 15 μm.

78. The antihypertensive pharmaceutical composition of claim 65 wherein said lercanidipine hydrochloride crystalline Forms (I) and (II) each have an average particle size of D (50%) 2-8 μm and D (90%) < 15 μm.

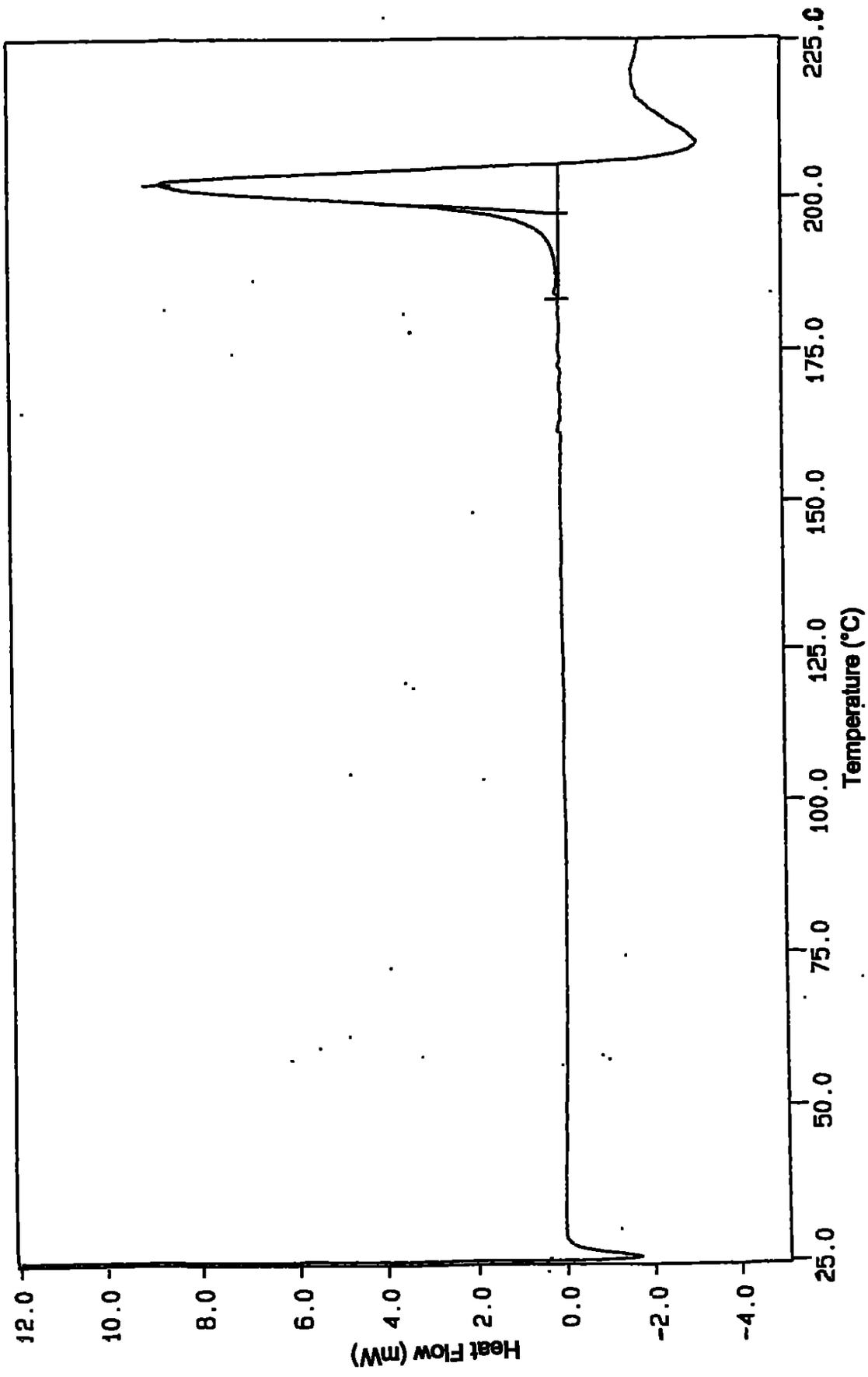

Heat Flow (mW)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
25.0
50.0
75.0
100.0
125.0
150.0
175.0
200.0
225.0
Temperature (°C)

FIG. 1

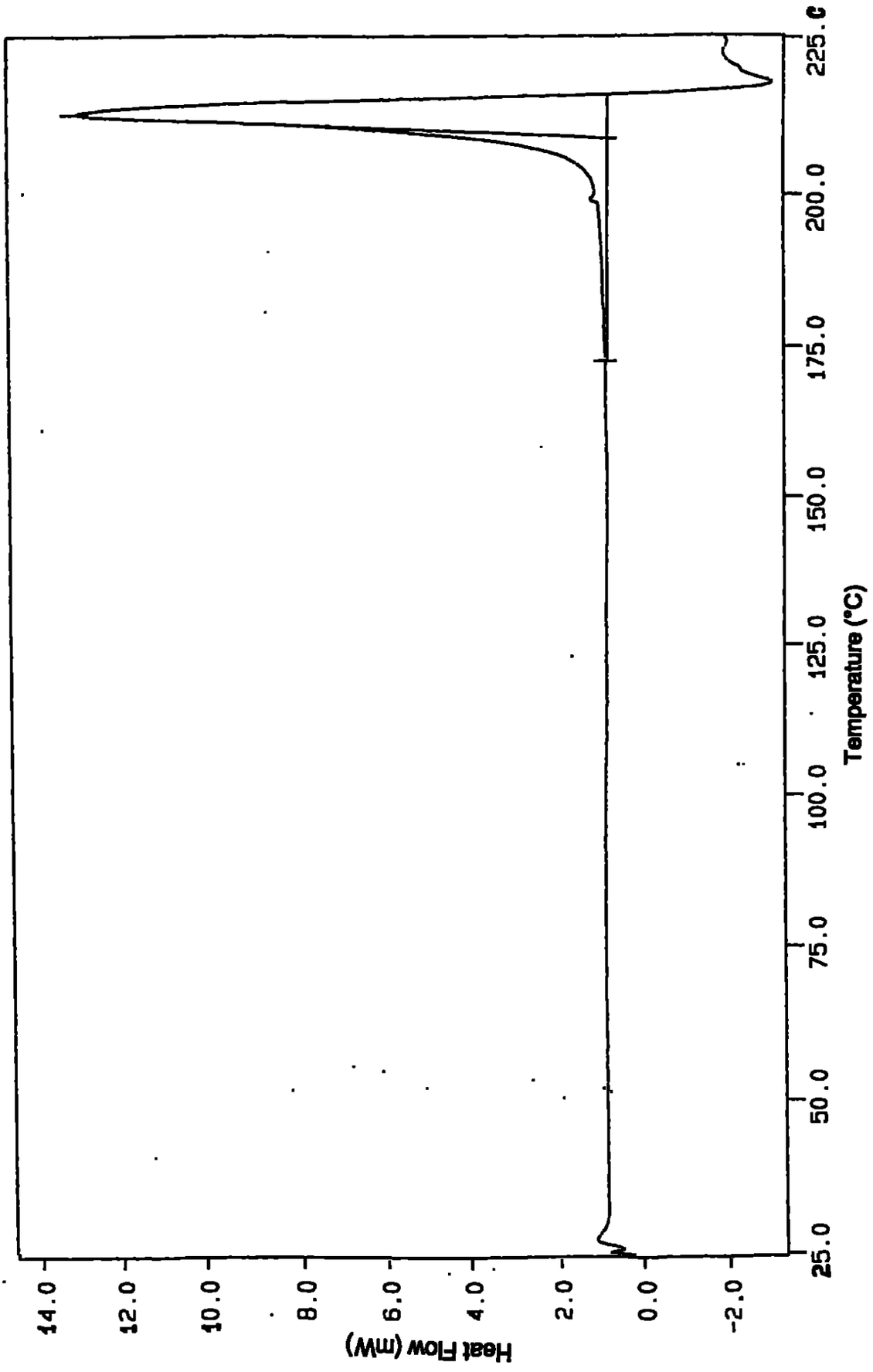

225.0
200.0
175.0
150.0
125.0
100.0
75.0
50.0
25.0
Temperature (°C)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
Heat Flow (mW)

FIG. 2

42

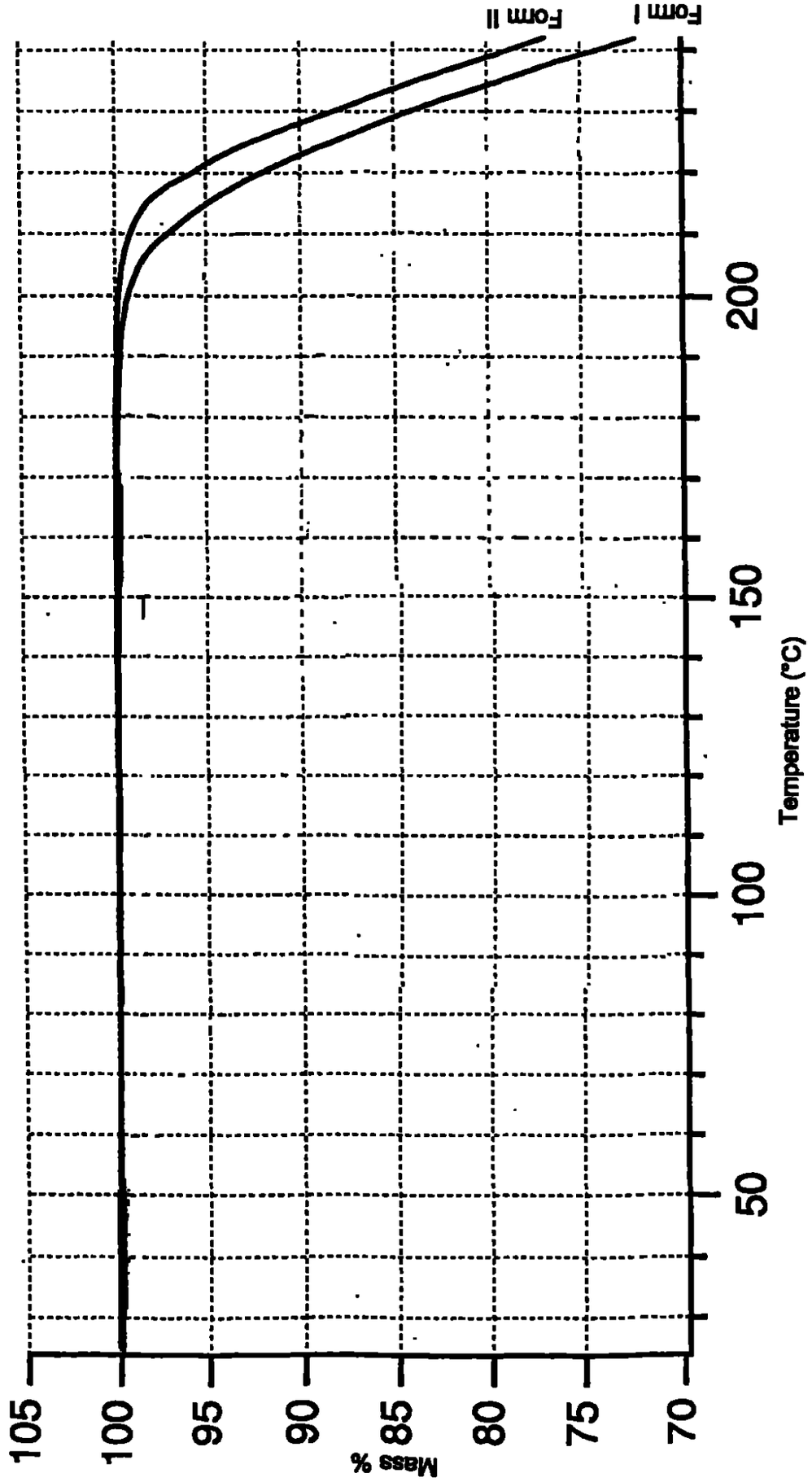

Form I
Form II
105
100
95
90
85
80
75
70
Mass %
50
100
150
200
Temperature (°C)

FIG. 3

SOLUBILITY IN WATER-ETHANOL

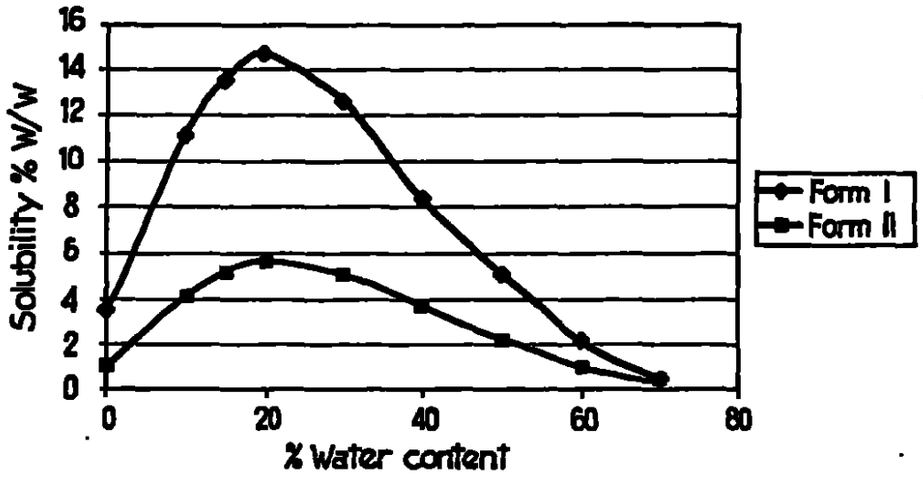


Fig. 4

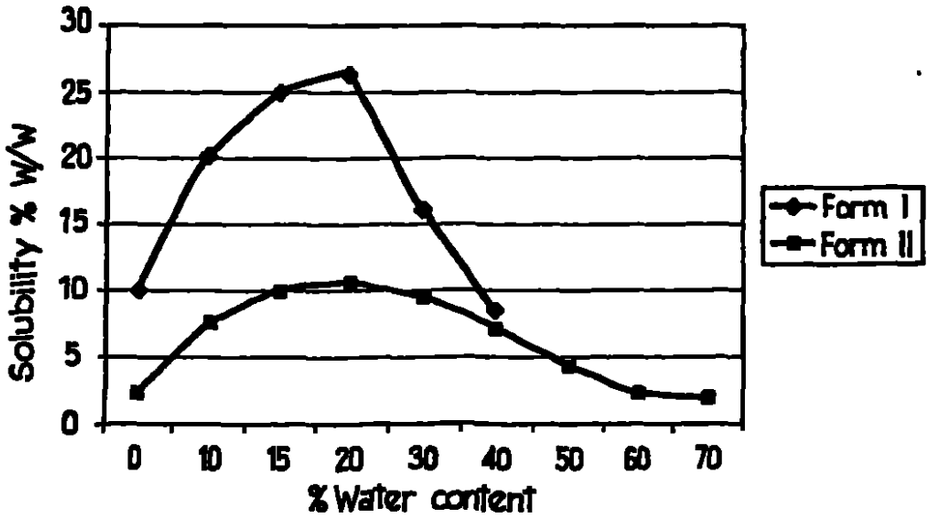


Fig. 5

49

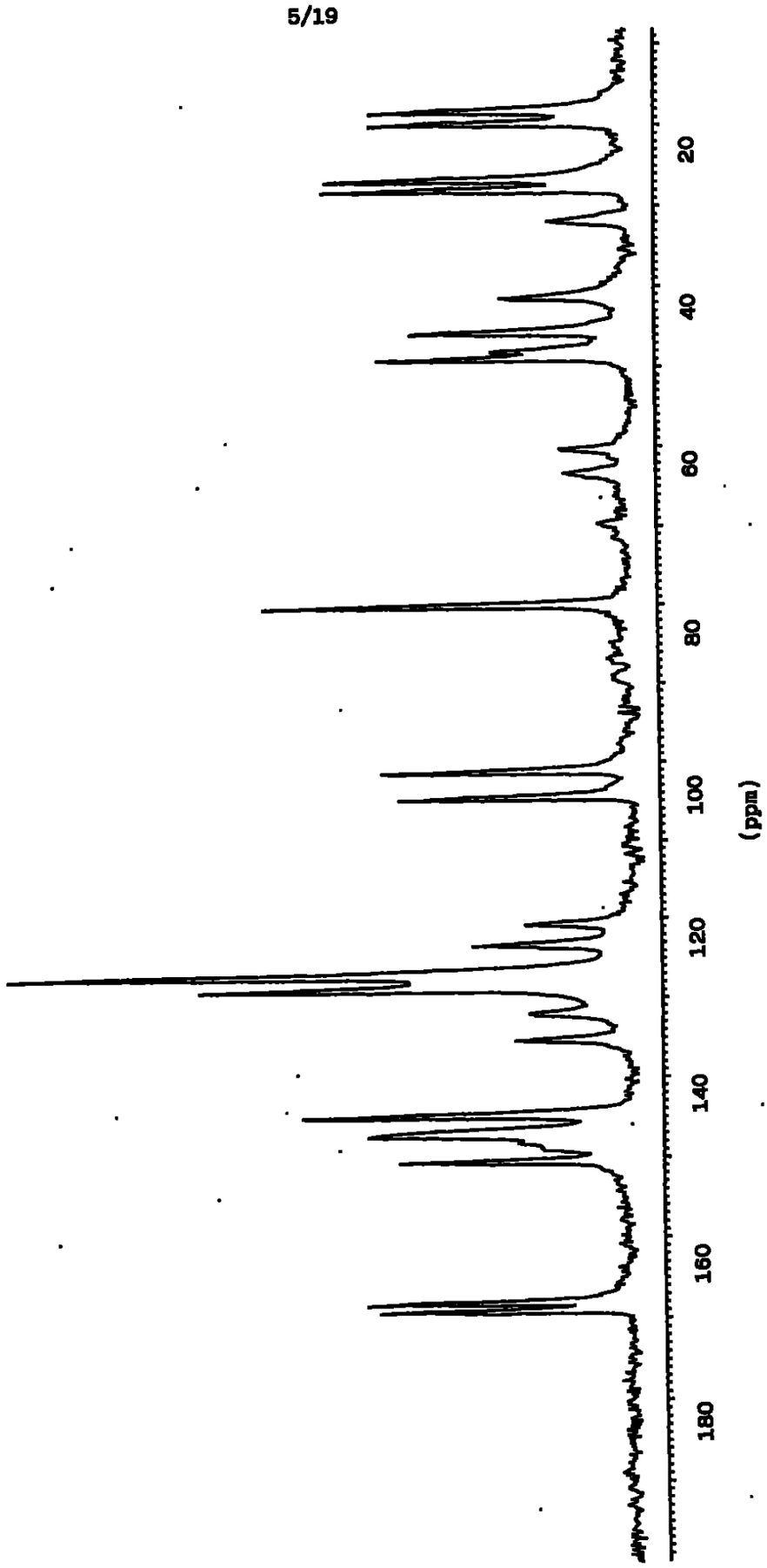


FIG. 8

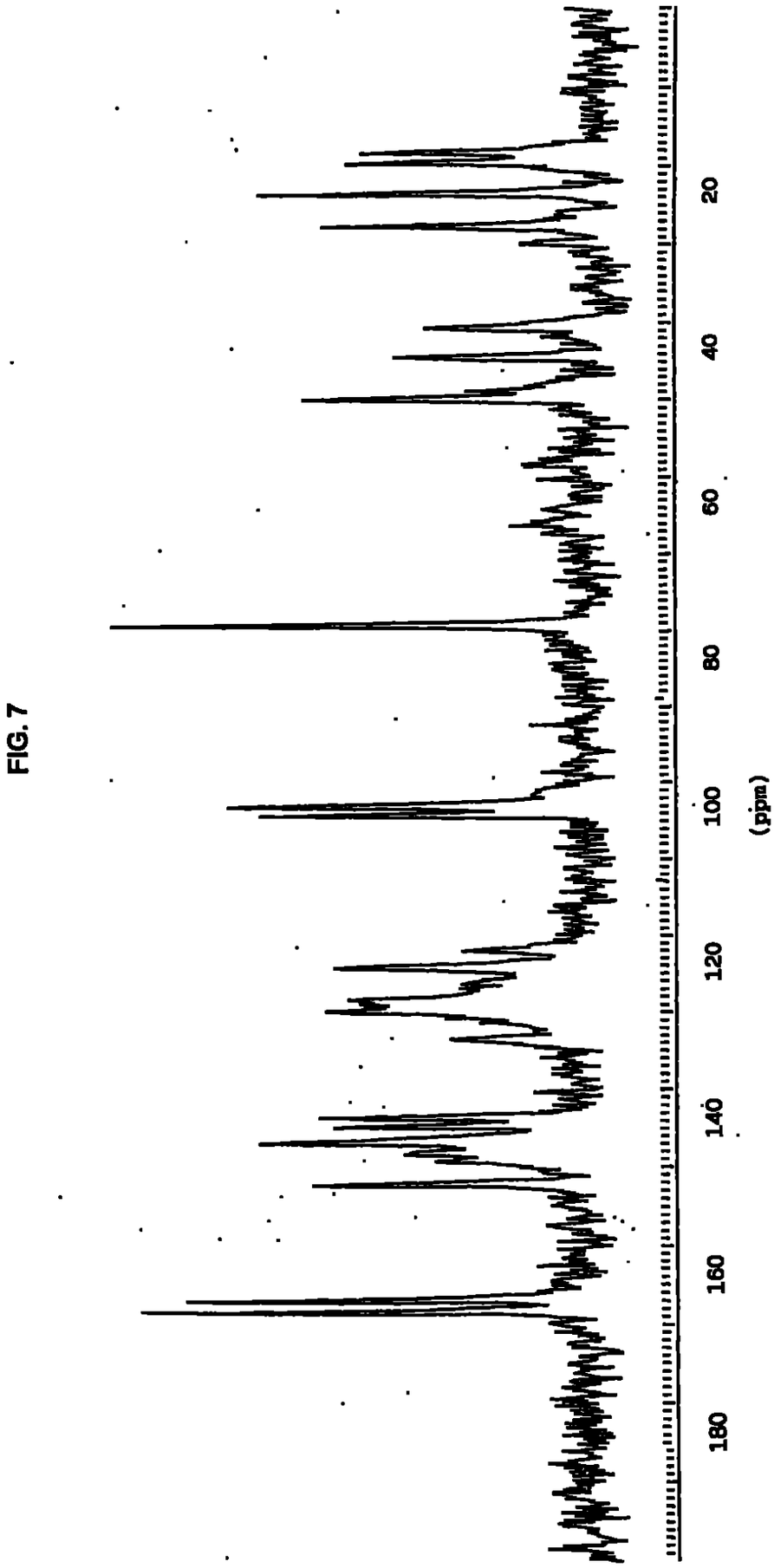


FIG. 7

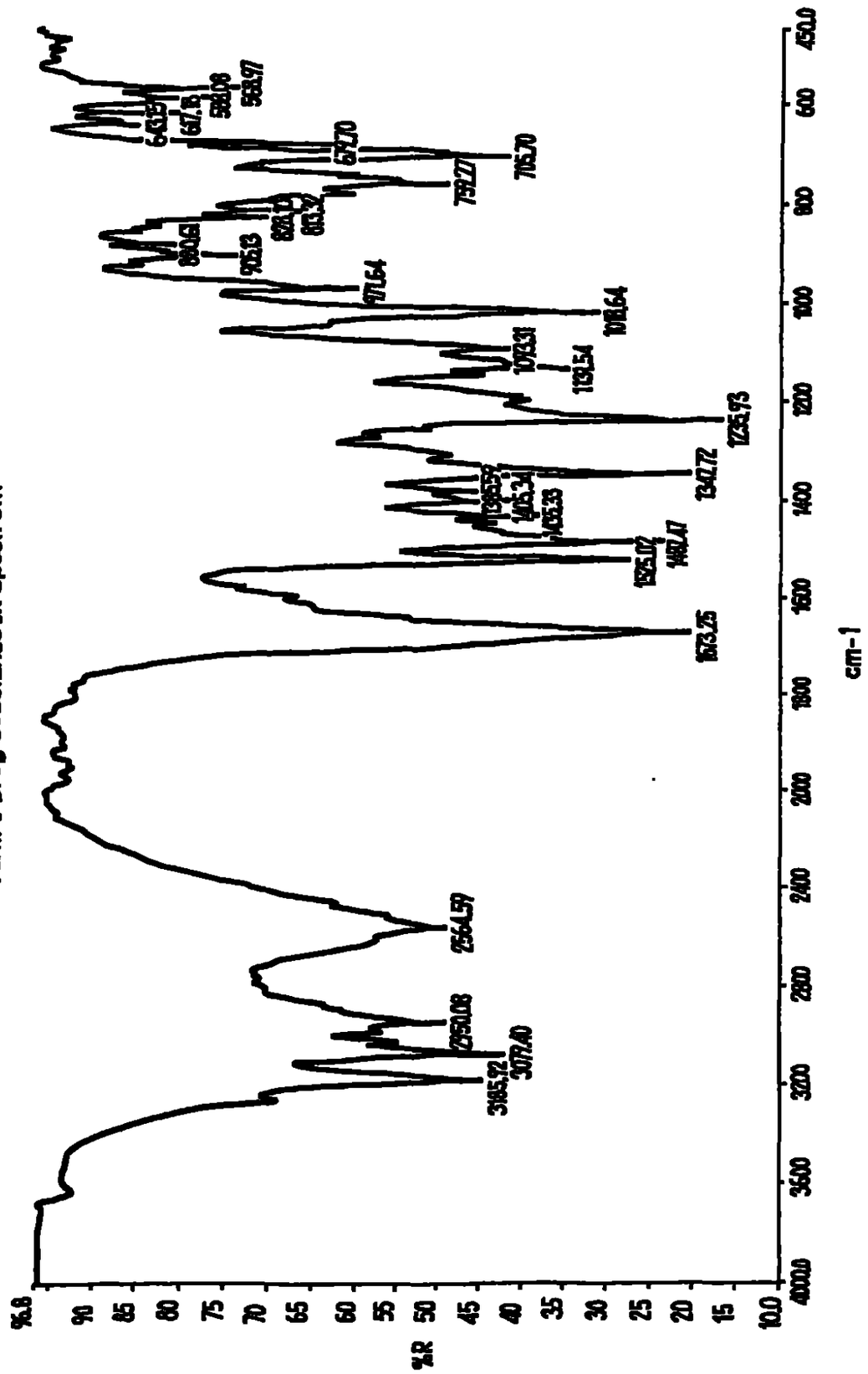


Fig. 8
Form I Drug Substance IR Spectrum

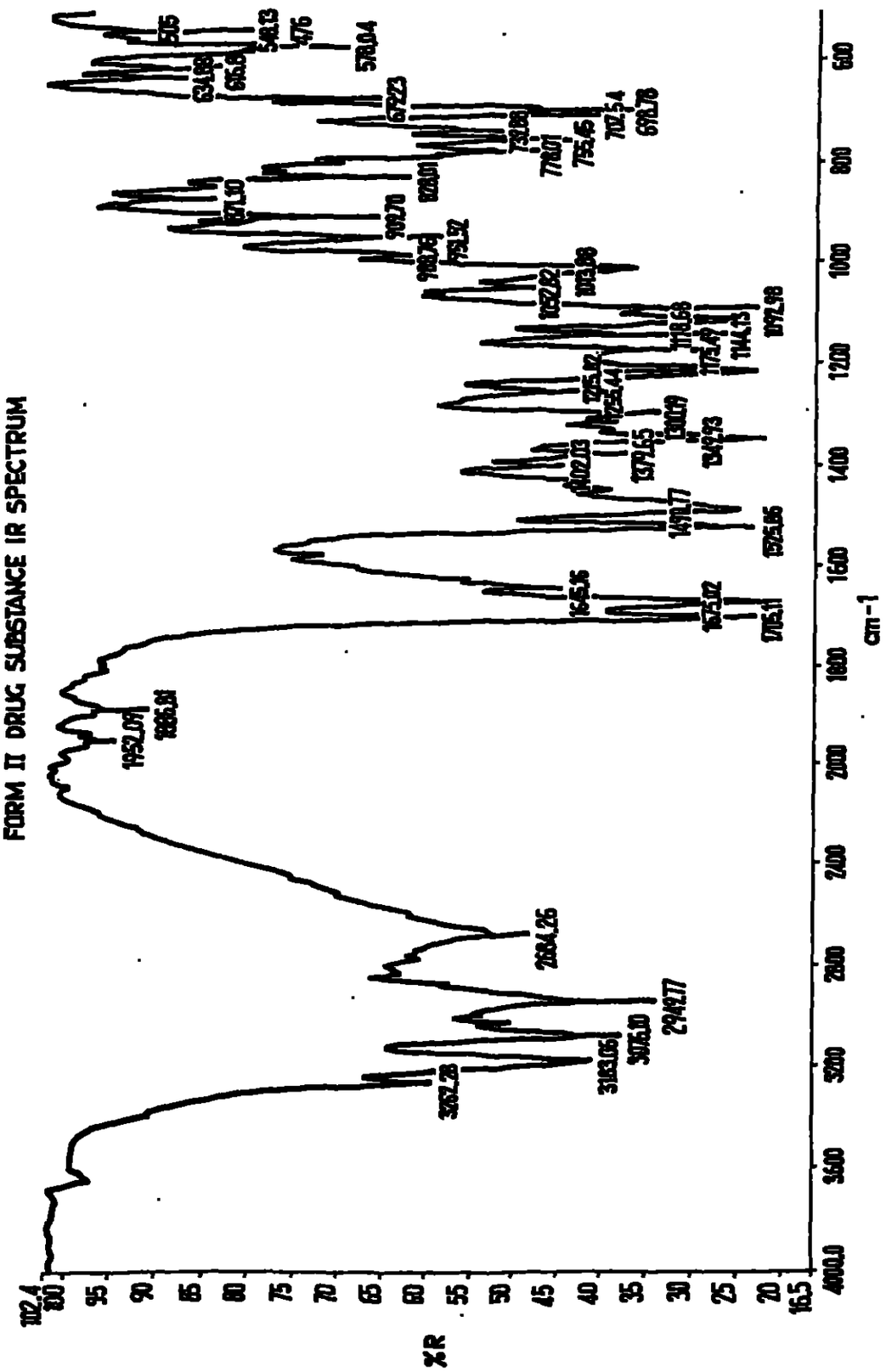


FIG. 9
FORM II DRUG SUBSTANCE IR SPECTRUM

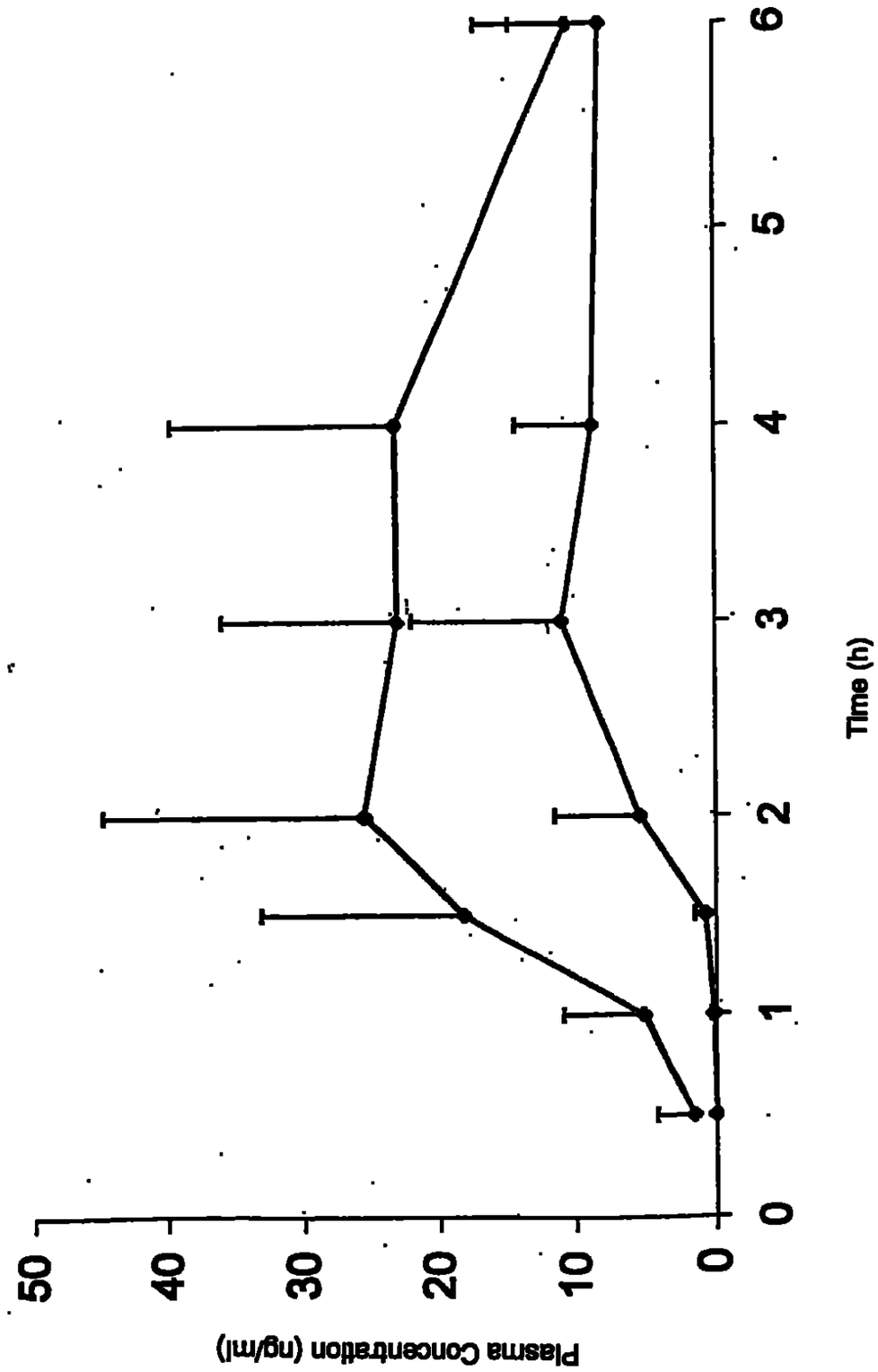
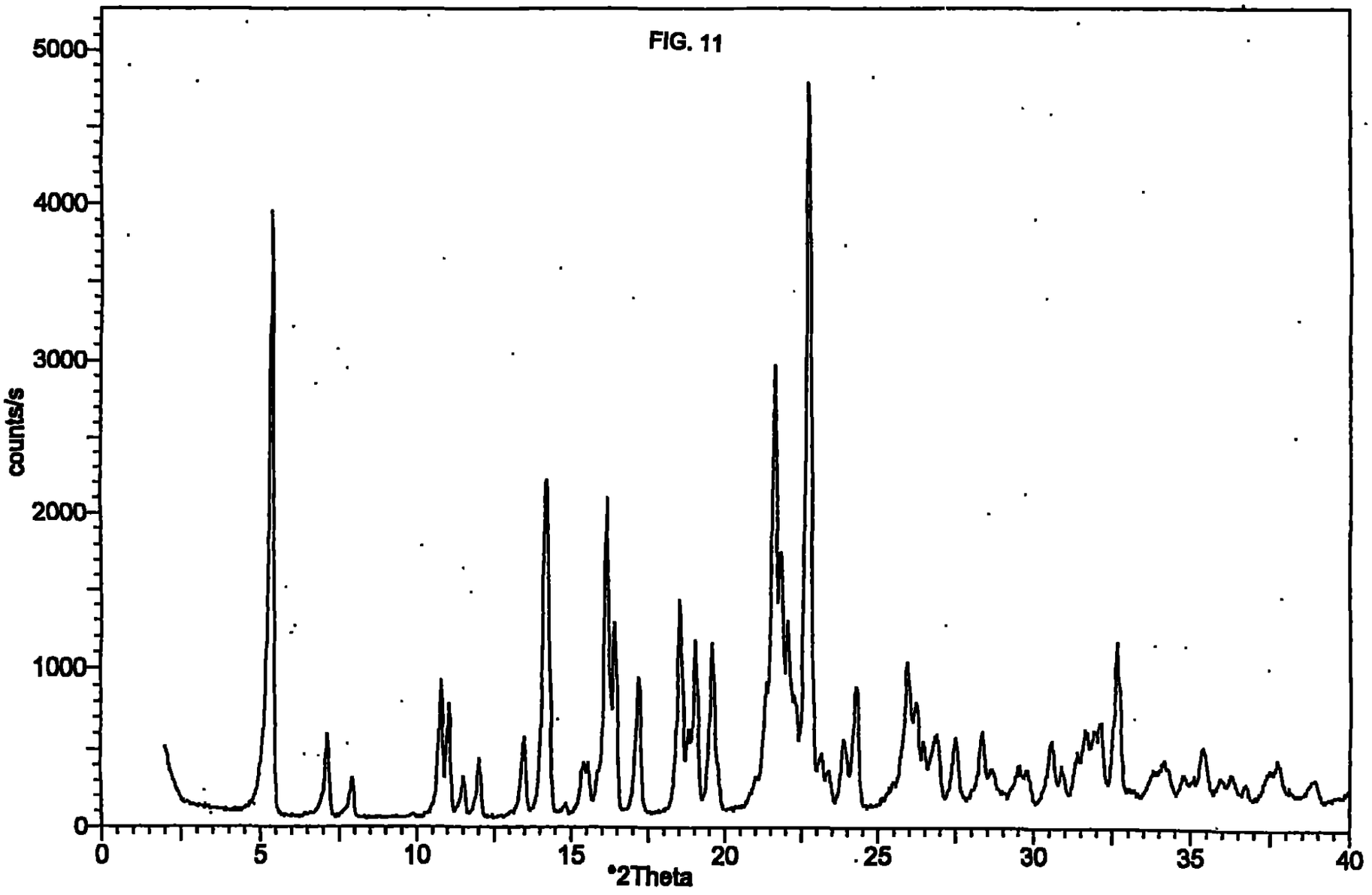


FIG. 10

FIG. 11


5000
4000
3000
2000
1000
0
counts/s
0
5
10
15
20
25
30
35
40
°2Theta

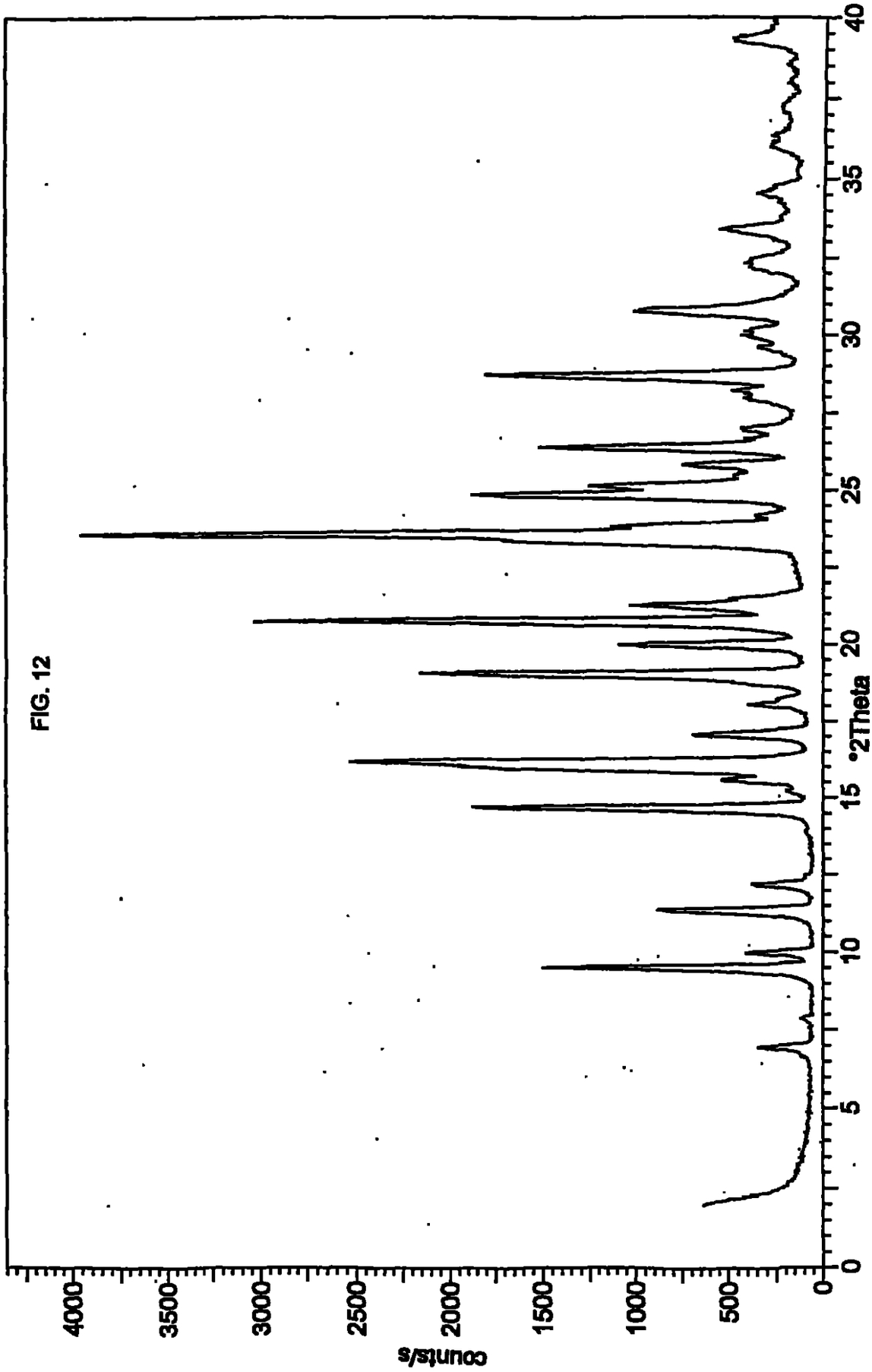
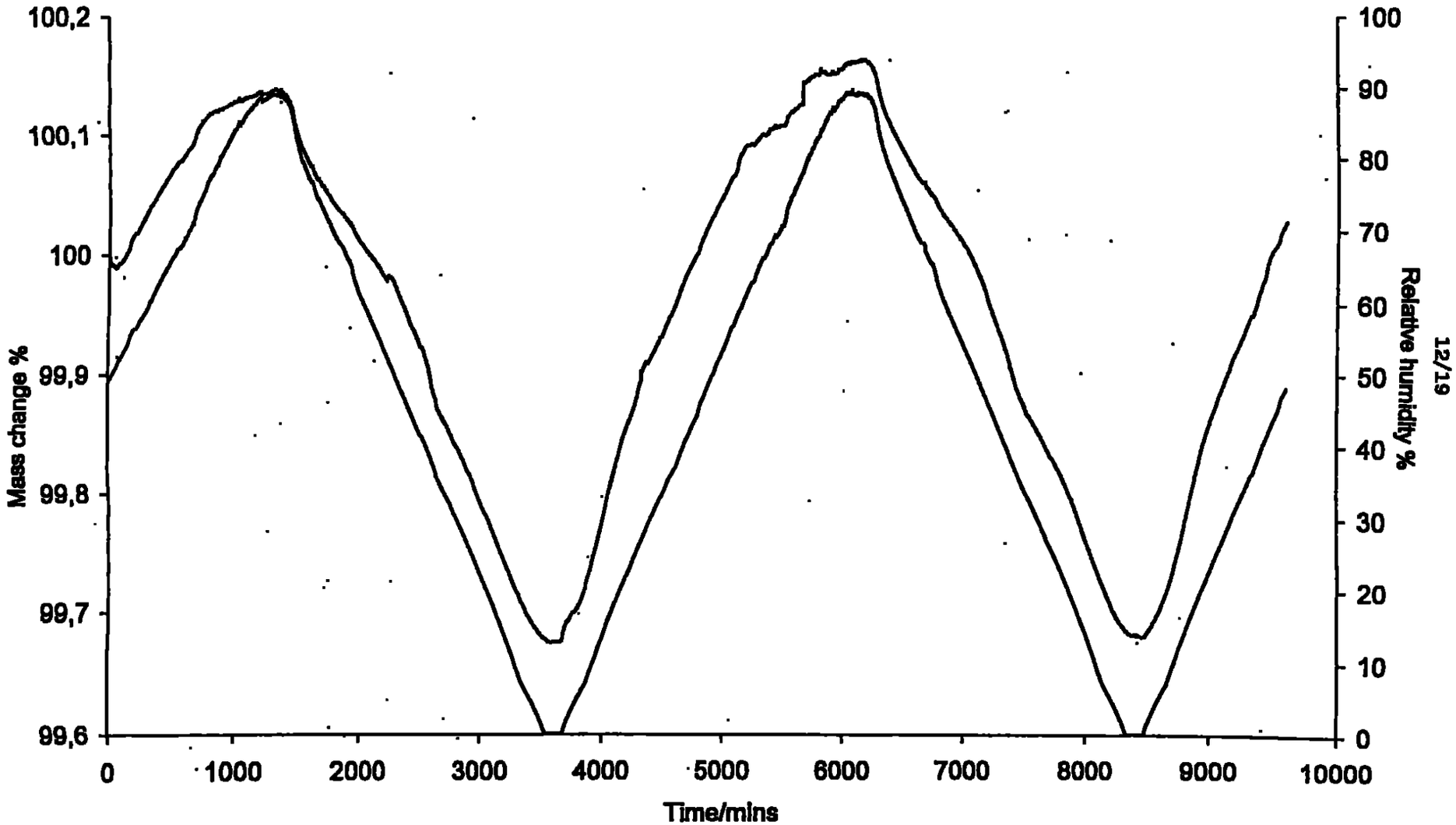

counts/s
4000
3500
3000
2500
2000
1500
1000
500
0
0
5
10
15
20
25
30
35
40
°2Theta

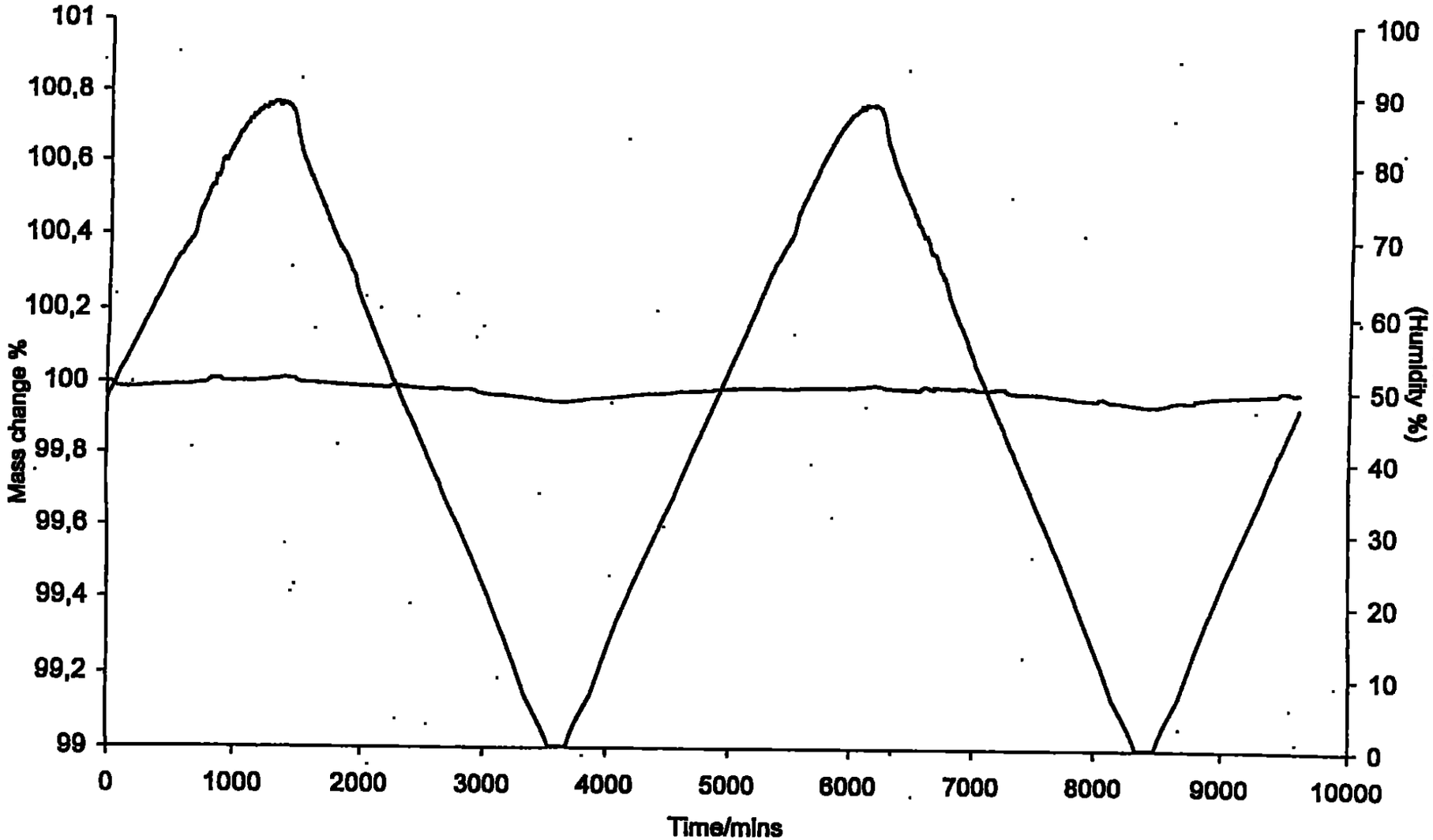
FIG. 12

FIG. 13



WO 03/014084
12/19
PCT/EP02/08699

FIG. 14



WO 03/014684
13/16
PCT/EP02/08699

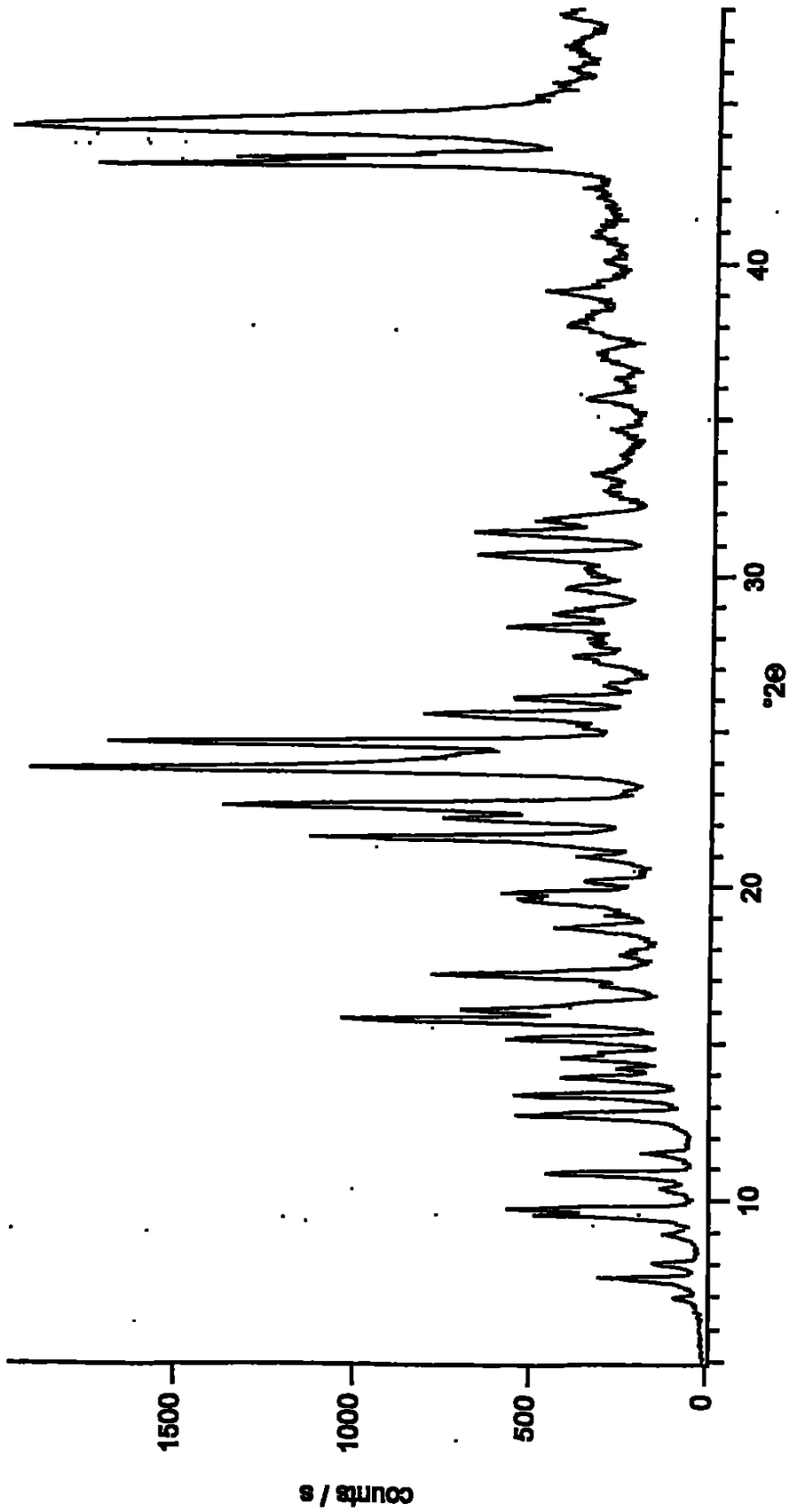


FIG. 15

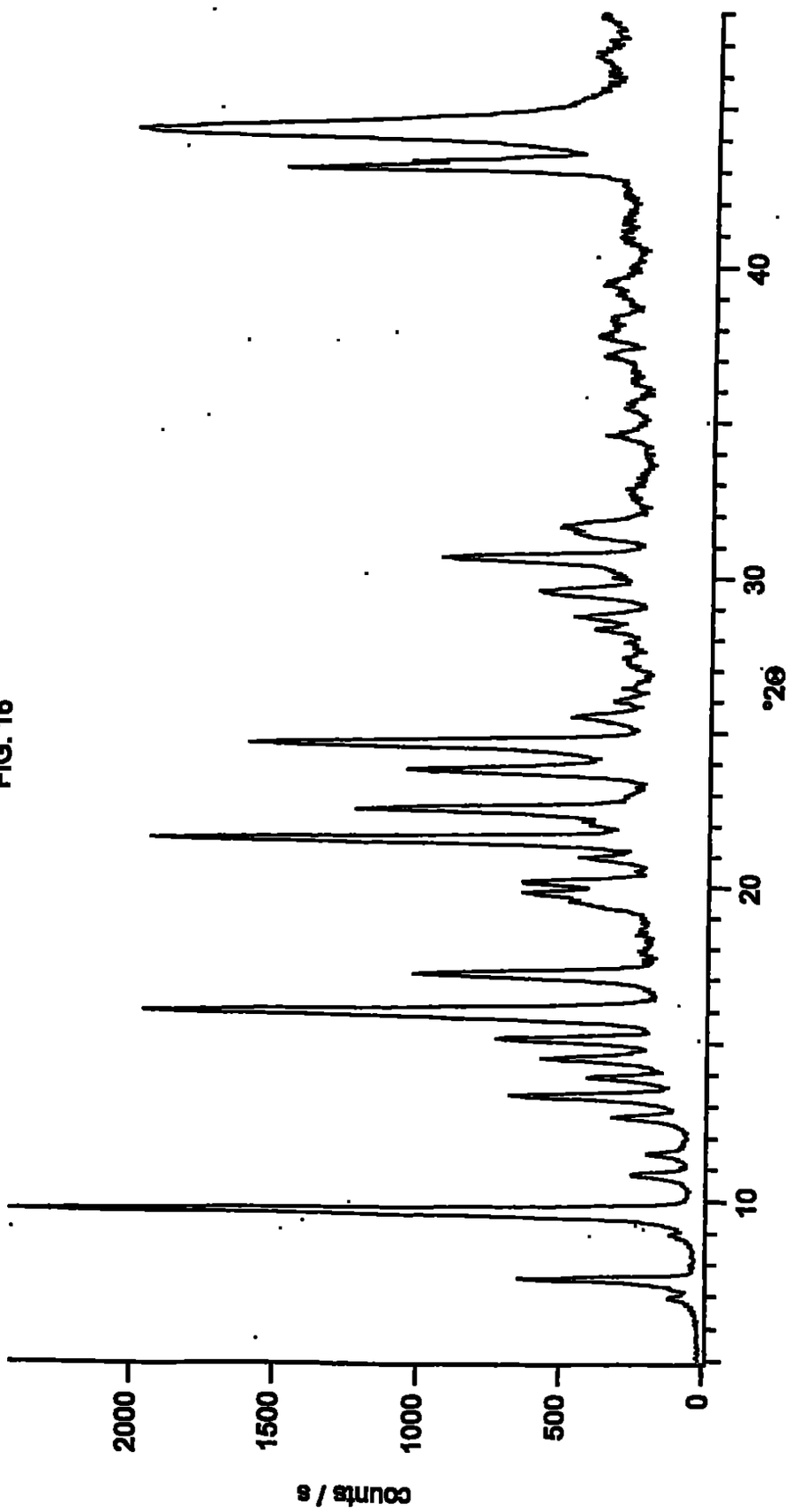


FIG. 16

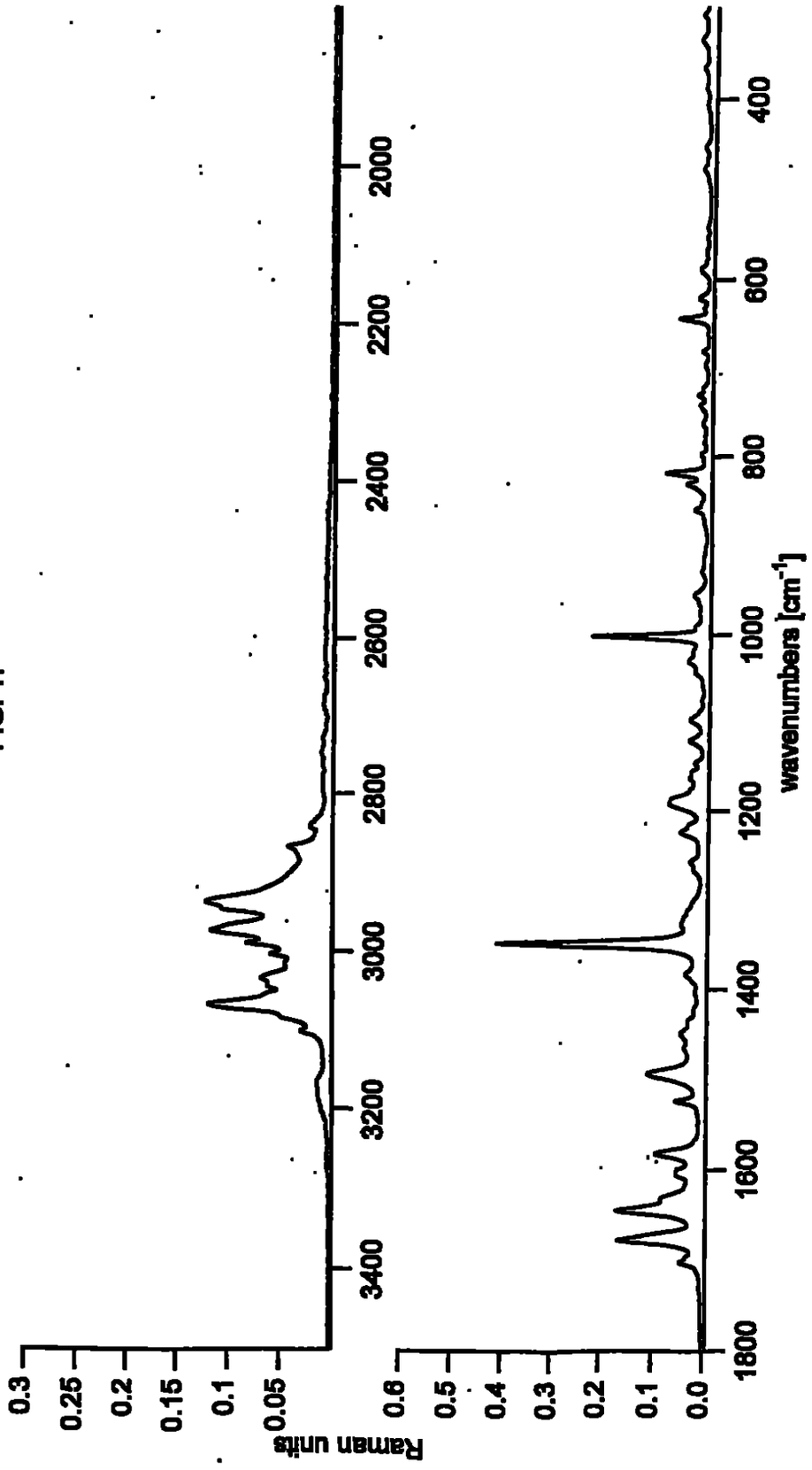

Raman units
0.3
0.25
0.2
0.15
0.1
0.05
3400
3200
3000
2800
2600
2400
2200
2000
0.8
0.5
0.4
0.3
0.2
0.1
0.0
1800
1600
1400
1200
1000
800
800
400
wavenumbers [cm-1]

FIG. 17

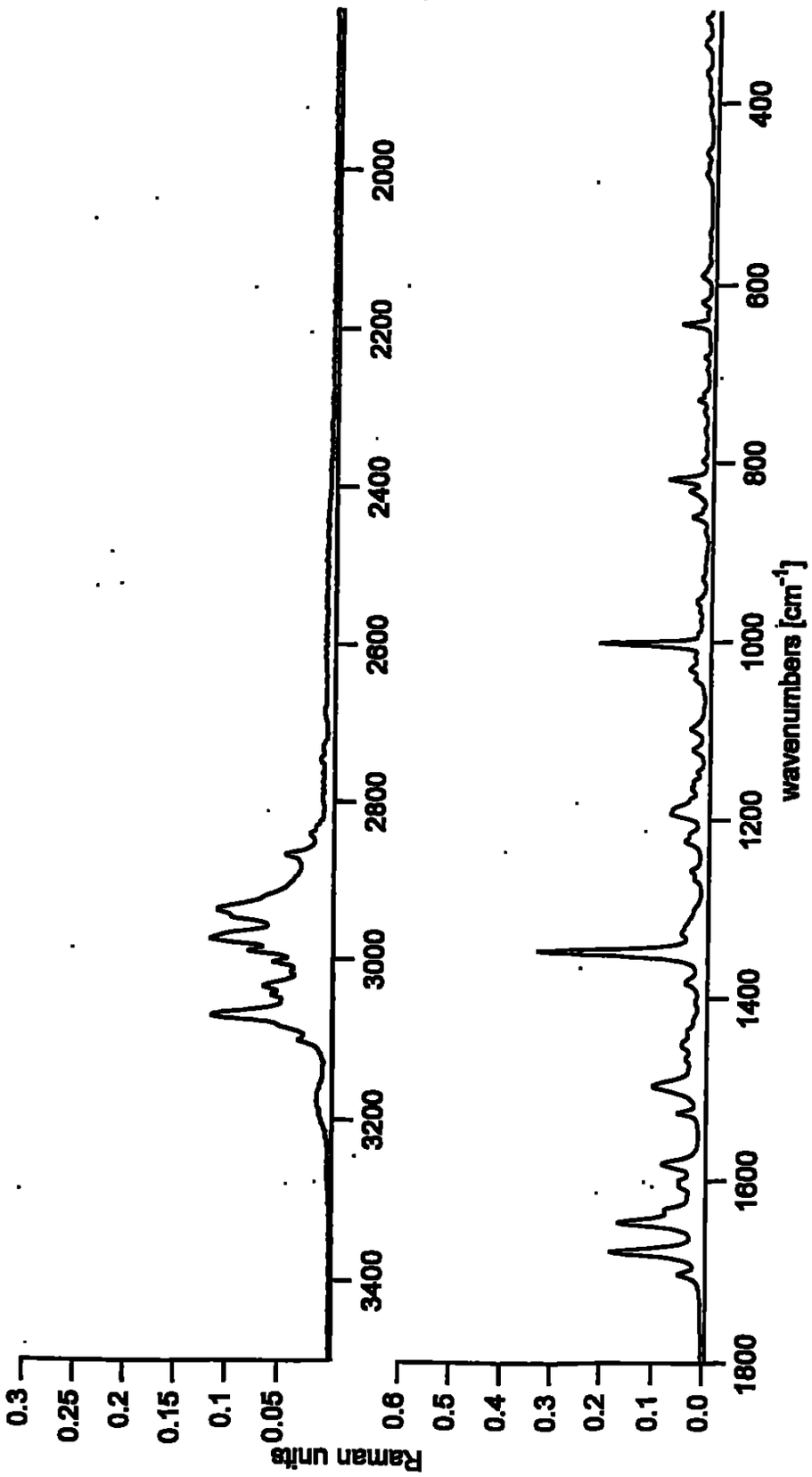

Raman units
0.3
0.25
0.2
0.15
0.1
0.05
3400
3200
3000
2800
2800
2400
2200
2000
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1800
1600
1400
1200
1000
800
600
400
wavenumbers [cm-1]

FIG. 18

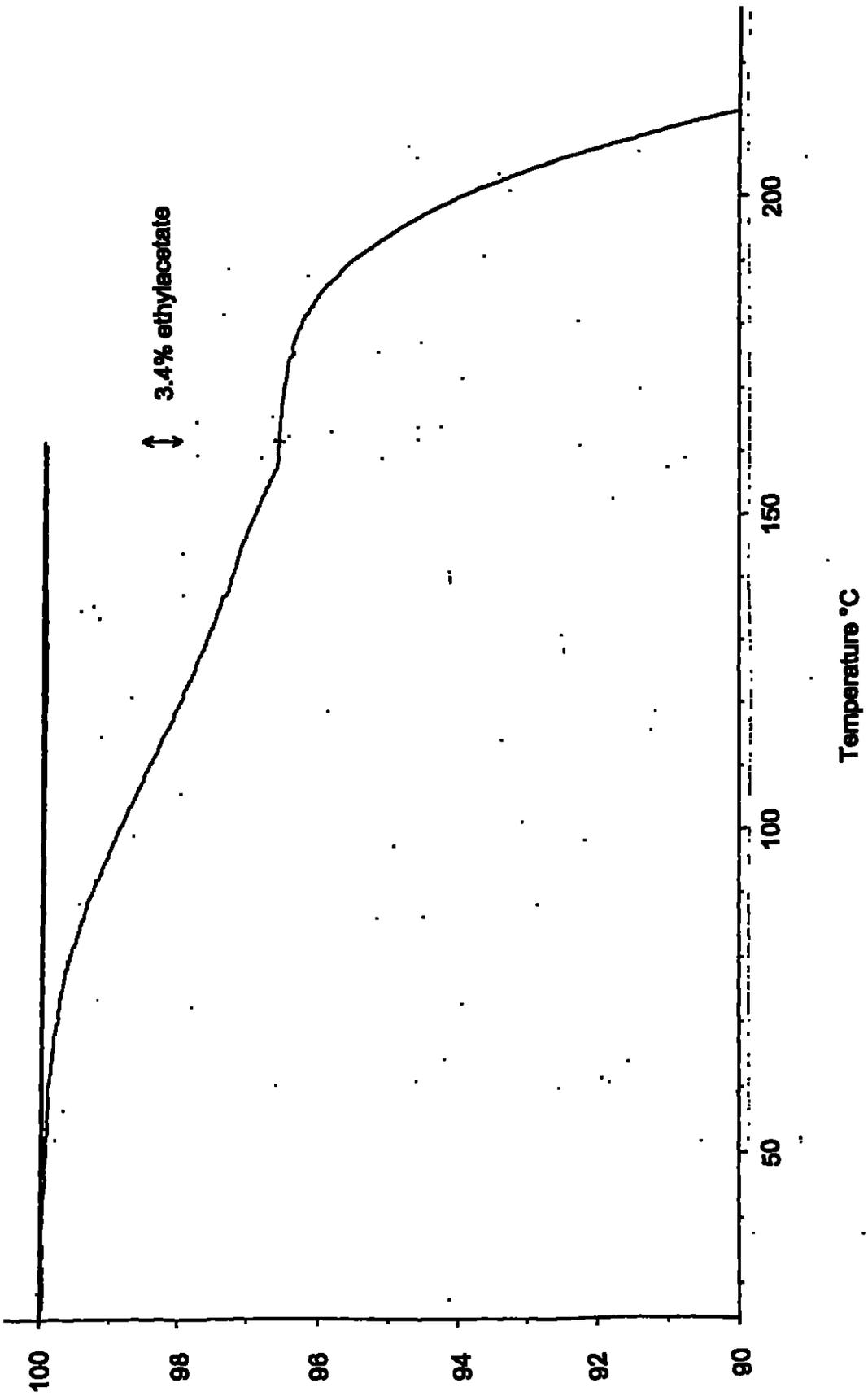

3.4% ethylacetate
Weight %
100
98
96
94
92
90
Temperature °C
50
100
150
200

Fig. 19

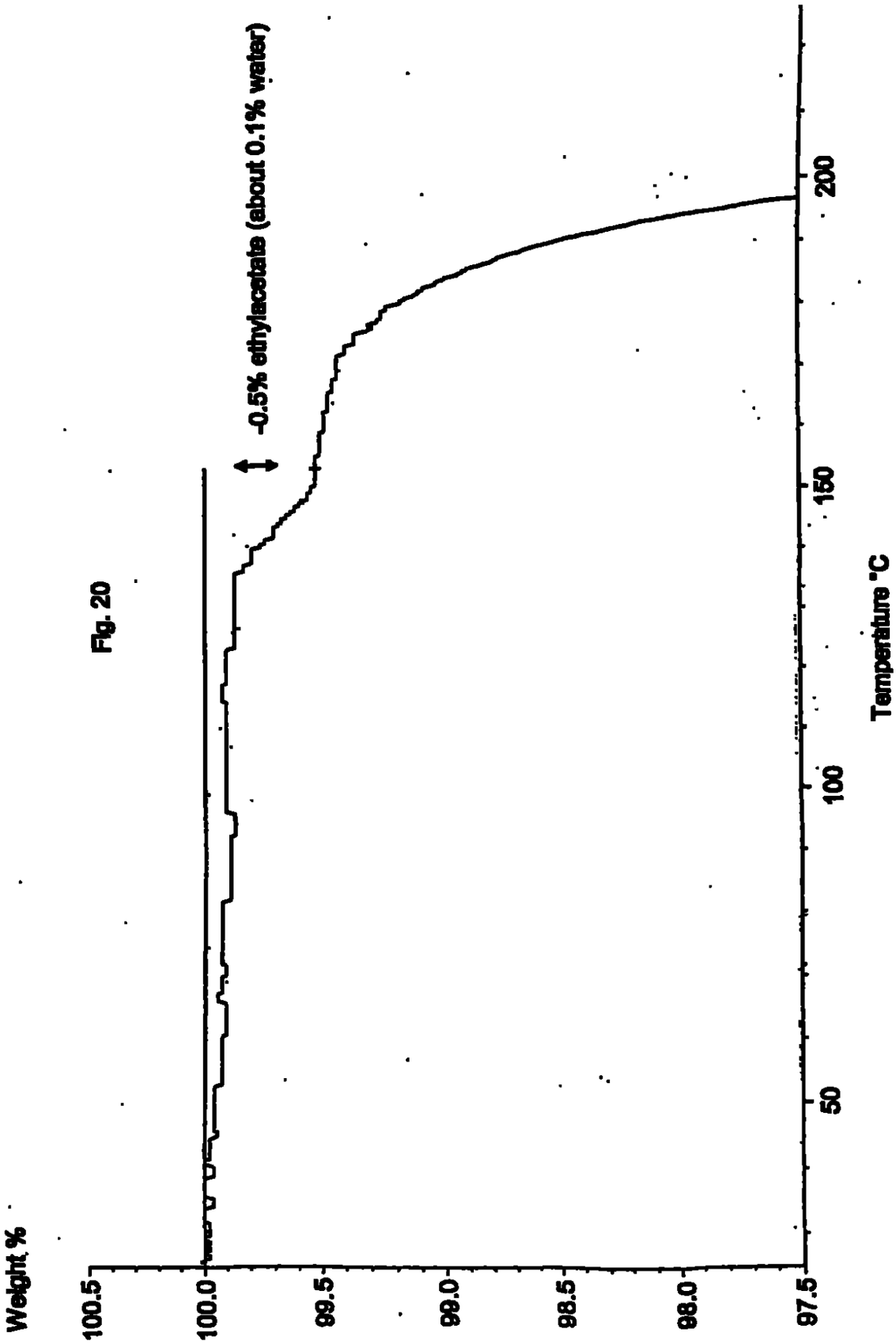

-0.5% ethylacetate (about 0.1% water)
Weight %
100.5
100.0
99.5
99.0
98.5
98.0
97.5
50
100
150
200
Temperature °C

Fig. 20

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/08699

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D211/90 A61K31/44 A61P9/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEONARDI, AMEDEO ET AL: "Asymmetric N-(3,3-diphenylpropyl)aminoalkyl esters of 4-aryl-2,6-dimethyl 1,4-dihydropyridine-3,5-dicarboxylic acids with antihypertensive activity" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY (1998), 33(5), 399-420 , XP004127371 see compound 9u in Table 1 conclusions p.405 method B2 p.415 and preparation of 9u, 9ul and 9v, p.416 -/-	1-78

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
4 November 2002	18/11/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Schuemacher, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 03669 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.;RECORDATI) 6 February 1997 (1997-02-06) reaction scheme 2, p.4 page 5; examples 1-3	1-78
X	WO 96 35668 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.;RECORDATI) 14 November 1996 (1996-11-14) the whole document	1-78
X	MCCLELLAN K J ET AL: "LERCANIDIPINE A REVIEW OF ITS USE IN HYPERTENSION" DRUGS, ADIS INTERNATIONAL LTD, AT, vol. 5, no. 60, November 2000 (2000-11), pages 1123-1140, XP008001723 ISSN: 0012-6667 see summary abstract p.1124	65-76
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Asymmetric *N*-(3,3-diphenylpropyl)aminoalkyl esters of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acids with antihypertensive activity

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Abstract – A series of asymmetric 4-aryl-1,4-dihydropyridine-3,5-dicarboxylates characterized by the presence of a 3,3-diphenylpropylamino moiety in one of the ester groups were synthesized. They exhibited remarkable antihypertensive activity in spontaneously hypertensive rats as well as affinity for the 1,4-dihydropyridines binding site labelled by ^3H-nitrendipine in the calcium channel. Introduction of this bulky and lipophilic amine confers to the whole series an elevated level of antihypertensive activity and a long duration of action, a structure-dependent modulation of the activity being found only in the subset characterized by the presence of a branched propylene bridge between the ester and the amino groups. The presence of the amino group is essential for oral activity. Out of this series, compound **9a** (Rec 15/2375-lercanidipine) was selected for clinical development and obtained marketing authorization as an antihypertensive in several countries. © Elsevier, Paris

1,4-dihydropyridine / antihypertensive / calcium antagonist / Rec 15 / 2375 / lercanidipine / calcium entry blocker

1. Introduction

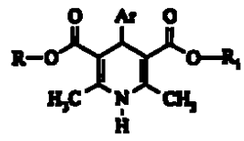
The presently available drugs classified as calcium antagonists (calcium entry blockers) are largely employed in a number of cardiovascular diseases, in particular hypertension and angina [1, 2]. The possibility of extending their use to other therapeutical indications, such as cerebral insufficiency and vasospasm, migraine, hypertrophic cardiomyopathy, ventricular and supraventricular tachyarrhytmia, atherosclerosis and others contributes to maintain an elevated interest for these agents [3–7].

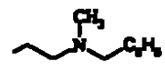
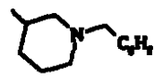
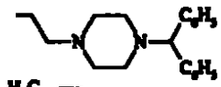
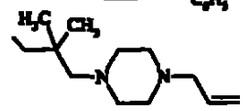
With regard to their structure, calcium antagonists can be roughly divided into two main groups: (a) the 4-aryl-1,4-dihydropyridine-3,5-dicarboxylic acid esters, and related derivatives, and (b) compounds containing one, or more, aryl moieties bearing a basic side chain as the essential active structure, such as verapamil, diltiazem, bepridil, cinnarizine and prenylamine [8–10].

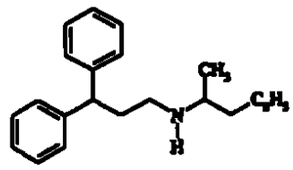
The dihydropyridines attracted the interest of many medicinal chemists, because of their high potency and selectivity of action, and many modifications of the original structure of nifedipine have been carried out, leading to new active compounds [11, 12].

Among the performed modifications, the introduction of bulky, lipophilic aralkylaminoalkyl chains as one of the two esterifying groups at position 3 or 5, led to the discovery of new, very potent calcium antagonists. The first compound of this type, nicardipine [13] is widely used as antianginal and antihypertensive. Other analogues, namely barnidipine [14], benidipine [15] and manidipine [16], recently entered the market or are in advanced clinical trials, such as NKY-722 [17] and palonidipine [18] (see *figure 1*).

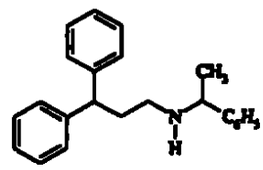
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Compd	R	Ar	R_1
Nicardipine	CH_3	3-$NO_2C_6H_4$	
Barnidipine (Mepirodipine)	CH_3	3-$NO_2C_6H_4$	
Benidipine (Nicardipine)	CH_3	3-$NO_2C_6H_4$	
Manidipine (Fenidipine)	CH_3	3-$NO_2C_6H_4$	
NKY-722	CH_3	3-$NO_2C_6H_4$	
Palonidipine	CH_3	2-F,5-$NO_2C_6H_3$	



Prenylamine



Fendiline

Figure 1. *Selected basic and lipophilic calcium-antagonists.*

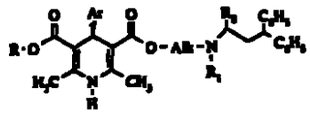
Following this approach, our group investigated new esters of 2,6-dimethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylic acid in which one estereal group represents a *N*-(3,3-diphenylpropyl)aminoalkyl moiety, which are reported here (*table I*).

The 3,3-diphenylpropylamino chain was chosen as a bulky, lipophilic group with the aim of improving the duration of action and also because it is present in other calcium antagonists such as prenylamine [19] and fendiline [20] (see *figure 1*).

Other structural variations were carried out, namely modification of the aryl group, of the non-basic ester chain and of the alkyl bridge between the carboxy and the amino functions, with the aim of possibly optimizing the pharmaco-toxicological properties of this series of compounds.

ENREGCID: <XP 4127371A | >

Table I. Alkyl *N*-[diphenylalkyl]aminoalkyl 4-aryl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates 9[a].



9

Compound	R	Ar	Alk	R_1	R_2	Method	Yield (%)	Eluents for base purification
9a	CH_3	2-NO_2-C_6H_4	$(CH_2)_2$	CH_3	H	A	34[b]	$CHCl_3$–EtOAc (100:0 to 97:3)
9b	CH_3	3-NO_2-C_6H_4	$(CH_2)_2$	CH_3	H	A	40	$CHCl_3$–EtOAc (85:15 to 1:1)
9c	CH_3	2,3-Cl_2-C_6H_3	$(CH_2)_2$	CH_3	H	A	40[c]	$CHCl_3$–EtOAc (4:1 to 3:2)
9d	CH_3	4-benzofurazanyl	$(CH_2)_2$	CH_3	H	A	32	$CHCl_3$–EtOAc (100:0 to 97:3)
9e	CH_3	3-NO_2-C_6H_4	$(CH_2)_2$	H	H	A	38	$CHCl_3$–EtOAc (4:1 to 1:1)
9f[d]	CH_3	3-NO_2-C_6H_4	$(CH_2)_2$	CH_3	CH_3	A	47	$CHCl_3$–EtOAc (100:0 to 85:15 to 0:100)
9g	C_2H_5	3-NO_2-C_6H_4	$(CH_2)_2$	CH_3	H	A	55	$CHCl_3$–EtOAc (100:0 to 7:3)
9h	$(CH_3)_2CH$	3-NO_2-C_6H_4	$(CH_2)_2$	CH_3	H	A	85	tol–EtOAc (4:1 to 3:2)
9i	$(CH_3)_2CHCH_2$	3-NO_2-C_6H_4	$(CH_2)_2$	CH_3	H	A	90	tol–EtOAc (3:1)
9j	$CH_3(CH_2)_2O(CH_2)_2$	3-NO_2-C_6H_4	$(CH_2)_2$	CH_3	H	A	99	tol–EtOAc (9:1 to 7:3)
9k	CH_3	3-NO_2-C_6H_4	$(CH_2)_3$	CH_3	H	A	35	$CHCl_3$–EtOAc (100:0 to 2:3)
9l[d]	CH_3	3-NO_2-C_6H_4	$(CH_2)_3$	CH_3	CH_3	A	48	$CHCl_3$–EtOAc (9:1 to 3:1)
9m	CH_3	3-NO2-C6H4	$(CH_2)_4$	CH_3	H	A	53[e]	$CHCl_3$–Me_2CO (95:5 to 7:3)
9n	CH_3	3-NO_2-C_6H_4	$CH(CH_3)CH_2$	CH_3	H	A	35	$CHCl_3$–EtOAc (95:5 to 7:3)
9n$_1$[f]						B_1	23[b]	$CHCl_3$–EtOAC (10:0 to 1:1)
9n$_2$[g]						B_1	29[e]	$CHCl_3$–EtOAc (10:0 to 1:1)
9o	$(CH_3)_2CH$	3-NO_2-C_6H_4	$CH(CH_3)CH_2$	CH_3	H	C	34	tol–EtOAc (9:1 to 0:10)
9p	$CH_3(CH_2)_2O(CH_2)_2$	3-NO_2-C_6H_4	$CH(CH_3)CH_2$	CH_3	H	C	30[e]	tol–EtOAc (4:1 to 1:1)
9q	$CH_3(CH_2)_2OCH_2C(CH_3)_2$	3-NO_2-C_6H_4	$CH(CH_3)CH_2$	CH_3	H	C	25[e]	tol–EtOAc (9:1 to 1:1)
9r	$(CH_3)_2CHCH_2$	2,3-Cl_2-C_6H_3	$CH(CH_3)CH_2$	CH_3	H	A	5	$CHCl_3$–EtOAc (4:1 to 3:2)
9s	$CH_3(CH_2)_2O(CH_2)_2$	2,3-Cl_2-C_6H_3	$CH(CH_3)CH_2$	CH_3	H	C	25	tol–EtOAc (4:1 to 1:1)
9t	CH_3	3-NO_2-C_6H_4	$C(CH_3)_2CH_2$	H	H	B_2	54	CH_2Cl_2–EtOAc (7:3)
9u	CH_3	3-NO_2-C_6H_4	$C(CH_3)_2CH_2$	CH_3	H	B_2	44	CH_2Cl_2–EtOAc (100:0 to 70:30)
9u$_1$						C	36[e]	petroleum ether–Me_2CO (90:10 to 85:15)
9v	CH_3	4-NO_2-C_6H_4	$C(CH_3)_2CH_2$	CH_3	H	B_2	57	$CHCl_3$–Me_2CO (100:0 to 97:3)
9w	C_2H_5	3-NO_2-C_6H_4	$C(CH_3)_2CH_2$	CH_3	H	C	35	petroleum ether–EtOAc (4:1 to 3:2)
9x	$(CH_3)_2CH$	3-NO_2-C_6H_4	$C(CH_3)_2CH_2$	CH_3	H	C	11[c]	$CHCl_3$–Me_2CO (100:0 to 95:5)
9y	$CH_3(CH_2)_2O(CH_2)_2$	3-NO_2-C_6H_4	$C(CH_3)_2CH_2$	CH_3	H	C	35[e]	$CHCl_3$–Me_2CO (100:0 to 94:6)
9z	$CH_3(CH_2)_2OCH_2C(CH_3)_2$	3-NO_2-C_6H_4	$C(CH_3)_2CH_2$	CH_3	H	C	40[e]	$CHCl_3$–Me_2CO (100:0 to 93:8)
9aa	CH_3	2,3-Cl_2-C_6H_3	$C(CH_3)_2CH_2$	CH_3	H	C	17[h]	$CHCl_3$–Me_2CO (100:0 to 95:5)
9ab	CH_3	3-NO_2-C_6H_4	$CH(CH_3)(CH_2)_2$	CH_3	H	B_1	51[b]	$CHCl_3$–EtOAc (10:0 to 0:10)
9ac	CH_3	3-NO_2-C_6H_4	$C(CH_3)_2(CH_2)_2$	CH_3	H	B_2	73	petroleum ether–Me_2CO (7:3 to 6:4)
9ad	CH_3	3-NO_2-C_6H_4	$CH_2C(CH_3)_2CH_2$	CH_3	H	B_2	60[e]	petroleum ether–EtOAc (7:3)
9ae	CH_3	2,3-Cl_2-C_6H_3	$CH_2C(CH_3)_2CH_2$	CH_3	H	B_2	34	petroleum ether–EtOAc (9:1 to 3:1)
9af	CH_3	3-NO_2-C_6H_4	$(CH_2)_2C(CH_3)_2$	CH_3	H	B_2	61[e]	petroleum ether–EtOAc (4:1 to 3:1)
9ag	CH_3	3-NO_2-C_6H_4	$C(CH_3)_2CH_2$	CH_3	=O	B_2	64	petroleum ether–EtOAc (3:2)

[a]Compounds 9a–f were isolated as HCl salts: [b]$\cdot H_2O$; [c]$\cdot 0.6H_2O$; [d]1,1-dimethyl-3,3-diphenylpropylamine used for the synthesis of these compounds was prepared according to the literature [21]; [e]$\cdot 0.5H_2O$; [f]diastereomer at lower R_f; [g]diastereomer at higher R_f; [h]$\cdot 0.75H_2O$.

2. Chemistry

The asymmetric monobasic diesters of 4-aryl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylic acids, listed in *table I*, were synthesized by three different routes described in *figure 2*.

The haloalkyl acetoacetates **2**, prepared starting from the corresponding haloalkyl alcohols **1**, were reacted with the suitable aldehydes **3** to give the haloalkyl 2-arylideneacetoacetates **4** as mixtures of the *E/Z* isomers. The suitable commercially available or synthesized aminocrotonates **5** were condensed with **4** to give the alkyl haloalkyl 4-aryl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates **6**. For the preparation of intermediate **6e** it was more convenient to carry out the esterification of 1,4-dihydro-2,6-dimethyl-3-methoxycarbonyl-4-(3-nitrophenyl)pyridine-5-carboxylic acid with 1,4-dibromobutane in anhydrous DMF and in the presence of K_2CO_3. The haloderivatives **6** were then used to perform the alkylation of the diphenylalkylamines **8** by the general *method A* to give the desired compounds **9a–n,r**.

The 3-alkoxycarbonyl-4-aryl-1,4-dihydro-2,6-dimethylpyridine-5-carboxylic acids **7** were used to esterify the *N*-(hydroxyalkyl)-diphenylalkylamines **10** by *method B*, alternatively using a condensation agent (DCC in the presence of DMAP) to give the final compounds **9n,ab** (method B_1) or via acyl chloride formation to give **9t–v,ac–ag** (method B_2).

Following the third pathway, some aminoalkyl-alcohols **10** were reacted with diketene to give the corresponding *N*-(diphenylalkyl)aminoalkyl acetoacetates **11** which were condensed with **3** to give the corresponding arylidene derivatives **12**. Subsequent cyclization with the suitable aminocrotonates **5** by *method C* gave the desired compounds **9o–q,s,u,w–aa**.

In steps i and vi diketene can be successfully replaced by its acetone adduct (2,2,6-trimethyl-1,3-dioxin-4-one) by operating in higher temperatures. In the reaction between intermediates **7** and **10** (method B_1), the formation of dicyclohexylcarbodiimide adduct occurred readily. But a long reaction time was needed in order to obtain the esters **9** in acceptable yields.

For the preparation of 2,3-dichlorobenzylidene-acetoacetates **4** and **12**, longer reaction times with respect to the other arylidene derivatives or heating with $CF_3COOH/CHCl_3$ was needed in order to accomplish the elimination reaction. Attempts to synthesize intermediates **12** in refluxing C_6H_6 in the presence of piperidinium acetate as catalyst resulted less satisfactory than using excess HCl in $CHCl_3$. This might be due to basic-assisted hydrolysis of the aminobenzylidene derivatives formed. Sometimes, the re-formation of small amounts of the corresponding **10** and **3** (5 to 10%) was noticed, after prolonged contact with water or silica gel of these compounds in the base form.

In some cases, the HCl-catalyzed condensation to **12** afforded also minor amounts of the addition compound of HCl to the benzylidene double bond. The aminoalcohols **10b,e,g** were synthesized by specific routes, as depicted in *figures 3* and *4*.

Addition of *N*-(3,3-diphenylpropyl)methylamine to 3-buten-2-one gave the 3-aminoketone **13** that was C-alkylated via methylmagnesium iodide, yielding the desired aminoalcohol **10e**. Intermediate **13** was also reduced with $NaBH_4$ to give the corresponding aminoalcohol **10d**.

In *figure 4*, esterification of 3-methylcrotonic acid with ethanol gave the corresponding ester **14**; the addition reaction of the commercially available 3,3-diphenylpropylamine to this ester gave the 3-aminobutyrate **15**. Alkylation of this secondary amine with *methyl iodide gave the tert-aminoester 16 that was* finally reduced with $LiAlH_4$ yielding **10g**.

Some of the 1,4-dihydropyridines **9** were isolated as hydrochloride salt in form of amorphous solids, because of difficulties in obtaining crystals. When the NMR spectrum of compound **9u** and some other **9** derivatives having only the C_4 chiral center, in the HCl form were recorded in $CDCl_3$ solution at high field (200–300 MHz) complex spectra were obtained, suggesting the presence of two different species, probably due to intramolecular interactions. This will be the subject of a separate publication.

The structure and main characteristics of the intermediates utilized are shown in *tables II–VIII*. The 'final' compounds are listed in *table I*.

3. Pharmacology

Radioreceptor binding studies were performed with ^{3}H-nitrendipine on rat brain membrane, according to the method previously reported [27]. Test compounds were assayed as solutions in ethanol and the affinity for the channel is reported as IC_{50} (concentration inhibiting by 50% the binding of ^{3}H-nitrendipine), in *table IX*.

The antihypertensive activity of new and known compounds was tested in 12–16 weeks old SHR/Cnl/BR rats (4 animals per dose). Test compounds were administered by gavage as suspensions in 0.5% methocel in saline. Conscious animals were prewarmed at 37 °C for 15 min before tail-cuff recording of systolic blood pressure [28]. The antihypertensive effects at peak time were expressed as ED_{25} (dose lowering by 25% the systolic blood pressure). The time of peak effect was generally about 3 h, with few exceptions reported in *table IX*.

Indicative acute toxicity was determined after intraperitoneal and oral administration of test

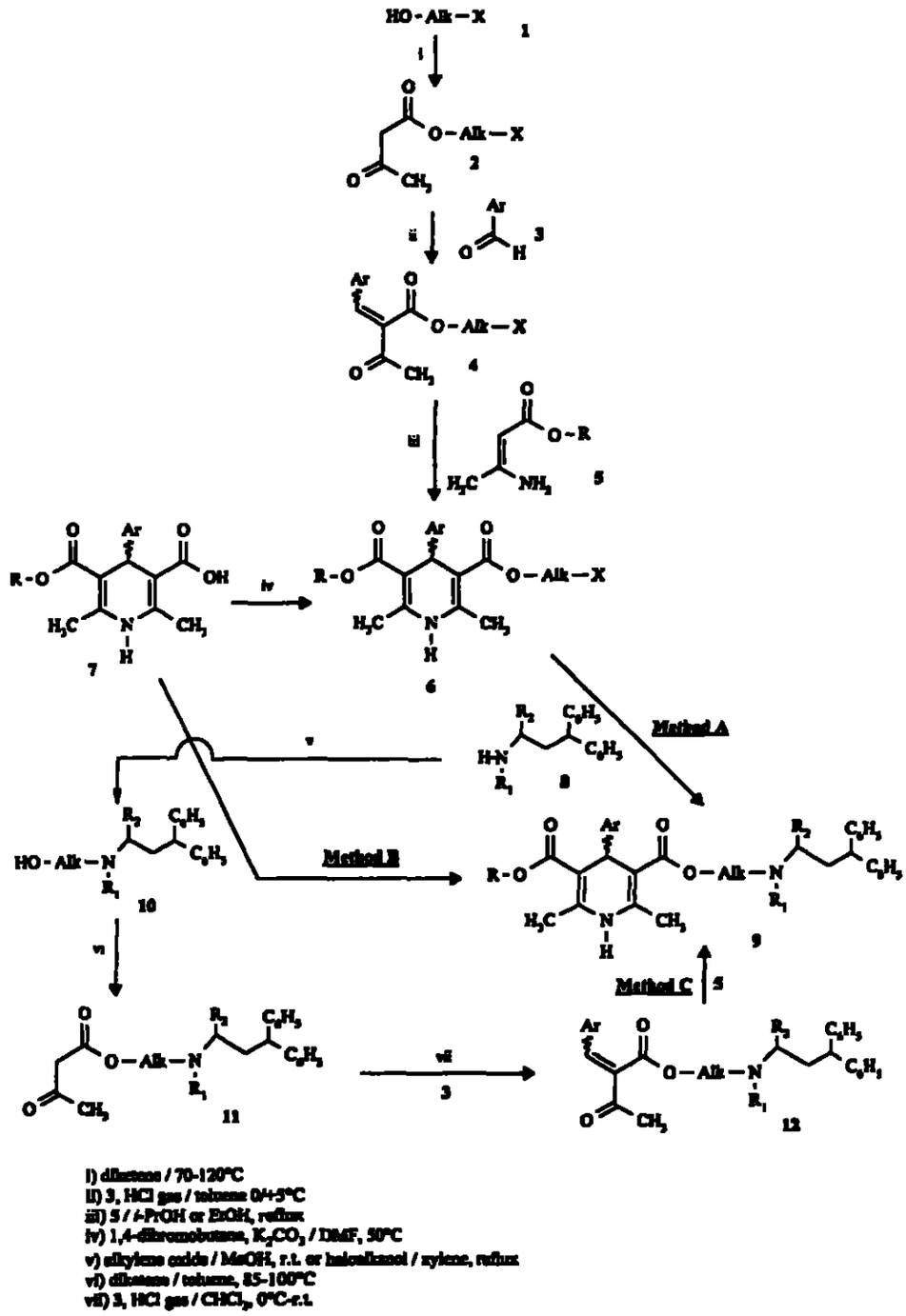
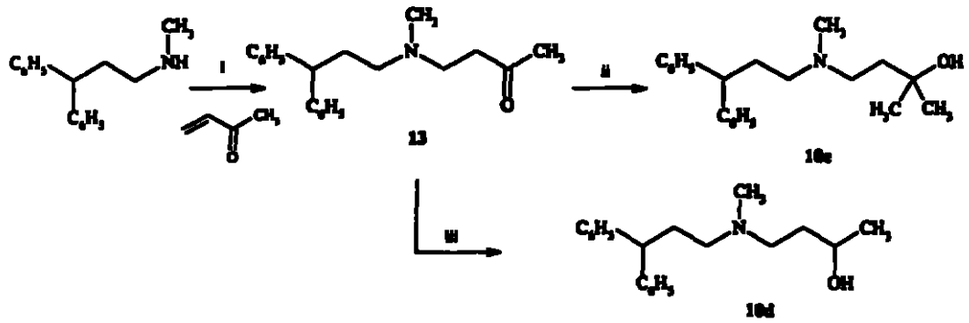
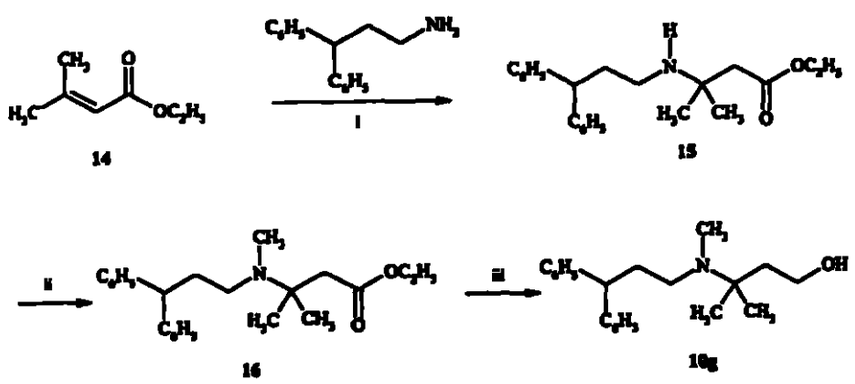


Figure 2. Synthesis of final 1,4-dihydropyridines **9**.



i) Et_2O, -5/+3°C
ii) CH_3MgI, Et_2O, -5/+10°C, r.t.
iii) $NaBH_4$, MeOH, 0°C

Figure 3. Synthetic pathway for aminoalcohol **10d** and **10e**.



i) EtOH, reflux
ii) CH_3I, K_2CO_3, DMF, r.t.
iii) $LiAlH_4$, Et_2O, reflux

Figure 4. Synthetic pathway for aminoalcohol **10g**.

compounds, as suspension in 0.5% methocel in saline, to CrlCD–I–(ICR)BR mice (4 animals per dose). For the interesting molecules, indicative acute toxicity was also determined in Sprague Dawley rats by intravenous administration of solutions in aqueous propylene glycol (4 animals per dose). The mortality rate was recorded over a 14-day period.

4. Results and discussion

As shown in *table IX*, most compounds exhibited antihypertensive activity and affinity for the calcium channel labelled by ^{3}H-nitrendipine comparable or better than the reference 1,4-DHPs. A trend to correlate was found between the in vitro and in vivo results (see *figure 5*), taking into account that the oral administration route was used for testing the antihypertensive activity ($Y = 5.49 - 0.53X$; $r = 0.723$; $n = 31$).

With regard to the structural modifications performed, the following comments can be made. With regard to the aromatic group at position 4 of the 1,4-DHP ring, Ar, the 3-nitrophenyl group showed the most desirable profile for pharmacological and toxicological properties.

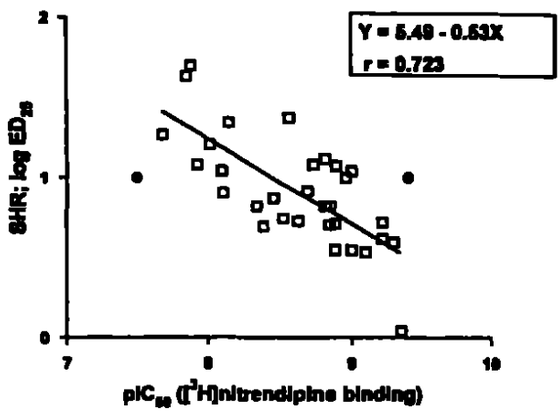


Figure 5. Correlation between affinity for the calcium channel binding site (pIC_{50}) and the antihypertensive activity in SHR (log ED_{25}). Points shown as closed circles were excluded from computation.

In agreement with the known SAR studies on this class of compounds [8, 29–31], the introduction of the 4-nitrophenyl group (compound **9v**) caused loss of activity. A tendency to a longer duration of the antihypertensive effect was noticed for compounds bearing the 2,3-dichlorophenyl group, in particular for **9c**.

As far as the non-basic ester group R is concerned, in the subset where Alk is an ethylene bridge (compounds **9b,g–j**) ethyl, *i*-propyl and *i*-butyl showed the best antihypertensive potency, whereas methyl group proved in general more potent when the ethylene bridge was branched (compounds **9n–s,u–z**).

Derivatives with R = propoxyalkyl resulted, on an average, less toxic in mouse. Since this loss of toxicity upon oral administration was not paralleled by a decrease in toxic effects after i.v. administration in rat, it is possible that lower toxicity in mouse is actually due to lower bioavailability in this species. Changing a hydrogen atom for a methyl group at R_1 or R_2 did not substantially affect the pharmaco-toxicological activity.

With regard to the alkylene spacer Alk, the propylene bridge of **9k** proved the best among linear chains. Branching with one or two methyl groups at the oxygen-bonded carbon in the ethylene subset enhanced potency and, in some cases, reduced toxicity in the rat.

The diastereoselectivity found with the 2-propylene derivatives $9n_1$ and $9n_2$ confirms the ability of this structural element in modulating, directly or indirectly, the affinity for the 1,4-DHP site in the calcium channel. This modulating role was more evident in the 1,3-propylene derivatives **9ac,ad,af**, where formal double methylation at position 2 of the chain afforded the most potent derivative in the series (**9ad**). Quite surprisingly, **9ac**, combining the desirable features of **9k** (linear propylene bridge) and of **9u** ($C(CH_3)_2CH_2$ in the bridge), proved significantly less potent than **9ad** and said analogues.

The absence of antihypertensive activity and toxic effects in the mouse seen with the 3,3-diphenylpropanamide derivative **9ag**, in presence of a strong affinity for the calcium channel, prompted us to evaluate its antihypertensive effect upon intravenous administration in catheterized SHR following the protocol described in [32]. In this case a strong antihypertensive effect was noticed (ED_{25} = 44 μg/kg, versus 16 μg/kg for **9u**), pointing out a very low systemic absorption for this compound upon oral administration and suggesting that the presence of the amino group in the whole series influences more the bioavailability of these compounds than their affinity for the calcium channel.

Inspection of data obtained for the new 4-(3-nitrophenyl)-1,4-DHPs suggests that the introduction of the 3,3-diphenylpropylamino moiety confers to the whole series an elevated and fairly constant level of antihypertensive activity irrespective of the structural variations of the groups R and Alk, the only feature which generates real differences in in vivo and in vitro pharmacological activity being the position of the gem-dimethyl group when the alkylene bridge is a propyl chain (compounds **9ac,ad,af**). In addition, the presence of this bulky and lipophilic moiety confers the new molecules a delayed peak of the antihypertensive effect, associated to a longer duration, with regard to the less lipophilic nifedipine.

5. Conclusions

Compound **9u** (Rec 15/2375-lercanidipine) was selected for development and obtained marketing authorization in several countries as an antihypertensive. The main characteristics of this drug are the slow onset and long duration of its action, although in the presence of low plasma levels and short plasma half life [33]. This behaviour is explained with the high partition coefficient, measured both in *n*-octanol/acidic buffer (log P = 6.0, pK_a' = 6.83, see experimental protocols) and in artificial cardiac phospholipids membranes (log $K_{p(mem)}$ = 6.1 at pH = 7.4) [34], which confers an elevated affinity for the lipidic bilayer of cells in the cardiovascular system.

Lercanidipine is also characterized by high vascular selectivity and absence of negative inotropic effects [35]. In agreement with the results obtained with most

chiral 1,4-dihydropyridines [36], (*S*)-lercanidipine proved to be the eutomer. The synthesis of lercanidipine enantiomers will be the subject of a separate publication. Full accounts on the pharmacological and clinical studies performed with lercanidipine are reported in references [37, 38].

6. Experimental protocols

6.1. Chemistry

Reagents, starting materials and solvents were purchased from commercial suppliers. Flash chromatography was performed on silica gel (Merck 70–230 mesh). Melting points were determined on a Buchi 535 apparatus and are uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 297 infrared spectrophotometer and were consistent with the structures. ^{1}H-NMR spectra were recorded on a Hitachi-Perkin Elmer R24A or on a Brucker AC 200. The chemical shifts (δ) are in ppm relative to tetramethylsilane. The purity of the compounds was checked by TLC (Merck silica gel 60, F254 0.25 mm). Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values.

6.2. Preparation of intermediates 1

6.2.1. 2-Methyl-1-propoxy-2-propanol 1a

0.1 g-atom (2.3 g) of sodium was added in small portions to 0.53 mol (32 g) of 1-propanol and the mixture was stirred until sodium dissolution (1.5 h). The temperature was lowered to 80 °C and 0.1 mol (10.76 g) of 1-chloro-2-methyl-2-propanol was added dropwise in 30 min. The mixture was stirred at reflux temperature for 10 h then cooled to room temperature, filtered and the filtrate distilled at normal pressure; the fraction collected at 145–148 °C gave 27.7 g (52%) of pure compound. Anal. $C_7H_{16}O_2$ (C, H). ^{1}H-NMR ($CDCl_3$): 3.5 (t, 2H, C–C–CH_2–O–C), 3.3 (s, 2H, O–CH_2–C–O), 2.65 (s, 1H, OH), 1.55 (m, 2H, C–CH_2–C–O), 1.25 (s, 6H, gem-CH_3s), 0.95 (t, 3H, CH_3–C–C–O).

6.3. Preparation of intermediates 2 (table II)

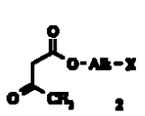
6.3.1. 2-Chloro-1-methylethyl acetoacetate 2c

0.42 mol (35.4 g) of diketene was added in 45 min to 0.40 mol (41.4 g) of 1-chloro-2-propanol stirred at 80 °C. The mixture was heated at 100 °C and after 45 min the temperature spontaneously rose to 120 °C for 20 min. Heating was continued at 100 °C for additional 3 h then the mixture was cooled to room temperature and distilled in vacuo. The fraction collected at 113–115 °C/16 mmHg gave 61.6 g (86%) of the pure compound. Anal. $C_7H_{11}ClO_3$ (C, H, Cl). ^{1}H-NMR ($CDCl_3$): 4.85–5.35 (m, 1H, CH), 3.4–3.7 (m, 4H, CH_2COO and CH_2Cl), 2.25 (s, 3H, CH_3–CO–C), 1.35 (d, 3H, COO–C–CH_3). The product contained about 8% of its enolic form whose main visible peaks are as follows: 12.2 (s, OH), 1.95 (s, CH_3–C=).

6.3.2. 3-Chloropropyl acetoacetate 2d

0.315 mol (26.5 g) of diketene was added in 45 min to 0.3 mol (28.35 g) of 3-chloropropanol stirred at 70 °C. During the addition the temperature was gradually raised to 120 °C and then was kept at 100 °C for 3 h. After cooling to room

Table II. Alkyl and haloalkyl acetoacetates 2. Acetoacetates 2e–h were reacted with ammonia to give the corresponding 3-aminocrotonates 5a–d (*table IV*).



2

Compound	Alk-X	Yield (%)	B.p. (°C)/mmHg	Anal.
2a	$(CH_2)_2Cl$	89	115–117/19[a]	–
2b	$(CH_2)_2Br$	61	83–88/0.4[b]	–
2c	$CH(CH_3)CH_2Cl$	86	113–115/16	C, H, Cl
2d	$(CH_2)_3Cl$	82	125–131/15	C, H, Cl
2e	$CH(CH_3)_2$	84	73–75/15[c]	–
2f	$CH_2CH(CH_3)_2$	83	93–95/15[d]	–
2g	$(CH_2)_2O(CH_2)_2CH_3$	92	125–128/16[e]	–
2h	$C(CH_3)_2CH_2O(CH_2)_2CH_3$	88	123–125/16	C, H

[a]100–102 °C/10 mmHg [13]; [b]124–127 °C/20 mmHg [22]; [c]66–69 °C/10 mmHg [23]; [d]100 °C/22 mmHg [24]; [e]130–137 °C/20 mmHg [13].

temperature the mixture was distilled in vacuo and the fraction collected at 125–131 °C/15 mmHg gave 43.9 g (82%) of the pure compound. Anal. $C_7H_{11}ClO_3$ (C, H, Cl). ^{1}H-NMR ($CDCl_3$): 4.2 (t, 2H, OCH_2–C–C), 3.65 (t, 2H, O–C–C–CH_2Cl), 3.5 (s, 2H, $COCH_2COO$), 2.2 (s, 3H, CH_3CO), 2.05 (m, 2H, O–C–CH_2–C–Cl). A peak at 12.3 (OH) and a peak at 5.0 (C=CHCOO) were present, attributed to about 5% of the enolic form.

6.3.3. 2-Methyl-1-propoxy-2-propyl acetoacetate 2h

0.347 mol (29.2 g) of diketene was dropped in 40 min into a stirred mixture of 0.316 mol (41.9 g) of 2-methyl-1-propoxy-2-propanol (1a) and 1 mmol (0.1 g) of triethylamine, preheated at 100 °C. Further heating lasted 4 h, without exceeding the temperature of 105 °C. After cooling to room temperature, the mixture was distilled in vacuo and the fraction collected at 123–125 °C/16 mmHg gave 60.6 (88%) of the pure compound. Anal. $C_{11}H_{20}O_4$ (C, H). ^{1}H-NMR ($CDCl_3$): 3.5 (s, 2H, $COCH_2$), 3.4 (t, 2H, O–CH_2–C–C), 3.35 (s, 2H, O–C–CH_2–O), 2.25 (s, 3H, CH_3–CO), 1.5 (m, 2H, O–C–CH_2–C), 1.45 (s, 6H, gem-CH_3s), 0.9 (t, 3H, O–C–C–CH_3).

6.4. Preparation of intermediates 3

6.4.1. Benzofurazan-4-carbaldehyde

A mixture of 0.01 mol (1.34 g) of 4-methylbenzofurazan [39] and 0.011 mol (1.21 g) of selenium dioxide was stirred at 160 °C for 8 h. After cooling to room temperature the mixture was crushed with EtOAc, the insoluble was removed by filtration and the filtrate evaporated to dryness in vacuo. The crude was purified by flash chromatography eluting with a $CHCl_3$–petroleum ether 9:1 mixture. Yield 82% (1.21 g), m.p. 108–109 °C after crystallization from cyclohexane. Anal. $C_7H_4N_2O_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 10.1 (s, 1H, CHO), 8.4–7.5 (m, 3H, aromatics). This compound was also obtained in lower yield starting from 4-bromomethylbenzofurazan through a Sommelet reaction [39].

6.5. Preparation of intermediates 4 (table III)

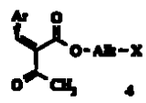
6.5.1. 2-Chloro-1-methylethyl 2-(3-nitrobenzylidene)acetoacetate 4c

A mixture of 0.08 mol (12.2 g) of 3-nitrobenzaldehyde and 0.08 mol (14.3 g) of 2-chloro-1-methylethyl acetoacetate 2c in 80 mL of toluene was saturated with HCl at 0/5 °C. The solution was kept two days at room temperature and after this period a stream of N_2 was bubbled to eliminate excess HCl. The solvent was evaporated in vacuo and the oil was dissolved into CH_2Cl_2, washed with H_2O until neutrality, dried over Na_2SO_4, filtered, and evaporated in vacuo to dryness. Crystallization from *i*-propanol gave 20.7 g (83%) of the pure compound (*E/Z* mixture). M.p. 95–96 °C. Anal. $C_{14}H_{14}ClNO_5$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.3–7.5 (m, 4H, aromatics), 7.55 (s, 1H, CH=C), 5.25 (m, 1H, COOCH), 3.55 (d, 2H, C–CH_2Cl), 2.45 (s, 3H, CH_3–CO–C), 1.36 (d, 3H, COO–C–CH_3).

6.5.2. 3-Chloropropyl 2-(3-nitrobenzylidene)acetoacetate 4d

A mixture of 0.17 mol (30.34 g) of 3-chloropropyl acetoacetate 2d and 0.17 mol (25.7 g) of 3-nitrobenzaldehyde in 170 mL of toluene was treated as described for 4c. The crude was purified by flash chromatography eluting with toluene. Yield 50.9 g (95%), m.p. 63–65 °C. Anal. $C_{14}H_{14}ClNO_5$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.50–7.50 (m, 5H, aromatics and CH=C), 4.45 (t, 2H, $COOCH_2$), 3.65 and 3.50 (2t, 2H, CH_2Cl of *E/Z* isomers), 2.50 (s, 3H, CH_3CO), 2.10 (m, 2H, COO–C–CH_2).

Table III. Haloalkyl 2-benzylideneacetoacetates 4 (*E/Z* mixtures).



4

Compound	Ar	Alk-X	Yield	M.p. (°C)	Anal.
4a	2-NO_2–C_6H_4	$(CH_2)_2Cl$	58	Oil[a]	–
4b	3-NO_2–C_6H_4	$(CH_2)_2Cl$	86	92–95[b]	–
4c	3-NO_2–C_6H_4	$CH(CH_3)CH_2Cl$	83	95–96	C, H, N, Cl
4d	3-NO_2–C_6H_4	$(CH_2)_3Cl$	95	63–65	C, H, N, Cl
4e	3-NO_2–C_6H_4	$(CH_2)_2Br$	69	83–91	C, H, N, Br
4f	2,3-Cl_2–C_6H_3	$(CH_2)_2Cl$	87	Oil	C, H, N, Cl
4g	2,3-Cl_2–C_6H_3	$CH(CH_3)CH_2Cl$	100	Oil	C, H, Cl
4h	4-benzofurazanyl	$(CH_2)_2Cl$	95	Oil	C, H, N, Cl

[a]Sample enriched in the isomer with higher R_f, m.p. 74–75 °C (*i*-PrOH) [25]; [b]sample crystallized from CCl_4, m.p. 94–97 °C (*i*-PrOH) [25].

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6.5.3. 2-Bromoethyl 2-(3-nitrobenzylidene)acetoacetate 4e

A mixture of 0.055 mol (8.3 g) of 3-nitrobenzaldehyde, 0.055 mol (11.5 g) of 2-bromoethyl acetoacetate 2b and 55 mL of toluene was saturated with anhydrous HBr at 0/5 °C. The solution was kept at room temperature for 7 days, bubbled with a N_2 stream and evaporated to dryness. The solution was diluted with Et_2O and washed with with H_2O until neutrality; the organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was dissolved into $CHCl_3$ (100 mL), added with trifluoroacetic acid (20 mL) and the solution refluxed for 20 h. After cooling to room temperature the solution was washed with H_2O until neutrality, dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give a crude (15.75 g) that was treated with boiling CCl_4 (300 mL). The insoluble was removed by filtration, the filtrate was evaporated to dryness and the residue washed with Et_2O (60 mL) to give 12.9 g (69%) of the pure compound. M.p. 83–91 °C. Anal. $C_{13}H_{12}BrNO_5$ (C, H, N, Br). ^{1}H-NMR ($CDCl_3$): 8.5–8.2 (m, 2H, 3-nitrophenyl–H_2–H_4), 8.05–7.6 (m, 3H, 3-nitrophenyl–H_5–H_6 and CH=C), 4.65 (t, 2H, $COOCH_2$), 3.65 and 3.55 (2t, 2H, CH_2Br of *E/Z* isomers), 2.5 (s, 3H, CH_3CO).

6.5.4. 2-Chloroethyl 2-(2,3-dichlorobenzylidene)acetoacetate 4f

A mixture of 0.1 mol (17.5 g) of 2,3-dichlorobenzaldehyde, 0.1 mol (16.45 g) of 2-chloroethyl acetoacetate 2a and 100 mL of toluene was saturated with HCl at 0/3 °C and the solution was kept at room temperature for 7 days. After this period a stream of N_2 was bubbled to eliminate HCl, the solution was diluted with Et_2O and washed with H_2O until neutrality. The organic phase was dried over anhydrous Na_2SO_4 and evaporated in vacuo to give an oily residue (35 g) that was dissolved into $CHCl_3$ (230 mL), added with trifluoroacetic acid (35 mL) and the solution refluxed for 20 min. After cooling to room temperature the solution was washed with H_2O until neutrality, dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give 28.5 g (87%) of the pure compound as a thick oil. Anal. $C_{13}H_{11}Cl_3O_3$ (C, H, Cl). ^{1}H-NMR ($CDCl_3$): 7.9 and 7.8 (2s, 1H, CH=C of *E/Z* isomers), 7.6–7.1 (m, 3H, aromatics), 4.5–4.2 (m, 2H, $COOCH_2$), 3.8–3.4 (m, 2H, CH_2Cl), 2.45 and 2.2 (2s, 3H, CH_3CO of *E/Z* isomers).

6.5.5. 2-Chloro-1-methylethyl 2-(2,3-dichlorobenzylidene)-acetoacetate 4g

A solution of 0.1 mol (17.5 g) of 2,3-dichlorobenzaldehyde in 70 mL of toluene was added at 20 °C to a solution of 0.1 mol (17.8 g) of 2-chloro-1-methylethyl acetoacetate 2c in 30 mL of toluene. The mixture was saturated with HCl at 0/3 °C and the solution was kept at room temperature for 7 days. After this period a stream of N_2 was bubbled to eliminate HCl, the solution was diluted with Et_2O and washed with H_2O until neutrality. The organic phase was dried over anhydrous Na_2SO_4 and evaporated in vacuo to give an oily residue that was dissolved in $CHCl_3$ (300 mL) and added with trifluoroacetic acid (35 mL), and the solution refluxed for 20 h. After cooling to room temperature the solution was washed with H_2O until neutrality, dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo to give 33.5 g (100%) of the pure compound as a *E/Z* isomers mixture in form of a thick oil. A sample of this mixture was flash chromatographed eluting with toluene and the residue crystallized from cyclohexane to give the pure isomer melting at 63–65 °C. Anal. $C_{14}H_{13}Cl_3O_3$ (C, H, Cl). ^{1}H-NMR ($CDCl_3$): 7.9 (s, 1H, CH=C–CO), 7.7–7.1 (m, 3H, aromatics), 5.5–4.9 (m, 1H, COOCH), 3.5 (d, 2H, CH_2Cl), 2.5 (s, 3H, CH_3CO), 1.25 (d, 3H, COO–C–CH_3).

6.5.6. 2-Chloroethyl 2-(4-benzofurazanylmethylidene)acetoacetate 4h

A mixture of 0.1 mol (16.2 g) of 2-chloroethyl acetoacetate, 0.1 mol (14.8 g) of benzofurazan-4-carbaldehyde and 200 mL of $CHCl_3$ was saturated with anhydrous HCl at 0 °C. The solution was kept at room temperature for 7 days, bubbled with a N_2 stream and evaporated to dryness. The residue was dissolved in EtOAc and the solution was washed with diluted aqueous $NaHCO_3$ solution followed by H_2O; the organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to give the oily pure compound. (TLC $CHCl_3$–EtOAc, 95:5). Yield 28 g (95%). This compound was directly used for the synthesis of the corresponding dihydropyridine 6g. Anal. $C_{13}H_{11}ClN_2O_4$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.2–7.35 (m, 4H, $H_{4,5,6,7}$ of benzofurazanyl ring and CH=C), 3.0–2.7 and 2.7–2.35 (2m, 2H, CH_2Cl of *E/Z* isomers), 2.8–2.45 and 2.4–2.05 (2m, 2H, $COOCH_2$ of *E/Z* isomers), 2.55 and 2.2 (2s, 3H, CH_3 of *E/Z* isomers).

6.6. Preparation of intermediates 5 (table IV)

6.6.1. 2-Methyl-1-propoxy-2-propyl 3-aminocrotonate 5d

A stream of anhydrous NH_3 (150 g) was bubbled in about 50 h into a solution of 0.05 mol (10.81 g) of 2-methyl-1-propoxy-2-propyl acetoacetate 2h in 40 mL of CH_2Cl_2. During this time the temperature was not allowed to exceed 15 °C and at the end the mixture was diluted with 50 mL of CH_2Cl_2, dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo at room temperature to give 10.1 g (94%) of the oily compound, which in some preparations contained from 5 to 10% of the starting material. This product was used without further purification for the synthesis of final compound 9aa. Anal. $C_{11}H_{21}NO_3$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.0–5.0 (bs, 2H, NH_2), 4.4 (s, 1H, CHCOO), 3.5 (s, 2H, COO–C–CH_2–O), 3.4 (t, 2H, O–CH_2–C–C), 1.8 (s, 3H, CH_3–C=C), 1.8–1.1 (m, 2H, O–C–CH_2–C), 1.45 (s, 6H, gem-CH_3s), 0.9 (t, 3H, O–C–C–CH_3).

6.7. Preparation of intermediates 6 (table V)

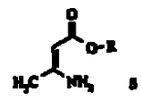
6.7.1. 2-Chloroethyl methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate 6a

A mixture of 0.075 mol (22.33 g) of 2-chloroethyl 2-(2-nitrobenzylidene)acetoacetate 4a, 0.075 mol (8.90 g) of methyl 3-aminocrotonate and 60 mL of 2-propanol was stirred at reflux temperature for 3 h. After cooling to room temperature, the solvent was evaporated in vacuo and the residue was purified by flash chromatography eluting with $CHCl_3$. The collected pure fractions (TLC $CHCl_3$–EtOAc, 9:1) were pooled and the solid residue obtained after solvent evaporation was washed with *i*-Pr_2O to give 11.2 g (38%) of pure 6a. M.p. 118–120 °C (*i*-Pr_2O). Anal. $C_{18}H_{19}ClN_2O_6$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 7.8–7.0 (m, 4H, aromatics), 6.1 (bs, 1H, NH), 5.75 (s, 1H, CH), 4.25 (t, 2H, $COOCH_2$), 3.55 (t, 2H, CH_2Cl), 3.55 (s, 3H, CH_3O), 2.35 and 2.25 (2s, 6H, 2,6 CH_3s).

6.7.2. 2-Chloro-1-methylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate 6c

Same procedure as for 6a. Yield 64% (after column chromatography, eluting with $CHCl_3$ and washing with H_2O); m.p. 115–119 °C. Anal. $C_{19}H_{21}ClN_2O_6$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.1–7.2 (m, 4H, aromatics), 6.05 (bs, 1H, NH), 5.05 and 5.1 (2s, 1H, pyridine–CH), 4.2–3.8 (m, 1H, COOCH), 3.65 (s, 3H, CH_3O), 3.5 (dd, 2H, CH_2Cl), 2.35 (s, 6H, 2,6 CH_3s), 1.25 (dd, 3H, COO–C–CH_3).

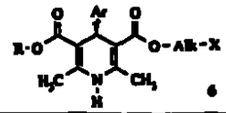
Table IV. Alkyl 3-aminocrotonates 5.



Compound	R	Yield (%)	M.p. or b.p. (°C)/mmHg	Anal.
5a	$CH(CH_3)_2$	95	106–107/20[a]	–
5b	$CH_2CH(CH_3)_2$	85	131/22[b]	–
5c	$(CH_2)_2O(CH_2)_2CH_3$	97	Oil[c,d]	–
5d	$C(CH_3)_2CH_2O(CH_2)_2CH_3$	94	Oil[d]	C, H, N

[a]102–103 °C/12 mmHg [23]; [b]116–118 °C/17 mmHg [26]; [c]120 °C/0.7 mmHg [13]; [d]not distilled by us since the crude showed acceptable purity (tlc).

Table V. Alkyl haloalkyl 4-aryl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates 6.



Compound	R	Ar	Alk-X	Yield (%)	M.p. (°C)	Anal.
6a	CH_3	2-NO_2–C_6H_4	$(CH_2)_2Cl$	38	118–120	C, H, N, Cl
6b	CH_3	3-NO_2–C_6H_4	$(CH_2)_2Cl$	62	136–138[a]	–
6c	CH_3	3-NO_2–C_6H_4	$CH(CH_3)CH_2Cl$	64	115–119	C, H, N, Cl
6d	CH_3	3-NO_2–C_6H_4	$(CH_2)_3Cl$	81	144–147	C, H, N, Cl
6e	CH_3	3-NO_2–C_6H_4	$(CH_2)_4Br$	84	124–125	C, H, N, Br
6f	CH_3	2,3-Cl_2–C_6H_3	$(CH_2)_2Cl$	44	167–169	C, H, N, Cl
6g	CH_3	4-benzofurazanyl	$(CH_2)_2Cl$	36	117–118	C, H, N, Cl
6h	CH_3CH_2	3-NO_2–C_6H_4	$(CH_2)_2Cl$	67	160–162	C, H, N, Cl
6i	$(CH_3)_2CH$	3-NO_2–C_6H_4	$(CH_2)_2Cl$	71	141–146[b]	–
6j	$(CH_3)_2CHCH_2$	3-NO_2–C_6H_4	$(CH_2)_2Cl$	69	155–157	C, H, N, Cl
6k[c]	$(CH_3)_2CHCH_2$	2,3-Cl_2–C_6H_3	$CH(CH_3)CH_2Cl$	48	Oil	C, H, N, Cl
6l	$CH_3(CH_2)_2O(CH_2)_2$	3-NO_2–C_6H_4	$(CH_2)_2Cl$	70	104–106	C, H, N, Cl

[a]130–131 °C [23]; [b]143–145 °C [23]; [c]unstable to heating and silicagel.

6.7.3. 3-Chloropropyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate 6d

The reaction was carried out as for **6a** but using ethanol, as reaction solvent, at reflux temperature for 6 h. The product crystallized from the reaction mixture. Yield 81%; m.p. 144–147 °C (EtOH). Anal. $C_{19}H_{21}ClN_2O_6$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.2–7.2 (m, 4H, aromatics), 6.5 (s, 1H, NH), 5.1 (s, 1H, CH), 4.2 (t, 2H, $COOCH_2$), 3.7 (s, 3H, CH_3O), 3.45 (t, 2H, CH_2Cl), 2.4 (s, 6H, 2,6 CH_3s), 2.05 (t, 2H, $COO-C-CH_2-C$).

6.7.4. 4-Bromobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate 6e

A suspension of 0.03 mol (9.96 g) of 1,4-dihydro-2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)pyridin-3-carboxylic acid [40], 0.09 mol (19.43 g) of 1,4-dibromobutane, 0.015 mol (2.07 g) of anhydrous K_2CO_3 and 60 mL of anhydrous DMF was stirred at 50 °C for 2.5 h. After cooling to room temperature, the mixture was poured into 400 mL of H_2O, extracted with Et_2O, the organic layer washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness in vacuo. The oily residue was purified by flash chromatography eluting with $CHCl_3$–EtOAc (100:0 to 97:3) gradient mixture, to give 11.75 g (84%) of the pure compound. M.p. 124–125 °C (*i*-PrOH or CCl_4). Anal. $C_{20}H_{23}BrN_2O_6$ (C, H, N, Br). ^{1}H-NMR ($CDCl_3$): 8.3–7.4 (m, 4H, aromatics), 6.45 (s, 1H, NH), 5.2 (s, 1H, CH), 4.3–4.0 (m, 2H, $COOCH_2$), 3.75 (s, 3H, OCH_3), 3.6–3.3 (m, 2H, CH_2Br), 2.4 (s, 6H, 2,6 CH_3s), 2.0–1.6 (m, 4H, $O-C-CH_2CH_2-C$).

6.7.5. 2-Chloroethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate 6f

Same procedure as for **6a**. The reaction mixture was refluxed for 8 h. The crude crystallized from the reaction mixture and was purified by flash chromatography eluting with $CHCl_3$–EtOAc (100:0 to 95:5) gradient mixture. Yield 44%; m.p. 167–169 °C (*i*-PrOH). Anal. $C_{18}H_{18}Cl_3NO_4$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 7.5–6.8 (m, 3H, aromatics), 6.05 (s, 1H, NH), 5.45 (s, 1H, CH), 4.25 (t, 2H, $COOCH_2$), 3.6 (s + t, 5H, CH_3O and CH_2Cl), 2.3 (s, 6H, 2,6 CH_3s).

6.7.6. 2-Chloroethyl methyl 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate 6g

Same procedure as for **6a**.The reaction mixture was refluxed for 13 h and the crude was purified by flash chromatography eluting with $CHCl_3$–EtOAc (100:0 to 95:5) gradient mixture. The residue was washed with *i*-Pr_2O and dried in vacuo at 40 °C for 2 h. Yield 36%; m.p. 117–118 °C. Anal. $C_{18}H_{18}ClN_3O_5$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.0–7.1 (m, 3H, aromatics), 6.8 (s, 1H, NH), 5.6 (s, 1H, CH), 4.3 (t, 2H, $COOCH_2$), 3.7 (s, 3H, CH_3O), 3.65 (t, 2H, CH_2Cl), 2.3 (s, 6H, 2,6 CH_3s).

6.7.7. 2-Chloroethyl ethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate 6h

Same procedure as for **6a**. The reaction was carried out in ethanol at reflux temperature for 7 h. Yield 67% (after column chromatography, eluent $CHCl_3$); m.p. 160–162 °C (*i*-PrOH). Anal. $C_{19}H_{21}ClN_2O_6$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.2–7.2 (m, 4H, aromatics), 6.35 (bs, 1H, NH), 5.1 (s, 1H, CH), 4.3 (t, 2H, $COOCH_2-C-Cl$), 4.08 (q, 2H, $COOCH_2-C$), 3.6 (t, 2H, CH_2Cl), 2.35 (s, 6H, 2,6 CH_3s), 1.2 (t, 3H, $COO-C-CH_3$).

6.7.8. 2-Chloroethyl 2-methylpropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate 6j

Same procedure as for **6a**. Yield 69% (after column chromatography, eluent $CHCl_3$); m.p. 155–157 °C (*i*-PrOH). Anal. $C_{21}H_{25}ClN_2O_6$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.1–7.2 (m, 4H, aromatics), 6.2 (s, 1H, NH), 5.05 (s, 1H, pyridine–CH), 4.25 (t, 2H, $COOCH_2-C-Cl$), 3.8 (d, 2H, $COOCH_2-C-C$), 3.6 (t, 2H, CH_2Cl), 2.3 (s, 6H, 2,6 CH_3s), 2.0–1.5 (m, 1H, *i*-butyl–CH), 0.95–0.85 (dd, 6H, gem-CH_3s).

6.7.9. 2-Chloro-1-methylethyl 2-methylpropyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate 6k

Same procedure as for **6a**. Yield 48% (after column chromatography, eluting with $CHCl_3$–EtOAc, 98:2). Anal. $C_{22}H_{25}Cl_3NO_4$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 7.7–7.1 (m, 3H, aromatics), 6.4 (m, 1H, NH), 5.5 (s, 1H, pyridine–CH), 5.3–4.8 (m, 1H, COOCH), 3.9 (d, 2H, $COOCH_2$), 3.65–3.45 (2d, 2H, CH_2Cl), 2.3 (s, 6H, 2,6 CH_3s), 2.3–1.7 (m, 1H, $COO-C-CH$), 1.3–1.1 (2d, 3H, $COO-C-CH_3$), 0.85–0.8 (2d, 6H, gem-CH_3s).

6.7.10. 2-Chloroethyl 2-propoxyethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate 6l

Same procedure as for **6a**. Yield 70% (after column chromatography, eluting with $CHCl_3$–EtOAc gradient, 100:0 to 97:3) m.p. 104–106 °C (*i*-PrOH or CCl_4). Anal. $C_{22}H_{27}ClN_2O_7$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.4–7.3 (m, 4H, aromatics), 6.3 (s, 1H, NH), 5.2 (s, 1H, CH), 4.5–4.1 (m, 4H, 2 $COOCH_2$), 3.9–3.5 (m, 4H, CH_2OCH_2), 3.45 (t, 2H, CH_2Cl), 2.4 (s, 6H, 2,6 CH_3s), 2.0–1.3 (m, 2H, $O-C-CH_2-C$), 0.95 (t, 3H, $O-C-C-CH_3$).

6.8. Preparation of intermediates 10 (table VI, figures 3 and 4)

6.8.1. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-propanol 10a

0.011 mol (0.63 g) of propylene oxide was added to a solution of 0.01 mol (2.25 g) of *N*-methyl-3,3-diphenylpropylamine [41] in 6 mL of MeOH and the reaction mixture was kept at 15–20 °C in a plugged round bottomed flask for 3 days. An additional amount of propylene oxide (0.11 g) was added and the mixture was kept in the same manner for 24 h. The solvent was evaporated in vacuo and the residue was purified by flash chromatography on silica gel eluting with $CHCl_3$–MeOH (100:0 to 95:5) gradient mixture, to give 2 g (70%) of the pure oily compound. The analytical sample was obtained after drying at 40 °C/0.5 mmHg. Anal. $C_{19}H_{25}NO$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.2 (s, 10H, aromatics), 3.95 (t, 1H, CH–C–C), 3.65 (m, 1H, CH–O), 3.6 (s, 1H, OH), 2.6–1.8 (m, 9H, NCH_3 and 3 CH_2s), 1.05 (d, 3H, $C-CH_3$).

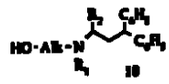
6.8.2. 1,N-(3,3-Diphenylpropyl)amino-2-methyl-2-propanol·HCl 10b

A mixture of 0.02 mol (4.23 g) of 3,3-diphenylpropylamine, 0.005 mol (0.54 g) of 1-chloro-2-methyl-2-propanol and 5 mL of xylene was stirred at reflux temperature for 8 h. After cooling to room temperature, the suspension was filtered, the filtrate was evaporated to dryness and the oily residue was purified by flash chromatography eluting with a $CHCl_3$–MeOH, 85:15 mixture to give 1.37 g of the pure base. This was converted into its HCl salt that was crystallized from a EtOAc–EtOH, 3:1 mixture to give 1.13 g (71%) of the compound. M.p. 199–200 °C. Anal. $C_{19}H_{25}NO·HCl$ (C, H, N, Cl). ^{1}H-NMR (DMSO-d_6): 9.5–9.0 (m, 1H, $^+$NH), 7.70–7.2 (m, 10H, aromatics), 5.35 (bs, 1H, OH), 4.25 (t, 1H, CH), 3.15–2.5 (m, 4H, CH_2CH_2N), 2.29 (bs, 2H, $N-CH_2-C-O$), 1.3 (s, 6H, gem-CH_3s).

6.8.3. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-methyl-2-propanol 10c

A mixture of 0.2 mol (45 g) of *N*-methyl-3,3-diphenylpropylamine, 0.08 mol (8.68 g) of 1-chloro-2-methyl-2-propanol and

Table VI. *N*-(Hydroxyalkyl) diphenylalkylamines 10.



Compound	Alk	R_1	R_2	Yield (%)	M.p. or b.p. (°C)/mmHg	Anal.
10a	$-CH(CH_3)CH_2-$	CH_3	H	70	Oil	C, H, N
10b	$-C(CH_3)_2CH_2-$	H	H	71	199–200[a]	C, H, N, Cl
10c	$-C(CH_3)_2CH_2-$	CH_3	H	80	145/0.2	C, H, N
10d	$-CH(CH_3)(CH_2)_2-$	CH_3	H	83	Thick oil	C, H, N
10e	$-C(CH_3)_2(CH_2)_2-$	CH_3	H	56	194–196[a]	C, H, N, Cl
10f	$-CH_2C(CH_3)_2CH_2-$	CH_3	H	65	147–148[a]	C, H, N, Cl
10g	$-(CH_2)_2C(CH_3)_2-$	CH_3	H	99	167–168[a]	C, H, N, Cl
10h	$-C(CH_3)_2CH_2-$	CH_3	=O	83	88–89	C, H, N

[a]As HCl salt.

70 mL of xylene was stirred at reflux temperature for 8.5 h. After cooling to room temperature, 450 mL of Et_2O was added, the suspension was filtered and the filtrate was evaporated to dryness. The oily residue was purified by flash chromatography on silica gel eluting with $CHCl_3$–MeOH (100:0 to 90:10) gradient mixture to give 19 g (80%) of the pure compound. A sample was distilled to give the analytical sample. B.p. 145 °C/0.2 mmHg. Anal. $C_{20}H_{27}NO$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.15 (s, 10H, aromatics), 3.9 (t, 1H, CH), 2.8 (bs, 1H, OH), 2.8–1.9 (m, 4H, CH_2CH_2N), 2.3 (s, 3H, NCH_3), 2.2 (s, 2H, N–CH_2–C–O), 1.05 (s, 6H, gem-CH_3s).

6.8.4. 4-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-butanone·HCl 13

0.045 mol (3.3 g) of 95% methyl vinyl ketone was dropped in 20 min into a stirred solution of 0.035 mol (7.87 g) of *N*-methyl-3,3-diphenylpropylamine in 7 mL of Et_2O at –5/+3 °C. Afterwards, the solvent was evaporated at room temperature to give 9.2 g (90%) of the pure base. A sample was converted to its HCl salt that was crystallized to give the analytical sample. M.p. 133–135 °C (Me_2CO or *i*-PrOH or MeCOEt). Anal. $C_{20}H_{25}NO$·HCl (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 13.3–12.5 (bs, 1H, $^+$NH), 7.3 (s, 10H, aromatics), 4.0 (t, 1H, CH), 3.4–2.9 (m, 4H, CH_2NCH_2), 2.9–2.4 (m, 4H, CH_2–C–N–C–CH_2), 2.7 (s, 3H, NCH_3), 2.15 (s, 3H, $COCH_3$).

6.8.5. 4-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-butanol 10d

0.024 mol (0.9 g) of $NaBH_4$ was added in small portions and at 0 °C into a solution of 0.031 mol (9.16 g) of 13 in 30 mL of MeOH. At the end of the addition (about 5 min), the solvent was evaporated at reduced pressure, the residue was treated with H_2O (100 mL) and extracted with Et_2O. The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to give 9.28 g of the oily product, that was dried in vacuo (about 0.4 mmHg at room temperature) to give 7.73 g (83%) of the pure compound. Anal. $C_{20}H_{27}NO$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.35–7.0 (m, 10H, aromatics), 5.6–5.2 (m, 1H, OH), 4.05–3.6 (m, 2H, 2 x CH), 2.6–1.9 (m, 9H, $CH_2CH_2N(CH_2)CH_3$), 1.55–1.15 (m, 2H, N–C–CH_2–C–O), 1.1 (d, 3H, CH_3–C–O).

6.8.6. 4-[N-(3,3-Diphenylpropyl-N-methylamino]-2-methyl-2-butanol·HCl 10e

A solution of 0.051 mol (7.23 g) of methyl iodide in 6 mL of anhydrous Et_2O (distilled from $LiAlH_4$) was slowly dropped (30 min) into a mixture of 0.051 mol (1.24 g) of Mg, 10 mg of I_2 and 5 mL of anhydrous Et_2O. Then, the mixture was heated at reflux temperature for 15 min until complete Mg dissolution and, after cooling to –5 °C, a solution of 0.042 mol (12.5 g) of 13 in 45 mL of anhydrous Et_2O was added dropwise in 25 min, without exceeding the temperature of 10 °C. After 18 h stirring at room temperature, the mixture was cooled, 10 mL of H_2O, 150 mL of EtOAc and 4.5 mL of 50% AcOH were added until complete dissolution. After separation of the organic layer, the aqueous phase was re-extracted with EtOAc and the collected organic layers were washed with 5% aqueous Na_2CO_3 solution followed with brine. The residue obtained from EtOAc evaporation was purified by flash chromatography, eluting with a $CHCl_3$–MeOH, 98:2 mixture, to give 7.4 g (56%) of the pure base. A sample was converted into its HCl salt to give the analytical sample. M.p. 194–196 °C (*i*-PrOH). Anal.

$C_{21}H_{29}NO$-HCl (C, H, N, Cl). ^{1}H-NMR (DMSO-d_6): 12.5–11.0 (bs, 1H, $^+$NH), 7.5 (s, 10H, aromatics), 4.7 (bs, 1H, OH), 4.4–4.0 (m, 1H, CH), 3.8–2.3 (m, 6H, $CH_2CH_2NCH_2$), 2.75 (s, 3H, NCH_3), 2.0–1.5 (m, 2H, CH_2C–O), 1.15 (s, 6H, gem-CH_3s).

6.8.7. 2,2-Dimethyl-3-[N-(3,3-diphenylpropyl)-N-methylamino]-propanol-HCl 10f

A mixture of 0.01 mol (2.61 g) of *N*-methyl-3,3-diphenylpropylamine-HCl, 0.011 mol (0.9 mL) of 37% HCHO and 1 mL of Ac_2O was stirred at 100 °C for 30 min. A solution of 0.012 mol (1.08 mL) of *i*-butyraldehyde in 1 mL of Ac_2O was dropped at the same temperature and heating was continued for additional 30 min. The solvent was almost completely distilled at 50 °C (1 mmHg), the residue was dissolved in H_2O and the solution was alkalinized and extracted with Et_2O. The crude obtained after solvent evaporation was purified by flash chromatography eluting with CH_2Cl_2–MeOH (100:3 to 100:4) gradient mixture to give 1.55 g (50%) of pure 3-[*N*-(3,3-diphenylpropyl)-*N*-methylamino]-2,2-dimethylpropionaldehyde. ^{1}H-NMR ($CDCl_3$): 9.5 (s, 1H, CHO), 7.3 (s, 10H, aromatics), 4.0 (t, 1H, CH), 2.7–1.9 (m, 9H, 3 CH_2s and NCH_3), 1.0 (s, 6H, gem-CH_3s).

0.065 mol (0.25 g) of $NaBH_4$ was added at 0/3 °C to a stirred solution of 0.005 mol (1.5 g) of the above aldehyde in 7 mL of MeOH. The mixture was stirred for 30 min at the same temperature and for 1 h at room temperature. The reaction could be monitored by TLC (toluene–Me_2CO, 7:3). 35 mL of H_2O was then added and the mixture was extracted with Et_2O. The organic phase was extracted with a solution of 0.6 g of oxalic acid in 25 mL of H_2O, the aqueous solution was washed twice with Et_2O, alkalinized with 1 mL of concentrated NaOH to give the base that was extracted with Et_2O. After drying and evaporation of the solvent, 1.38 g (80%) of the pure oily base was obtained. This was converted into its HCl salt that was crystallized from Me_2CO to give 1.26 g (65%) of **10f**. M.p. 147–148 °C. Anal. $C_{21}H_{29}NO$-HCl (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 10.8–9.9 (m, 1H, $^+$NH), 7.7–7.2 (m, 10H, aromatics), 5.0–4.4 (m, 1H, OH), 4.1 (t, 1H, CH), 3.7 (s, 2H, CH_2O), 3.5–2.5 (m, 9H, $CH_2CH_2N(CH_3)CH_2$), 1.1 (s, 6H, gem-CH_3s).

6.8.8. Ethyl 3-[N-(3,3-diphenylpropyl)amino]-3-methylbutyrate-HCl 15

A mixture of 0.088 mol (11.27 g) of ethyl 3-methylcrotonate 14 [42], 0.08 mol (17.43 g) of 3,3-diphenylpropylamine and 60 mL of EtOH was refluxed for 57 h. The reaction was monitored by GLC, that showed a 50% maximum rate of 15 formation. The mixture was evaporated to dryness in vacuo and the residue was purified by flash chromatography eluting with $CHCl_3$–CH_3OH (100:0 to 98:2) gradient mixture. Yield 9.8 g (36%) as base. A sample was converted into its hydrochloride salt. M.p. 126–130 °C (EtOAc). Anal. $C_{22}H_{29}NO_2$-HCl (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 10.0–9.3 (bs, 2H, $^+NH_2$), 7.3 (s, 10H, aromatics), 4.2–4.0 (m, 1H, CH), 4.15 (q, 2H, OCH_2–C), 3.0–2.6 (m, 4H, CH_2CH_2N), 2.7 (s, 2H, CH_2COO), 1.4 (s, 6H, gem-CH_3s), 1.2 (t, 3H, O–C–CH_3).

6.8.9. Ethyl 3-[N-(3,3-diphenylpropyl)-N-methylamino]-3-methylbutyrate 16

A mixture of 0.036 mol (12.35 g) of 15, 0.04 mol (5.7 g) of methyl iodide, 0.0547 mol (7.56 g) of anhydrous K_2CO_3 and 48 mL of anhydrous DMF (dried on 4 Å molecular sieves) was stirred at room temperature for 6 h. Afterwards, the mixture was poured into 350 mL H_2O and the separated oil was extracted with Et_2O (4 x 70 mL). The residue obtained after solvent evaporation was purified by flash chromatography eluting with a petroleum ether–Me_2CO, 95:5 mixture. Yield 9.6 g (74%). A sample was distilled to give the analytical sample; b.p. 190–195 °C/0.7 mmHg. Anal. $C_{23}H_{31}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.35 (s, 10H, aromatics), 4.15 (q, 2H, $COOCH_2$), 4.2–3.9 (m, 1H, CH), 2.4–2.15 (m, 6H, CH_2CH_2N–C–CH_2), 2.25 (s, 3H, NCH_3), 1.25 (t, 3H, COO–C–CH_3), 1.1 (s, 6H, gem-CH_3s).

6.8.10. 3-[N-(3,3-Diphenylpropyl)-N-methylamino]-3-methylbutanol-HCl 10g

A solution of 0.017 mol (6 g) of 16 in 20 mL of Et_2O (distilled from $LiAlH_4$) was dropped in 15 min into a stirred suspension of 0.0255 mol (0.97 g) of $LiAlH_4$ in 20 mL of Et_2O, mantaining a gentle reflux. After 3 h at 25 °C, 1 mL of H_2O, 1 mL of 35% NaOH and 3 mL of H_2O were cautiously added and the mixture was stirred for 15 min. The suspension was filtered, washed with Et_2O and the solvent was evaporated to dryness to give 5.25 g (99%) of **10g** base. A sample of this thick oil was converted into its HCl salt to give the analytical sample. M.p. 167–168 °C (Me_2CO). Anal. $C_{21}H_{29}NO$-HCl (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 11.0–10.5 (bs, 1H, $^+$NH), 7.55 (s, 10H, aromatics), 5.3–5.0 (bs, 1H, OH), 4.4–3.8 (m, 3H, CH and CH_2O), 3.0–2.5 (m, 4H, CH_2CH_2N), 2.8 (s, 3H, NCH_3), 2.1–1.8 (m, 2H, N–C–CH_2–C–O), 1.6–1.3 (m, 6H, gem-CH_3s).

6.8.11. 3,3-Diphenyl-N-(2-hydroxy-2-methylpropyl)-N-methylpropanamide 10h

A solution of 0.05 mol (12.2 g) of 3,3-diphenylpropionyl chloride [43] in 30 mL of 1,4-dioxane was added at 30–35 °C to a stirred solution of 0.05 mol (5.15 g) of 1-methylamino-2-methyl-2-propanol [44] in 0.0525 mol (25 mL) of 2.1 N NaOH. The mixture was stirred at room temperature for 3 h and then poured into 200 mL of H_2O kept at 5–10 °C. The precipitate was extracted with 200 mL of Et_2O, the ethereal solution evaporated to dryness and the residue redissolved in $CHCl_3$. This solution was dried ($CaCl_2$), filtered, evaporated to dryness and the residue was washed repeatedly with petroleum ether to give 12.9 g (83%) of the pure compound. A little amount was crystallized from cyclohexane to give the analytical sample. M.p. 88–89 °C. Anal. $C_{20}H_{25}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.2 (s, 10H, aromatics), 4.7 (t, 1H, CH), 3.9 (s, 1H, OH), 3.3 (s, 2H, NCH_2), 3.05 (d, 2H, CH_2CO), 3.0 (s, 3H, NCH_3), 1.0 (s, 6H, gem-CH_3s).

6.9. Preparation of intermediates 11 (table VII)

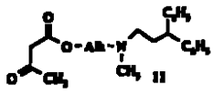
6.9.1. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-propyl acetoacetate 11a

0.084 mol (7 g) of diketene was dropped at 95 °C into a stirred solution of 0.08 mol (22.67 g) of 1-[*N*-(3,3-diphenylpropyl)-*N*-methylamino]-2-propanol **10a** in 22 mL of toluene. After cooling to room temperature, the solvent was evaporated in vacuo and the residue was purified by flash chromatography eluting with EtOAc to give 20 g (68%) of pure compound containing about 10% of the enolic form. The analytical sample was obtained by repeating flash chromatography. Anal. $C_{23}H_{29}NO_3$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.1 (s, 10H aromatics), 5.15–4.7 (m, 1.1H, CH–O and enolic CHCOO), 4.1–3.8 (m, 1H, N–C–C–CH), 3.3 (s, 1.8H, CH_2COO), 2.55–1.8 (m, 12H, NCH_2, $CH_2NCH_2CH_2$, CH_2CO), 1.2 (d, 3H, COO–C–CH_3).

6.9.2. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-methyl-2-propyl acetoacetate 11b

0.046 mol (3.8 g) of diketene was dropped in 10 min at 85–100 °C into a stirred solution of 0.044 mol (13.1 g) of

Table VII. *N*-(3,3-Diphenylpropyl)methylaminoalkyl acetoacetates **11**.



Compound	Alk	Yield	M.p. (°C)	Anal.
11a	$-CH(CH_3)CH_2-$	68	Oil	C, H, N
11b	$-C(CH_3)_2CH_2-$	72	Oil	C, H, N

1-[*N*-(3,3-diphenylpropyl)-*N*-methylamino]-2-methyl-2-propanol **10c** in 10 mL of toluene. After 2 h stirring at 80 °C, the mixture was cooled to room temperature, the solvent was evaporated in vacuo and the residue was purified by flash chromatography, eluting with $CHCl_3$–Me_2CO (100:0 to 98:2) gradient mixture, to give 12.3 g (72%) of the ester. Anal. $C_{24}H_{31}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 7.35 (s, 10H, aromatics), 4.0 (t, 1H, CH), 3.2 (s, 2H, $COCH_2$), 2.55 (s, 2H, COO–C–CH_2), 2.55–2.1 (m, 4H, N–CH_2CH_2), 2.3 (s, 3H, NCH_3), 2.2 (s, 3H, CH_3CO), 1.45 (s, 6H, gem-CH_3s).

6.10. Preparation of intermediates 12 (table VIII)

6.10.1. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-propyl 2-(3-nitrobenzylidene)acetoacetate·HCl 12a

A mixture of 0.01 mol (1.51 g) of 3-nitrobenzaldehyde and 0.01 mol (3.67 g) of 1-[*N*-(3,3-diphenylpropyl)-*N*-methylamino]-2-propyl acetoacetate **11a** in 14.5 mL of $CHCl_3$ was saturated with HCl at 0 °C. After 3 days at 15 °C, the mixture was diluted with $CHCl_3$, washed with diluted NaOH until neutrality and the organic phase dried over $CaCl_2$. The residue obtained after solvent evaporation was dissolved in Et_2O and an excess of 3.2 N HCl in Et_2O was added at 0 °C. The precipitate obtained after Et_2O addition was decanted and washed repeatedly with Et_2O to give 4.3 g (80%) of the product as *E/Z* isomers mixture of the HCl salt as a glassy solid. Anal. $C_{30}H_{32}N_2O_5$·HCl (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 8.35 and 8.2 (2s, 1H, CH–C–CO), 8.0–6.9 (m, 14H, aromatics), 5.8–5.2 (m, 1H, COOCH), 4.2–3.8 (m, 1H, N–C–C–CH), 3.6–2.2 (m, 12H, $CH_2N(CH_3)CH_2CH_2$ and CH_3CO), 1.4 and 1.2 (2d, 3H, COO–C–CH_3).

6.10.2. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-methyl-2-propyl 2-(3-nitrobenzylidene)acetoacetate 12b

Same procedure as for **12a**, but using **11b**. The analytical sample was obtained by treatment of the hydrochloride salt with diluted NaOH, extraction with EtOAc and purifing the crude base by flash chromatography eluting with a $CHCl_3$–EtOAc, 95:5 mixture. Yield 91%, m.p. 65–80 °C. Anal. $C_{31}H_{34}N_2O_5$ (C, H, N). ^{1}H-NMR ($CDCl_3$): 8.5–8.2 (m, 2H, aromatics at positions 2,4 of $C_6H_4NO_2$), 8.0–7.5 (m, 3H, aromatics at positions 5,6 of $C_6H_4NO_2$ and CO–C–CH), 7.3 (s, 10H, other aromatics), 4.1–3.8 (m, 1H, N–C–C–CH), 2.7–2.1 (m, 12H, $CH_2N(CH_3)CH_2CH_2$ and $COCH_3$), 1.52 and 1.48 (2s, 6H, gem-CH_3s).

6.10.3. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-propyl 2-(2,3-dichlorobenzylidene)acetoacetate 12c

A mixture of 0.01 mol (3.67 g) of 1-[*N*-(3,3-diphenylpropyl)-*N*-methylamino]-2-propyl acetoacetate **11a**, 0.01 mol (1.75 g) of 2,3-dichlorobenzaldehyde in 12 mL of $CHCl_3$ was saturated with HCl at 0 °C. After 1 day at room temperature, the solvent was evaporated to dryness, the residue was dissolved into CH_2Cl_2 and the solution was neutralized by washing with diluted $NaHCO_3$ aqueous solution. Evaporation of the organic layer gave 4.88 g (93%) of the compound as a glassy solid as *E/Z* isomers mixture. Anal. $C_{30}H_{31}Cl_2NO_3$ (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 7.9 and 7.8 (2s, 1H, CO–C–CH), 7.7–7.0 (m, 13H, aromatics), 5.4–4.8 (m, 1H, COOCH), 4.3–3.8 (m, 1H, N–C–C–CH), 2.8–1.9 (m, 12H, $CH_2N(CH_3)CH_2CH_2$ and CH_3CO), 1.25 and 1.1 (2d, 3H, COO–C–CH_3).

6.10.4. 1-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-methyl-2-propyl 2-(2,3-dichlorobenzylidene)acetoacetate·HCl 12d

Same procedure as for **12c**, but using **11b**. The residue after $CHCl_3$ evaporation was treated with *i*-Pr_2O until solidification. The solid was washed with a *i*-Pr_2O–EtOAc, 95:5 mixture and dried in desiccator to give the hydrochloride salt as a glassy solid. Yield 100%. Anal. $C_{31}H_{33}Cl_2NO_3$·HCl (C, H, N, Cl). ^{1}H-NMR ($CDCl_3$): 10.8 (bs, 1H, $^+$NH), 7.9–7.6 (m, 1H, CO–C–CH), 7.55–7.0 (m, 13H, aromatics), 4.2–3.7 (m, 1H, N–C–C–CH), 3.6–2.0 (m, 12H, $CH_2N(CH_3)CH_2CH_2$ and CH_3CO), 1.6 and 1.5 (2s, 6H, gem-CH_3s).

6.11. General procedure for the synthesis of 1,4-dihydropyridines 9 (table I)

6.11.1. Method A

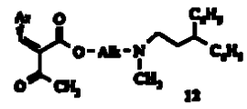
A mixture of the monohaloalkyl ester of general formula **6** (0.02 mol), a diphenylalkylamine **8** (0.04–0.06 mol) and toluene or xylene (15–30 mL) was stirred at reflux temperature for 1–24 h. After cooling to room temperature, the mixture was diluted with Et_2O in order to precipitate the formed **8**·HCl. The filtrate was evaporated in vacuo and the residue was purified by flash chromatography. The purified base was converted into its ·HCl salt by usual methods; the product was isolated as amorphous material or washed or crystallized from a suitable solvent or solvents mixture. The following compounds were synthesized by this route.

6.11.1.1. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·H₂O 9a: m.p. 118–125 °C (EtOAc or Me_2CO); Anal. $C_{34}H_{37}N_3O_6$·HCl·H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR (DMSO-d_6): 11.3–10.0 (bs, 1H, $^+$NH), 9.45 (s, 1H, pyridine–NH), 8.0–7.3 (m, 14H, aromatics), 5.65 (s, 1H, pyridine–H_4), 4.7–3.9 (m, 3H, N–C–C–CH and $COOCH_2$), 3.65 (s, 3H, $COOCH_3$), 2.75 (s, 3H, NCH_3), 2.4 (s, 6H, 2,6 CH_3s), 3.7–2.2 (m, 8H, $CH_2NCH_2CH_2$ and H_2O).

6.11.1.2. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9b: m.p. 108–113 °C (amorphous); Anal. $C_{34}H_{37}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 12.3 (bs, 1H, $^+$NH), 8.0 (s, 1H, pyridine–NH), 8.1–7.0 (m, 14H, aromatics), 5.0 (s, 1H, pyridine–H_4), 4.48 (m, 2H, $COOCH_2$), 3.98 (m, 1H, N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 3.4–2.2 (m, 6H, $CH_2NCH_2CH_2$), 2.69 (s, 3H, NCH_3), 2.39 (s, 6H, 2,6 CH_3s).

6.11.1.3. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate·HCl·0.6H₂O 9c: m.p. 161 °C (EtOH + Et_2O); Anal. $C_{34}H_{36}Cl_2N_2O_4$·HCl·0.6 H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR ($CDCl_3$): 7.85 (bs, 1H, pyridine–NH), 7.4–6.8 (m, 13H, aromatics), 5.2 (s, 1H, pyridine–H_4), 4.5–4.1 (m, 2H,

Table VIII. *N*-(3,3-Diphenylpropyl)methylaminoalkyl 2-arylideneacetoacetates **12**.



12

Compound	Ar	Alk	Yield	M.p. (°C)	Anal.
12a	3-NO_2–C_6H_4	–$CH(CH_3)CH_2$–	80[a]	Glassy	C, H, N, Cl
12b	3-NO_2–C_6H_4	–$C(CH_3)_2CH_2$–	91	65–80	C, H, N
12c	2,3-Cl_2–C_6H_3	–$CH(CH_3)CH_2$–	93	Glassy	C, H, N, Cl
12d	2,3-Cl_2–C_6H_3	–$C(CH_3)_2CH_2$–	100[a]	Glassy	C, H, N, Cl

[a]As HCl salt.

$COOCH_2$), 4.0–3.7 (m, 1H, N–C–C–CH), 3.5 (s, 3H, $COOCH_3$), 2.45 (s, 3H, NCH_3), 2.3 (s, 6H, 2,6 CH_3s), 3.3–2.1 (m, 7H, $CH_2NCH_2CH_2$ and H_2O).

6.11.1.4. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl methyl 4-(benzofurazan-4-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate·HCl 9d: m.p. 161–166 °C (MeOAc or MeCN); Anal. $C_{34}H_{39}N_4O_5$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 11.5–10.5 (bs, 1H, $^+$NH), 8.0–7.2 (m, 13H, aromatics and benzofurazan–H_5, H_6, H_7), 6.6 (s, 1H, pyridine–NH), 5.6 (s, 1H, pyridine–H_4), 4.3–3.9 (m, 3H, $COOCH_2$ and N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 2.7–1.9 (m, 15H, 2,6 CH_3s and $CH_2N(CH_3)CH_2CH_2$).

6.11.1.5. 2-[N-(3,3-Diphenylpropylamino)]ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9e: m.p. 128–132 °C (*i*-PrOAc or EtOAc); Anal. $C_{33}H_{35}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$) of the base: 8.35–7.25 (m, 14H, aromatics), 6.55 (s, 1H, pyridine–NH), 5.35 (s, 1H, pyridine–H_4), 4.4–4.0 (m, 3H, $COOCH_2$ and N–C–C–CH), 3.8 (s, 3H, $COOCH_3$), 3.0–2.0 (m, 6H, $CH_2NCH_2CH_2$), 2.45 (s, 6H, 2,6 CH_3s), 1.55 (s, 1H, NH).

6.11.1.6. 2-[N-(4,4-Diphenyl-2-butyl)-N-methylamino]ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9f: m.p. 113–120 °C (amorphous); Anal. $C_{34}H_{39}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 12.2 (bs, 1H, $^+$NH), 8.3–7.0 (m, 14H, aromatics), 6.8 (bs, 1H, pyridine–NH), 4.9 (bs, 1H, pyridine–H_4), 4.7–4.3 (m, 2H, $COOCH_2$), 4.2–3.8 (m, 1H, N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 3.4–2.8 (m, 4H, O–C–CH_2–N–C–CH_2–C), 2.6 (s, 3H, NCH_3), 2.4 (s, 6H, 2,6 CH_3s), 2.2–1.8 (m, 1H, N–CH–C), 1.6–1.1 (m, 3H, N–C–CH_3).

6.11.1.7. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl ethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9g: m.p. 164–167 °C (Me_2CO); Anal. $C_{33}H_{39}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 11.3 (bs, 1H, $^+$NH), 9.35 (bs, 1H, pyridine–NH), 8.0–7.3 (m, 14H, aromatics), 5.0 (s, 1H, pyridine–H_4), 4.5–3.9 (m, 5H, 2 x $COOCH_2$ and N–C–C–CH), 3.6–2.25 (m, 6H, $CH_2NCH_2CH_2$), 2.75 (s, 3H, NCH_3), 2.35 (s, 6H, 2,6 CH_3s), 1.2 (t, 3H, COO–C–CH_3).

6.11.1.8. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl 1-methylethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9h: m.p. 160–164 °C (Me_2CO); Anal. $C_{34}H_{41}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 12.0–11.5 (bs, 1H, $^+$NH), 9.5 (s, 1H, pyridine–NH), 8.3–7.6 (m, 4H, aromatics of 3-nitrophenyl ring), 7.5 (s, 10H, other aromatics), 5.1 (s, 1H, pyridine–H_4), 5.2–4.8 (m, 1H, COOCH), 4.7–4.3 (m, 2H, $COOCH_2$), 4.3–3.9 (m, 1H, N–C–C–CH), 3.8–2.2 (m, 6H, $CH_2NCH_2CH_2$), 2.8 (s, 3H, NCH_3), 2.4 (s, 6H, 2,6 CH_3s), 1.25 and 1.15 (2d, 6H, COO–C–$(CH_3)CH_3$).

6.11.1.9. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl 2-methylpropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9i: m.p. 123–124 °C (EtOAc or EtOAc + Me_2CO); Anal. $C_{37}H_{43}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 11.7–11.25 (bs, 1H, $^+$NH), 9.55 (s, 1H, pyridine–NH), 8.35–7.65 (m, 4H, aromatics of 3-nitrophenyl ring), 7.5 (s, 10H, other aromatics), 5.15 (s, 1H, pyridine–H_4), 4.7–4.05 (m, 3H, $COOCH_2$–C–N and N–C–C–CH), 4.05–3.75 (d, 2H, $COOCH_2$–C–$(C)_2$), 3.65–2.25 (m, 6H, $CH_2NCH_2CH_2$), 2.8 (s, 3H, NCH_3), 2.45 and 2.4 (2s, 6H, 2,6 CH_3s), 2.05–1.65 (m, 1H, COO–C–CH), 1.9 and 0.85 (2d, 6H, COO–C–$C(CH_3)_2$).

6.11.1.10. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]ethyl 2-propoxyethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9j: m.p. 124–129 °C (EtOAc); Anal. $C_{36}H_{45}N_3O_7$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 11.8–11.3 (bs, 1H, $^+$NH), 9.5 (s, 1H, pyridine–NH), 8.3–7.7 (m, 4H, aromatics of 3-nitrophenyl ring), 7.45 (s, 10H, other aromatics), 5.1 (s, 1H, pyridine–H_4), 4.7–4.0 (m, 5H, 2 x $COOCH_2$ and N–C–C–CH), 3.8–3.2 (m, 8H, CH_2NCH_2 and CH_2OCH_2), 3.2–2.3 (m, 2H, N–C–CH_2–C), 2.8 (s, 3H, NCH_3), 2.4 (s, 6H, 2,6 CH_3s), 1.8–1.2 (m, 2H, O–C–CH_2–C), 0.85 (t, 3H, O–C–C–CH_3).

6.11.1.11. 3-[N-(3,3-Diphenylpropyl)-N-methylamino]propyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9k: m.p. 100–107 °C (amorphous); Anal. $C_{33}H_{39}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 8.0–6.8 (m, 16H, aromatics, $^+$NH and pyridine–NH), 5.0 (s, 1H, pyridine–H_4), 4.2–3.8 (m, 3H, N–C–C–CH and $COOCH_2$), 3.47 (s, 3H, $COOCH_3$), 3.1–2.2 (m, 8H, $CH_2CH_2NCH_2CH_2$), 2.62 (s, 3H, NCH_3), 2.35 (s, 6H, 2,6 CH_3s).

6.11.1.12. 3-[N-(4,4-Diphenyl-2-butyl)-N-methylamino]propyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9l: m.p. 113–118 °C (H_2O); Anal. $C_{36}H_{41}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 8.15–7.1 (m, 15H, aromatics and pyridine–NH), 5.05 (s, 1H, pyridine–H_4), 4.25–3.8 (m, 3H, N–C–C–CH and $COOCH_2$), 3.61 (s, 3H, $COOCH_3$), 2.6 (s, 3H, NCH_3), 2.4 (s, 6H, 2,6 CH_3s), 2.4–1.7 (m, 7H, O–C–CH_2CH_2 and $NCHCH_2$), 1.5–1.2 (m, 3H, N–C–CH_3).

6.11.1.13. 4-[N-(3,3-Diphenylpropyl)-N-methylamino]butyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·0.5H_2O 9m: the reaction was carried out in DMF at room temperature for 16 h. M.p. 91–103 °C (amorphous); Anal. $C_{36}H_{41}N_3O_6$·HCl ·0.5H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR ($CDCl_3$): 11.6–11.2 (bs, 1H, *NH), 9.3 (s, 1H, pyridine–NH), 8.5–8.1 (m, 4H, aromatics of 3-nitrophenyl ring), 7.35 (s, 10H, other aromatics), 5.05 (s, 1H, pyridine–H_4), 4.2–3.8 (m, 3H, $COOCH_2$ and N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 3.4 (s, 1H, H_2O), 3.3–2.0 (m, 6H, $CH_2NCH_2CH_2$), 2.8 (s, 3H, NCH_3), 2.35 (s, 6H, 2,6 CH_3s), 1.7–1.4 (m, 4H, O–C–CH_2CH_2).

6.11.1.14. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1-methylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9n: m.p. 114–123 °C (amorphous); Anal. $C_{36}H_{41}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 8.1–7.0 (m, 16H, aromatics, *NH and pyridine–NH), 5.3 (m, 1H, COOCH), 5.05 and 5.00 (2s, 1H, pyridine–H_4), 4.1–3.5 (m, 1H, N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 3.5–2.2 (m, 9H, $CH_2N(CH_2)CH_2CH_2$), 2.4 (s, 6H, 2,6 CH_3s), 1.6–1.0 (m, 3H, COO–C–CH_3).

This compound was also synthesized by *method* B_1 and the diastereoisomers were separated by repeated flash chromatography, eluting with $CHCl_3$ containing increasing amounts of EtOAc (0 to 50%). In these conditions $9n_2$ base was collected before $9n_1$. Each crude base was dissolved into Et_2O–Me_2CO mixture and HCl in Et_2O was added to the solution. Each hydrochloride salt was collected by filtration and crystallized from EtOAc.

6.11.1.15. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1-methylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·H_2O 9n$_1$ (diastereomer at lower R_f in $CHCl_3$–5 N methanolic NH_3, 99:1 eluent mixture): m.p. 143–147 °C (EtOAc); Anal. $C_{36}H_{41}N_3O_6$·HCl·H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR (DMSO-d_6): 11.5–10.0 (bs, 1H, *NH), 9.25 (s, 1H, pyridine–NH), 8.2–7.5 (m, 4H, aromatics of 3-nitrophenyl ring), 7.35 (s, 10H, other aromatics), 5.2 (m, 1H, COOCH), 5.05 (s, 1H, pyridine–H_4), 4.0 (m, 1H, N–C–C–CH), 3.55 (s, 3H, $COOCH_3$), 3.7–3.2 (m, 4H, H_2O and COO–C–CH_2–N), 2.75 (s, 3H, NCH_3), 2.35 (s, 6H, 2,6 CH_3s), 3.2–2.2 (m, 4H, N–CH_2CH_2), 1.05 (d, 3H, COO–C–CH_3).

6.11.1.16. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1-methylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·0.5H_2O 9n$_2$ (diastereomer at higher R_f in $CHCl_3$–5 N methanolic NH_3, 99:1 eluent mixture): m.p. 196–198 °C (dec.) (EtOAc); Anal. $C_{36}H_{41}N_3O_6$·HCl·0.5H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR (DMSO-d_6): 11.0–10.0 (bs, 1H, *NH), 9.35 (s, 1H, pyridine–NH), 8.1–7.5 (m, 4H, aromatics of 3-nitrophenyl ring), 7.35 (s, 10H, other aromatics), 5.25 (m, 1H, COOCH), 5.0 (s, 1H, pyridine–H_4), 4.0 (m, 1H, N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 3.7–3.0 (m, 3H, H_2O and COO–C–CH_2–N), 3.0–2.1 (m, 7H, $CH_2CH_2NCH_3$), 2.35 (s, 6H, 2,6 CH_3s), 1.25 (d, 3H, COO–C–CH_3).

The analysis of the diastereoisomers $9n_1$ and $9n_2$ was performed also by HPLC method, under the following conditions:

Column:	Nova-pak C-18 4μ (Waters); 150 x 3.9 mm
Mobile phase:	CH_3CN (63 vol) – NORMEX buffer pH 7.4 (37 vol)
Elution:	isocratic
Flow:	1.2 mL/min
Temperature:	room temperature
Detector:	UV (240 nm)
Attenuation:	0.02 AUFS

Under these conditions a good separation was observed: $9n_1$, $R_T \approx 11.10$ min and $9n_2$, $R_T = 11.85$ min.

6.11.1.17. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1-methylethyl 2-methylpropyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate·HCl 9r: m.p. 162–164 °C (EtOAc); Anal. $C_{36}H_{41}Cl_2N_2O_4$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 7.85 (s, 1H, pyridine–NH), 7.6–7.0 (m, 13H, aromatics), 5.6–5.1 (m, 1H, COOCH), 5.35 (s, 1H, pyridine–H_4), 4.2–3.7 (m, 3H, $COOCH_2$ and N–C–C–CH), 3.6–1.4 (m, 16H, $CH_2N(CH_3)CH_2CH_2$, 2,6 CH_3s and COO–C–CH), 0.95 (d, 3H, COO–C–CH_3), 0.8 (2d, 6H, COO–C–$C(CH_3)_2$).

6.11.2. Method B_1

A mixture of the appropriate 4-aryl-1,4-dihydropyridine-3-carboxylic acid 7 (0.01 mol), aminoalcohol 10 (0.01 mol), dicyclohexylcarbodiimide (0.011 mol), 4-dimethylaminopyridine (0.0011 mol) and DMF (8.5–20 mL) was stirred under N_2 atmosphere at 75 °C for 8–48 h. After cooling to room temperature the mixture was diluted with Et_2O, the precipitated *N,N'*-dicyclohexylurea was filtered and the filtrate was washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to give the crude that was purified by flash chromatography. The purified base was converted into ·HCl salt by usual methods; the product was obtained as amorphous material, then was washed or crystallized from a suitable solvent or solvent mixtures. The following compound was synthesized by this route.

6.11.2.1. 4-[N-(3,3-Diphenylpropyl)-N-methylamino]-2-butyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·H_2O 9ab: m.p. 93–110 °C (amorphous); Anal. $C_{36}H_{41}N_3O_6$·HCl·H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR (DMSO-d_6): 11.5–10.8 (bs, 1H, *NH), 9.35 (s, 1H, pyridine–NH), 8.3–7.6 (m, 4H, aromatics of 3-nitrophenyl ring), 7.4 (s, 10H, other aromatics), 5.1 (s, 1H, pyridine–H_4), 5.2–4.7 (m, 1H, COOCH), 4.4–3.9 (m, 1H, N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 3.5 (s, 2H, H_2O), 3.3–2.2 (m, 6H, $CH_2NCH_2CH_2$), 2.65 (s, 3H, NCH_3), 2.35 (s, 6H, 2,6 CH_3s), 2.2–1.7 (m, 2H, COO–C–CH_2), 1.4–0.9 (m, 3H, COO–C–CH_3).

6.11.3. Method B_2

A mixture of the appropriate 4-aryl-1,4-dihydropyridine-3-carboxylic acid 7 (0.01 mol), anhydrous CH_2Cl_2 (35 mL) and anhydrous DMF 9 mL) was stirred under N_2 atmosphere at –10 °C and added with $SOCl_2$ (0.012 mol). After 1 h stirring at 0/5 °C, a solution of the suitable aminoalcohol 10 (0.02 mol) in anhydrous CH_2Cl_2 (10 mL) was dropped into the mixture in about 10 min without exceeding 3 °C. After 3 h at 0 °C, the solvent was evaporated, the residue dissolved in EtOAc and washed repeatedly with brine, 1 N HCl, 10% Na_2CO_3, H_2O. The residue obtained after solvent evaporation was purified by flash chromatography. The purified base was converted into ·HCl salt by usual methods; the product was isolated as amor-

phous material or washed or crystallized from a suitable solvent or solvents mixture. The following compounds were synthesized by this route.

6.11.3.1. 2-[N-(3,3-Diphenylpropyl)amino]-1,1-dimethylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl 9t: m.p. (118) 130 °C dec. (amorphous); Anal. $C_{33}H_{39}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 9.55–9.15 (m, 3H, $^+NH_2$ and pyridine–NH), 8.25–7.55 (m, 4H, aromatics of 3-nitrophenyl ring), 7.4 (s, 10H, other aromatics), 5.05 (s, 1H, pyridine–H_4), 4.3–3.9 (m, 1H, N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 3.5–3.2 (m, 2H, N–C–CH_2–C), 3.0–2.4 (m, 4H, CH_2NCH_2), 2.35 and 2.3 (2s, 6H, 2,6 CH_3s), 1.42 (s, 6H, COO–C(CH_3)$_2$).

6.11.3.2. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl 9u: m.p. 185–190 °C (EtOH); Anal. $C_{34}H_{41}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 10.8–9.4 (bb, 1H, ^+NH), 9.5 (bs, 1H, pyridine–NH), 8.30–8.05 (m, 2H, 3-nitrophenyl–H_2–H_4), 7.85–7.60 (m, 2H, 3-nitrophenyl–H_5–H_6), 7.55–7.20 (m, 10H, other aromatics), 5.05 (s, 1H, pyridine–H_4), 4.15–3.35 (m, 6H, N–C–CH_2–CH, $COOCH_2$), 3.20–2.15 (m, 13H, $CH_2N(CH_3)CH_2$ and 2,6 CH_3s), 1.50 (s, 6H, COO–C(CH_3)$_2$).

This compound was also prepared by method C giving 36% yield, after crystallization from H_2O containing a slight excess of hydrochloric acid.

6.11.3.3. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl-0.5H_2O 9u$_1$: m.p. 119–123 °C (H_2O); Anal. $C_{34}H_{41}N_3O_6$·HCl·0.5H_2O (C, H, N, Cl, H_2O).

6.11.3.4. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(4-nitrophenyl)pyridine-3,5-dicarboxylate-HCl 9v: m.p. 119–121 °C (amorphous); Anal. $C_{34}H_{41}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 10.1–10.4 (bs, 1H, ^+NH), 9.5 (bs, 1H, pyridine–NH), 8.3 and 7.6 (2dd, 4H, 4-nitrophenyl AA'BB' system), 7.43 (s, 10H, other aromatics), 5.05 (s, 1H, pyridine–H_4), 4.3–3.7 (m, 1H, N–C–C–CH), 3.67 (s, 3H, $COOCH_3$), 3.0–2.2 (m, 6H, $CH_2NCH_2CH_2$), 2.75 (s, 3H, NCH_3), 2.4 and 2.3 (2s, 6H, 2,6 CH_3s), 1.5 (s, 6H, COO–C(CH_3)$_2$).

6.11.3.5. 3-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylpropyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl 9ac: m.p. 201–203 °C (EtOH); Anal. $C_{35}H_{43}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 12.0–11.0 (bs, 1H, ^+NH), 9.4 (bs, 1H, pyridine–NH), 8.2–7.9 (m, 2H, 3-nitrophenyl–H_2–H_4), 7.8–7.5 (m, 2H, 3-nitrophenyl–H_5–H_6), 7.4 (s, 10H, other aromatics), 5.05 (s, 1H, pyridine–H_4), 4.3–3.9 (m, 1H, N–C–C–CH), 3.6 (s, 3H, $COOCH_3$), 2.7 (s, 3H, NCH_3), 2.35 (s, 6H, 2,6 CH_3s), 3.2–1.9 (m, 8H, $CH_2CH_2NCH_2CH_2$), 1.35 (bs, 6H, COO–C(CH_3)$_2$).

6.11.3.6. 3-[N-(3,3-Diphenylpropyl)-N-methylamino]-2,2-dimethylpropyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl-0.5H_2O 9ad: m.p. (119) 130–140 °C (amorphous); Anal. $C_{35}H_{43}N_3O_6$·HCl·0.5H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR ($CDCl_3$): 10.5–9.5 (m, 1H, ^+NH), 8.6–7.2 (m, 15H, aromatics and pyridine–NH), 5.2 (s, 1H, pyridine–H_4), 4.1 (s, 2H, OCH_2), 4.2–3.7 (m, 1H, N–C–C–CH), 3.7 (s, 3H, $COOCH_3$), 3.2–2.2 (m, 9H, NCH_3 and $CH_2NCH_2CH_2$), 2.5 and 2.4 (2s, 6H, 2,6 CH_3s), 1.2 and 1.0 (2s, 6H, COO–C–C(CH_3)$_2$).

6.11.3.7. 3-[N-(3,3-Diphenylpropyl)-N-methylamino]-2,2-dimethylpropyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate-HCl 9ae: m.p. 117–119 °C (Me_2CO); Anal. $C_{35}H_{41}Cl_2N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 10.5 (m, 1H, ^+NH), 9.4 (s, 1H, pyridine–NH), 7.7–7.1 (m, 13H, aromatics), 5.4 (s, 1H, pyridine–H_4), 4.2–3.8 (m, 3H, N–C–C–CH and OCH_2), 3.6 (s, 3H, $COOCH_3$), 3.2–2.2 (m, 15H, $CH_2N(CH_3)CH_2CH_2$ and 2,6 CH_3s), 1.0 (s, 6H, COO–C–C(CH_3)$_2$).

6.11.3.8. 3-[N-(3,3-Diphenylpropyl)-N-methylamino]-3,3-dimethylpropyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl-0.5H_2O 9af: m.p. 112–117 °C (amorphous); Anal. $C_{37}H_{45}N_3O_6$·HCl·0.5H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR (DMSO-d_6): 11.2–10.6 (bs, 1H, ^+NH), 9.5 (s, 1H, pyridine–NH), 8.3–8.0 (m, 2H, 3-nitrophenyl–H_2–H_4), 7.8–7.6 (m, 2H, 3-nitrophenyl–H_5–H_6), 7.45 (s, 10H, other aromatics), 5.1 (s, 1H, pyridine–H_4), 4.5–3.9 (m, 3H, N–C–C–CH and OCH_2), 3.65 (s, 3H, $COOCH_3$), 3.55 (s, 1H, 0.5 H_2O), 3.2–2.2 (m, 7H, $CH_2CH_2N(CH_3)_2$), 2.4 (s, 6H, 2,6 CH_3s), 2.2–1.8 (m, 2H, O–C–CH_2), 1.3 (s, 6H, N–C(CH_3)$_2$).

6.11.3.9. 2-[N-(3,3-Diphenyl-1-oxopropyl)-N-methylamino]-1,1-dimethylethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate 9ag: m.p. 158 °C (EtOH 95); Anal. $C_{36}H_{39}N_3O_7$ (C, H, N); ^{1}H-NMR ($CDCl_3$): 8.4–7.2 (m, 14H, aromatics), 6.6 (s, 1H, pyridine–NH), 5.2 (s, 1H, pyridine–H_4), 4.75 (t, 1H, N–C–C–CH), 3.85 (s, 2H, NCH_2), 3.7 (s, 3H, $COOCH_3$), 3.1 (d, 2H, $NCOCH_2$), 2.8 (s, 3H, NCH_3), 2,3 (s, 6H, 2,6 CH_3s), 1.2 (s, 6H, COO–C(CH_3)$_2$).

6.11.4. Method C

A mixture of the appropriate aminoalkyl 2-arylideneacetoacetate of general formula 12 (0.01 mol), alkyl 3-aminocrotonate 5 (0.01 mol) and *i*-PrOH (10–40 mL) was stirred at reflux temperature for 3–6 h under N_2 atmosphere. After cooling to room temperature the solvent was evaporated and the residue was purified by flash chromatography. When 12 was used as $^+$HCl salt, the residue was dissolved in a suitable solvent (CH_2Cl_2 or EtOAc) and the solution was washed with 20% Na_2CO_3 aqueous solution followed by H_2O. The organic layer was dried, filtered and evaporated to dryness to give the residue for flash chromatography. The following compounds were synthesized by this route.

6.11.4.1. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1-methylethyl 1-methylethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl 9o: m.p. 108–110 °C (amorphous); Anal. $C_{37}H_{43}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 12.00–11.50 (bs, 1H, ^+NH), 8.4–7.2 (m, 15H, aromatics and pyridine–NH), 5.6–4.8 (m, 2H, 2 x O–CH), 5.05 (s, 1H, pyridine–H_4), 4.2–3.7 (m, 1H, N–C–C–CH), 3.4–2.2 (m, 6H, $CH_2NCH_2CH_2$), 2.8 (s, 6H, 2,6 CH_3s), 1.5–1.0 (m, 9H, O–C(CH_3)$_2$ and O–C(CH_3)–C).

6.11.4.2. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1-methylethyl 2-propoxyethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate-HCl-0.5H_2O 9p: m.p. 85–92 °C (amorphous); Anal. $C_{39}H_{47}N_3O_7$·HCl·0.5 H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR ($CDCl_3$): 8.2–7.0 (m, 14H, aromatics), 6.0 (s, 1H, pyridine–NH), 5.3–4.6 (m, 1H, COOCH), 5.1 (s, 1H, pyridine–H_4), 4.3–3.7 (m, 3H, N–C–C–CH and $COOCH_2$), 3.7–3.15 (m, 4H, CH_2OCH_2), 2.6–1.85 (m, 9H, $CH_2N(CH_3)$-

CH_2CH_3), 2.3 (s, 6H, 2,6 CH_3s), 1.75–1.3 (m, 2H, O–C–CH_2–C), 1.2 and 1.0 (2d, 3H, O–C–CH_3), 0.85 (t, 3H, O–C–C–CH_3).

6.11.4.3. 1,1-Dimethyl-2-propoxyethyl 2-[N-(3,3-diphenylpropyl)-N-methylamino]-1-methylethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·0.5H_2O 9q: m.p. 86–95 °C (amorphous); Anal. $C_{41}H_{51}N_3O_7$·HCl·0.5H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR ($CDCl_3$): 11.0–10.5 (bs, 1H, $^+$NH), 8.1–7.0 (m, 14H, aromatics), 5.7 (s, 1H, pyridine–NH), 5.2–4.7 (m, 1H, COOCH), 5.0 (s, 1H, pyridine–H)$_4$), 4.1–3.6 (m, 1H, N–C–C–CH), 3.4 (s, 2H, OCH_2–C–C), 3.25 (t, 2H, OCH_2–C(C_2), 2.5–1.9 (m, 10H, $CH_2N(CH_3)CH_2CH_2$ and 0.5H_2O), 2.25 (s, 6H, 2,6 CH_3s), 1.75–1.2 (m, 2H, O–C–CH_2–C), 1.35 (s, 6H, COO–C(CH_3)$_2$), 1.2 and 1.0 (2d, 3H, COO–C–CH_3), 0.8 (t, 3H, O–C–C–CH_3).

6.11.4.4. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1-methylethyl 2-propoxyethyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate·HCl 9s: m.p. 108–118 °C (amorphous); Anal. $C_{39}H_{48}Cl_2N_2O_5$·HCl (C, H, N, Cl); ^{1}H-NMR ($CDCl_3$): 12.0–11.5 (bs, 1H, $^+$NH), 8.0 (s, 1H, pyridine–NH), 7.8–7.1 (m, 13H, aromatics), 5.7–5.3 (m, 1H, COOCH), 5.45 (s, 1H, pyridine–H_4), 4.4–3.8 (m, 3H, N–C–C–CH and $COOCH_2$), 3.8–2.1 (m, 13H, $CH_2N(CH_3)$–CH_2CH_2 and CH_2OCH_2), 2.4 (s, 6H, 2,6 CH_3s), 1.8–1.2 (m, 5H, COO–C–CH_3 and O–C–CH_2–C), 1.0–0.7 (t, 3H, O–C–C–CH_3).

6.11.4.5. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylethyl ethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl 9w (the reaction was carried out in anhydrous DMF at 80 °C for 5 h): m.p. 135–145 °C (EtOAc); Anal. $C_{37}H_{43}N_3O_6$·HCl (C, H, N, Cl); ^{1}H-NMR (DMSO-d_6): 11.0–10.0 (bs, 1H, $^+$NH), 9.45 (s, 1H, pyridine–NH), 8.2–7.6 (m, 4H, aromatics of 3-nitrophenyl ring), 7.4 (s, 10H, other aromatics), 5.0 (s, 1H, pyridine–H_4), 4.3–3.8 (m, 3H, N–C–C–CH and $COOCH_2$), 3.8–3.3 (m, 2H, O–C–CH_2–N), 2.4 and 2.3 (2s, 6H, 2,6 CH_3s), 3.2–2.1 (m, 7H, $N(CH_3)CH_2CH_2$), 1.45 (s, 6H, COO–C(CH_3)$_2$), 1.2 (t, 3H, COO–C–CH_3).

6.11.4.6. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylethyl 1-methylethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·0.5H_2O 9x: m.p. 100–115 °C (amorphous); Anal. $C_{38}H_{45}N_3O_6$·HCl·0.5H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR (DMSO-d_6): 11.2–10.5 (bs, 1H, $^+$NH), 9.45–9.25 (s, 1H, pyridine–NH), 8.3–7.6 (m, 4H, aromatics of 3-nitrophenyl ring), 7.45 (s, 10H, other aromatics), 5.05 (s, 1H, pyridine–H_4), 5.2–4.75 (m, 1H, COOCH), 4.2–3.8 (m, 1H, N–C–C–CH), 3.5 (s, 1H, 0.5H_2O), 3.8–2.0 (m, 6H, $CH_2NCH_2CH_2$), 2.7 (s, 3H, NCH_3), 2.4 and 2.3 (2s, 6H, 2,6 CH_3s), 1.5 (s, 6H, COO–C(CH_3)$_2$–C), 1.2 (t, 6H, COO–C(CH_3)$_2$).

6.11.4.7. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylethyl 2-propoxyethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·0.5H_2O 9y: m.p. 87–92 °C (amorphous); Anal. $C_{39}H_{49}N_3O_7$·HCl·0.5H_2O (C, H, N, Cl, H_2O); ^{1}N-NMR (DMSO-d_6): 10.6–10.1 (bs, 1H, $^+$NH), 9.3 (s, 1H, pyridine–NH), 8.2–7.5 (m, 4H, aromatics of 3-nitrophenyl ring), 7.35 (s, 10H, other aromatics), 5.0 (s, 1H, pyridine–H_4), 4.3–3.8 (m, 3H, N–C–C–CH and $COOCH_2$), 3.8–2.1 (m, 20H, $CH_2N(CH_3)CH_2CH_2$, 2,6 CH_3s, CH_2OCH_2 and 0.5 H_2O), 1.6–1.2 (m, 8H, O–C–CH_2–C and COO–C(CH_3)$_2$), 0.8 (t, 3H, O–C–C–CH_3).

6.11.4.8. 1,1-Dimethyl-2-propoxyethyl 2-[N-(3,3-diphenylpropyl)-N-methylamino]-1,1-di-methylethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylate·HCl·0.5H_2O 9z: m.p. 88–92 °C (amorphous); Anal. $C_{40}H_{53}N_3O_7$·HCl·0.5H_2O (C, H, N, Cl, H_2O); ^{1}H-NMR (DMSO-d_6 at 80 °C): 10.5–9.3 (bs, 1H, $^+$NH), 9.25 (s, 1H, pyridine–NH), 8.4–7.7 (m, 4H, aromatics of 3-nitrophenyl ring), 7.6 (s, 10H, other aromatics), 5.3 (s, 1H, pyridine–H_4), 4.5–4.1 (m, 1H, N–C–C–CH), 3.8 (s, 3H, COO–C–CH_2–O and 0.5H_2O), 3.6 (t, 2H, COO–C–C–O–CH_2), 2.9 (s, 3H, NCH_3), 2.6 and 2.55 (2s, 6H, 2,6 CH_3s), 3.5–2.3 (m, 6H, $CH_2NCH_2CH_2$), 1.65 and 1.6 (2s, 12H, 2 COO–C–(CH_3)$_2$), 1.8–1.3 (m, 2H, O–C–CH_2–C), 0.95 (t, 3H, O–C–C–CH_3).

6.11.4.9. 2-[N-(3,3-Diphenylpropyl)-N-methylamino]-1,1-dimethylethyl methyl 4-(2,3-di-chlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate·HCl·0.75H_2O 9aa: m.p. 124–140 °C (amorphous); Anal. $C_{36}H_{40}Cl_2N_2O_4$·HCl·0.75H_2O (C, H, N, Cl, H_2O). ^{1}H-NMR (DMSO-d_6): 10.7–10.2 (bs, 1H, $^+$NH), 9.35 (s, 1H, pyridine–NH), 7.55 (s, 13H, aromatics), 5.45 (s, 1H, pyridine–H_4), 4.2–3.9 (m, 1H, N–C–C–CH), 3.65 (s, 3H, $COOCH_3$), 3.5 (s, 1.5H, 0.75H_2O), 3.3–2.1 (m, 9H, $CH_2N(CH_3)CH_2CH_2$), 3.35 and 3.25 (2s, 2,6 CH_3s), 1.4 (s, 6H, COO–C(CH_3)$_2$).

6.11.5. Determination of apparent dissociation constant for 9u

The apparent dissociation constant (pK_a') of 9u was determined by potentiometric titration according to Albert and Serjeant [45]. 10^{-3} M Ethanolic–aqueous solutions (80:20 to 50:50) of the compound were titrated in a double run with 0.02 N NaOH at 37 °C using a glass–calomel electrode system. Only the pK_a values obtained in the range of 20–80% of titrating solution were taken into account and used to determine the regression line, which gave the extrapolated pK_a' of 9u in pure water. The value found was pK_a' = 6.83.

By the same method a pK_a' value of 7.05 was obtained for nicardipine, in agreement with the value of 7.0 from the literature [46].

6.11.6. Determination of the partition coefficient for 9u

Log *P* determination of 9u was carried out at 25 °C in *n*-octanol–phosphate buffer using a modified procedure based on the shake-flask method [47]. *n*-Octanol and aqueous phosphate buffers (pH 2.5 and 3) were mutually saturated by stirring equal volumes of each component at 25 °C for 2 h, and the two layers were separated after standing for 1 h.

Isotonic phosphate buffer solutions at pH 2.5 and 3 were prepared using 0.05 M KH_2PO_4 solutions adjusted to desired pH by H_3PO_4 addition. A shaking time of 5 min was used, followed by centrifugation at 3000 rpm for 10 min to completely separate the two layers, and the concentration of compound 9u was determined by HPLC on the aqueous phase.

The determinations were made using 10^{-3} M solutions of 9u base in *n*-octanol and volumetric *n*-octanol/aqueous buffer ratios 1:1 and 1:10 for each pH value. The concentration of compound 9u in the aqueous layer was quantitated by HPLC using a Waters 3.9 x 300 mm reverse-phase μ-Bondapack C-18 column with UV detection at 240 nm and with MeCN–0.15 M $NaClO_4$ aqueous solution adjusted to pH 3 by $HClO_4$ addition (61:39, v/v) as the mobile phase at a flow rate of 1.5 mL/min (isocratic elution at 25 °C).

The distribution coefficient *D* was calculated from the equation

$$D = \frac{\text{(total concentration} - \text{concentration in aqueous layer)}}{\text{concentration in aqueous layer}}$$

Table IX. Results from pharmaco-toxicological screening for 1,4-dihydropyridines 9.

Compound	^{3}H-Nitrendipine binding, IC_{50} (nM)	SHR	Indicative toxicity, LD_{50} (μmol/kg)		
		ED_{25} p.o. (μmol/kg)	i.p. (mouse)	p.o. (mouse)	i.v. (rat)
9a	9.6	16.0	114	396	
9b	0.4	10.0	200	250	11
9c	7.1	22.0	149	570	
9d	2.6	23.6[a]	116	275	
9e	1.8	12.0	112	460	
9f	1.5	13.0	270	180	
9g	1.4	5.0[a]	99	200	
9h	0.6	4.1	77	179	7
9i	1.3	5.1	95	605	16
9j	1.1	10.0	320	3170	18
9k	2.3	5.3	270	400	
9l	7.8	8.0	170	290	
9m	1.0	11.0	150	750	11
9n	1.0	3.5	113	310	7
9n$_1$	0.3	3.4			5
9n$_2$	14.0	43.0			32
9o	3.4	7.4	110	190	11
9p	1.5	6.6	94	75	6
9q	8.0	11.0	375	> 4000	40
9r	4.0	4.9		> 770	19
9s	4.5	6.6		1860	10
9t	2.0	8.2	81	312	
9u	0.5	3.9	114	440	39
9v	380.0	> 154	710	> 4000	
9w	1.3	3.5[a]	520	4530	
9x	1.3	11.8	320		39
9y	0.6	5.2	71	515	23
9z	31.6	10.0	132	990	100
9aa	11.7	12.0	218	> 4000	28
9ab	1.4	6.6	150	230	
9ac	13.3	49.8	759	> 4000	
9ad	0.45	1.1	18	89	38
9ae	2.9	5.5	104	> 4000	
9af	20.8	18.4	320	> 4000	
9ag	4.3	> 160	> 1600	> 4800	
Nifedipine	2.3	18.0[a]	660	810	56
Nicardipine·HCl	0.4	14.0	390	890	48
Felodipine	0.2	16.0	350	320	22
Prenylamine lactate	470.0	> 100			

[a]Peak effect at 1 hour after administration.

The partition coefficient was calculated by the formula

$$\log P = \log D - \log [1/1 + (10^{pKa - pH})]$$

and proved to be 6.0–6.1.

6.12. *Pharmaco-toxicology*

6.12.1. *^{3}[H]nitrendipine binding*

Radioreceptor binding studies on 1,4-dihydropyridines binding site in the Ca^{2+} channels were carried out in membranes of rat brain. Male Sprague Dawley rats (200–300 g, Charles River, Italy) were killed by cervical dislocation and brains (minus cerebella) were excised and homogenized (2 x 20 sec) in 50 vol. of cold Tris-HCl buffer pH 7.7, using a Politron homogenizer (speed 7). Homogenates were centrifuged at 1000 *g* for 10 min and supernatants recentrifuged at 48000 *g* for 10 min; pellets were resuspended in 50 vol. of the same buffer and centrifuged once more.

The final pellets were suspended in 75 vol. of 50 mM Tris-HCl buffer pH 7.7; membranes were incubated in a final volume of 1 mL for 1 h at 25 °C with 0.2–0.5 nM ^{3}[H]nitrendipine, in the absence or presence of competing drugs; non-specific binding was determined in the presence of 1 μM nifedipine.

The incubation was stopped by addition of ice-cold Tris-HCl buffer and rapid filtration through Whatman GF/B. The filters were then washed with ice-cold buffer and the radioactivity retained on the filters was counted by liquid scintillation spectrometry. The inhibition of specific binding of the radioligands by the tested drugs was analyzed to estimate the IC_{50} value by using the non-linear curve-fitting program Allfit [48].

6.12.2. *Effects on blood pressure in Spontaneously Hypertensive Rats (SHR)*

Male spontaneously hypertensive rats of the Okamoto Strain, age 12–16 weeks, weighing 250–300 g were obtained from Charles River Italia, Calco, Como. Animals were housed with free access to food and water and maintained under standard conditions of temperature and humidity with a 12 h light–dark cycle, at least for 5 days before the experiment. Systolic arterial pressure (SBP) was non-invasively recorded (tail-cuff method) using a W&W 8006 BP Recorder and a piezoelectric pulse transducer (U. Basile, Italy). The animals were trained to comply unstressfully to the recording procedure for three days before the beginning of the experiment and only those having SBP values over 200 mmHg were used.

Animals were pre-warmed for 15 min at 37 °C before recording SBP. Compounds were dissolved or suspended in an aqueous solution of 0.5% Methocel A4C Premium, and administered by oral gavage in a volume of 5 mL/kg; the effects on blood pressure were monitored at 1, 3, 5 and 7 h after administration. The decrease in SBP recorded at different times were calculated as % changes versus the basal values, and the ED_{25} values (extrapolated dose inducing 25% decrease of SBP) were evaluated by linear regression analysis at time of peak effect.

6.12.3. *Acute toxicity in mice and rats*

Female Albino Swiss mice (28–34 g), and male Sprague–Dawley rats (180–220 g), obtained from Charles River Italia, Calco, Como, were used. Animals were housed with free access to food and water and maintained on standard conditions of temperature and humidity with a 12 h light–dark cycle, at least for 5 days before the experiment. The acute toxicity of tested compounds in mice was evaluated after intraperitoneal and oral administration, in groups of 3 mice (fasted for 4 h) at the maximal dose of 2000 mg/kg/30 mL (oral) or 500 mg/kg/20 mL (intraperitoneal). Compounds were dissolved or suspended in an aqueous solution of 0.5% Methocel A4C Premium. When necessary, logarithmic scaled doses of the compounds were administered to different groups of mice. Mortality was recorded for 14 days after the administration. LD_{50} values were evaluated by the method of Weil [49].

Intravenous toxicity in rats was evaluated as reported above. Compounds were dissolved in polypropylene glycol 400–water 1:1 and administered in the caudal vein (1 mL/kg).

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(57) Abstract

1,4-Dihydropyridines have been found to counter several processes which play a role in the development of atherosclerotic vascular lesions, such as myocytes proliferation and migration, cholesterol metabolism in macrophages and oxidative modification of low density lipoproteins. They are therefore useful in the manufacture of medicaments for preventing, arresting and reversing atherosclerotic degradation in the arterial walls of humans. The preferred 1,4-dihydropyridines for this purpose are lercanidipine, (S)-lercanidipine and (R)-lercanidipine.

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USE OF 1,4-DIHYDROPYRIDINE DERIVATIVES IN THE PREVENTION AND THERAPY OF ATHEROSCLEROTIC DEGRADATION OF ARTERIAL WALLS.

The invention relates to the use of 1,4-dihydropyridines to counter several processes which play a role in the development of atherosclerotic vascular lesions, such as myocytes proliferation and migration, cholesterol metabolism in macrophages and oxidative modification of low density lipoproteins. Beneficial effects on the above biological processes can be considered the basis for prevention of atherosclerotic degradation in the arterial walls of humans. The invention also relates to the use of 1,4-dihydropyridines in the manufacture of medicaments for preventing, arresting and reversing atherosclerotic degradation in the arterial walls of humans.

Arteriosclerosis, a generic term for thickening and hardening of the arterial wall, is responsible for very many deaths in the United States and other westernized societies. One type of arteriosclerosis is atherosclerosis, the disorder of the larger arteries that underlies most coronary artery disease, aortic aneurysm, and arterial disease of the lower extremities and also plays a major role in cerebrovascular disease. Atherosclerosis is by far the leading cause of death in the United States, both above and below age 65.

It is now recognized that atherosclerosis is a multifactorial process which, when leading to clinical sequelae, is based on extensive proliferation of migrated smooth muscle cells within the intima of the affected artery. The atherosclerotic plaque formation can be considered the result of three fundamental biological processes. These are:

1) migration and proliferation of intimal smooth muscle cells, together with variable numbers of accumulated macrophages and T-lymphocytes;
2) formation by the proliferated smooth muscle cells of large amounts of connective tissue matrix, including collagen, elastic fibres, and proteoglycans; and
3) accumulation of lipid, principally in the form of cholesteryl esters and free cholesterol within the cells as well as in the surrounding connective tissues.

In addition, a number of experimental reports suggest a key role for the oxidative modification of low density lipoproteins (LDL) in the early stages of atherosclerosis in humans, where hypercholesterolemia represents the major risk factor associated with the increased incidence of the disease. Available data suggest that LDL undergoes oxidative modification, and that oxidized LDL may promote atherogenesis by a number of mechanisms, including their enhanced uptake in tissue macrophages which leads to lipid accumulation and chemotactic activity for monocytes, and cytotoxicity to arterial

wall endothelial cells.

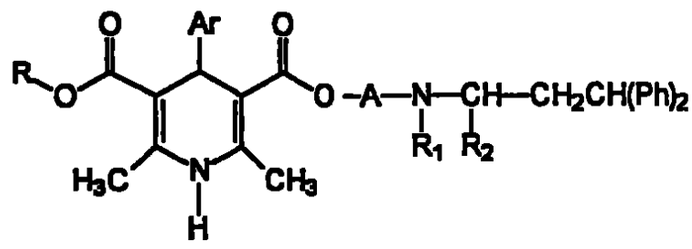
It has now surprisingly been found that certain 1,4-dihydropyridines, known from US Patent 4705797 for their coronary dilating and antihypertensive activity, are able to counteract many of the biological processes leading to atherosclerotic lesions and therefore can be used in humans to prevent and cure the atherosclerotic degradation of the arterial wall, hypercholesterolemia and various diseases caused by these, for example, ischemic heart diseases such as myocardial infarction and cerebrovascular diseases such as cerebral infarction and cerebral apoplexy.
The compounds of the invention can also be useful for the inhibition of restenosis following percutaneous transluminal coronary angioplasty (PTCA) and for suppressing the progression of vascular hypertrophy associated with hypertension.

Particularly preferred among these 1,4-dihydropyridine derivatives are lercanidipine, its enantiomers, and pharmaceutically acceptable salts thereof. Lercanidipine is methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl--4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate.

On the one hand, the (S)-enantiomer and the racemate of lercanidipine, both being endowed with antihypertensive activity, can be used in patients in need of treatment for both hypertension and diseases related to atherosclerotic phenomena.

On the other hand, (R)-lercanidipine, being practically devoid of antihypertensive activity, can be used for treating conditions involving smooth muscle cell migration and proliferation without any concomitant cardiovascular effect. This enantiomer is therefore applicable to those patients for whom reduction of blood pressure would be undesirable.

The invention provides the use of a compound having the general formula I



(I)

wherein
Ph represents a phenyl group,

Ar represents a 2-nitrophenyl, 3-nitrophenyl, 2,3-dichlorophenyl or benzofurazan-4-yl group,

A represents a branched chain alkylene group having from 3 to 6 carbon atoms,

R represents a straight or branched chain alkyl group having from 1 to 6 carbon atoms, optionally mono-substituted by an alkoxy group having from 1 to 6 carbon atoms,

R_1 represents a hydrogen atom, a hydroxy group or an alkyl group having from 1 to 4 carbon atoms, and

R_2 represents a hydrogen atom or a methyl group,

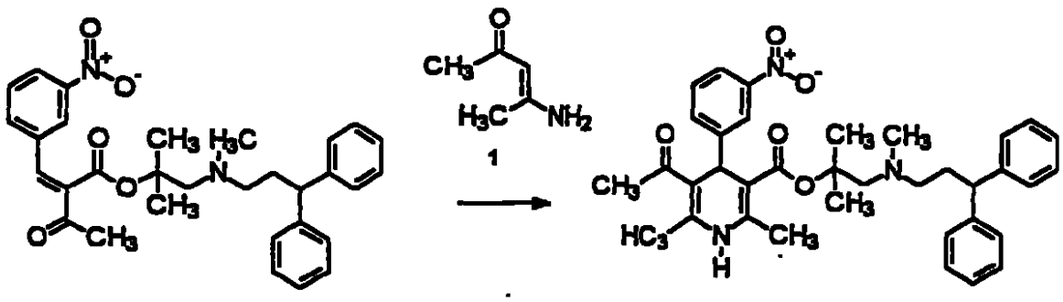
or of a salt, enantiomer, hydrate or solvate of such a compound, for the preparation of a medicament for the prevention, arrest or reversal of atherosclerotic degradation in the arterial walls of a patient.

The invention further provides a method for the prevention, arrest or reversal of atherosclerotic degradation in the arterial walls of a patient, the method comprising administering to the patient a therapeutically effective amount of a compound of the general formula I or of a salt, enantiomer, hydrate or solvate of such a compound.

The preferred 1,4-dihydropyridine derivatives I for administration to a patient, or for use in the preparation of a medicament for administration to a patient, are lercanidipine and its (R)- and (S)-enantiomers.

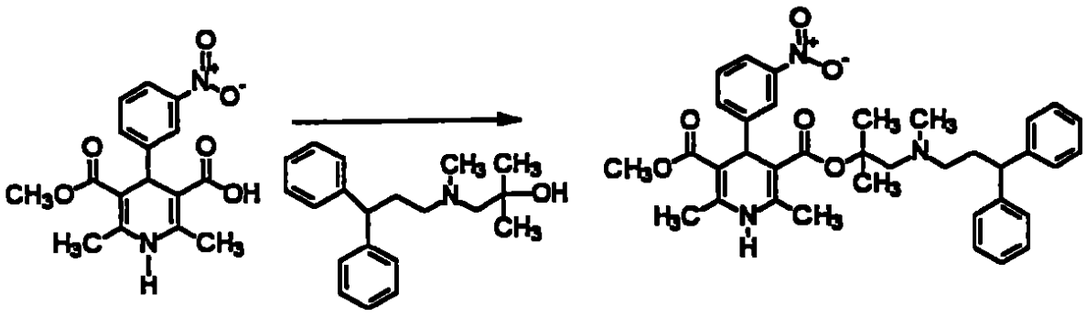
Lercanidipine may be prepared by Hantzsch cyclization of methyl 3-aminocrotonate (1) with 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl α-acetyl-3-nitrocinnamate (2) according to the method shown in Reaction Scheme 1 below and more fully described in US 4707797.

REACTION SCHEME 1



Lercanidipine may alternatively be prepared by esterification of 1,4-dihydro--2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-pyridine-3-carboxylic acid (3) with 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol (4) according to the method shown in Reaction Scheme 2 below and more fully described in Example 3 below.

REACTION SCHEME 2



3 4 LERCANIDIPINE

Lercanidipine enantiomers can be prepared by the esterification method reported in Reaction Scheme 2 above by using the appropriate homochiral acids which, when present in 1:1 ratio, constitute the above cited acid 3. These homochiral acids, hereinbelow for convenience called acid 5 [the (R)-enantiomer] and acid 6 [the (S)-enantiomer], can readily be prepared by resolution of the racemic acid 3 according to the methods reported by A. Ashimori et al., *Chem. Pharm. Bull.* 39, 108 (1991).

The esterification of Reaction Scheme 2 may be performed in the presence of a coupling agent, such as dicyclohexylcarbodiimide, N,N'-carbonyldiimidazole or diethyl cyanophosphonate, and optionally in the presence of a promoting agent, such as N-hydroxysuccinimide or 4-dimethylaminopyridine, in aprotic or chlorinated solvents, e.g. dimethylformamide or chloroform, at temperatures ranging from -10 to 140°C according to well known synthetic methods: Albertson, *Org. React.* 12, 205 (1982); Doherty et al., *J. Med. Chem.* 35, 2 (1992); Staab et al., *Newer Methods Prep. Org. Chem.* 5, 81 (1988); Ishihara, *Chem. Pharm. Bull.* 39, 3238 (1991).

Alternatively, lercanidipine enantiomers may be prepared by first reacting the acid 5 (or 6) with alkyl chloroformate in presence of a tertiary amine such as triethylamine, and then adding the intermediate (4) at 0-80°C. Optionally, a promoting agent such as 1-hydroxypiperidine may be added before the addition of the intermediate (4), see Albertson, *Org. React.* 12, 157 (1982)).

ENSDOCID: <WO 9703669A1 I >

The lercanidipine enantiomers may be also prepared by conversion of the acid 5 (or 6) into the corresponding acyl halide using an inorganic acid halide, such as phosphorus pentachloride, oxalyl chloride, phosphorus trichloride, phosphorous oxychloride or thionyl chloride, in a chlorinated solvent, e.g. chloroform, dichloroethane, dichloromethane or 1,1,1-trichloroethane, optionally in the presence of a promoting agent such as dimethylformamide, at a temperature of from -10 to 85°C. The acyl halides may, but need not, be isolated before the addition of the intermediate (4).

The lercanidipine enantiomers, howsoever obtained, may be purified according to methods known in the art, either as bases (e.g. by column chromatography) or as salts (e.g. by reprecipitation or recrystallization). The above methods can also be used for all the other compounds of the general formula I.

According to the invention, the 1,4-dihydropyridine derivative I may be administered to the patient as such, or in the form of any of its pharmaceutically acceptable salts, hydrates or solvates. Preferred pharmaceutically acceptable acid addition salts include those formed with hydrochloric, sulphuric, maleic, succinic, citric, methanesulphonic and toluenesulphonic acids; they may be prepared from the free bases in conventional manner. Whatever the form (base, salt, hydrate or solvate), the active ingredient will usually be administered in admixture with a pharmaceutically acceptable carrier.

For oral administration, the 1,4-dihydropyridine derivatives may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gum and the like. These preparations should contain at least 0.5% of active compounds, but the amount of active ingredient may be varied depending upon the particular form and may conveniently be from about 5% to about 70% of the weight of the unit. The amount of active compound in such compositions is such that a suitable dosage will be obtained, although the desired dosage can be obtained by administering a plurality of dosage forms. The compounds of the invention may be administered at the oral dosage of 0.1 to 400 mg, the 1 to 200 mg dosage range being preferred. The tablets, pills, capsules, troches and the like may also contain, for example, the following ingredients: a binder such as microcrystalline cellulose, tragacanth gum or gelatin; an excipient such as starch or lactose; a disintegrating agent such as alginic acid, sodium starch glycolate, corn starch and the like; a lubricant such as magnesium stearate or hydrogenated castor oil; and a glidant such as colloidal silicon dioxide. Sweetening agents such as sucrose or saccharin can be included, as can flavouring agents such as peppermint, methyl salicylate or orange flavouring. When the

dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil. Oral dosage units may contain various other materials which modify the physical form of the dosage unit, for example, as coatings. Thus, tablets or pills may be coated with sugar, shellac, or other enteric coating agents. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes, colouring and flavours. Materials used in preparing these various compositions should be pharmaceutically pure and non-toxic in the amounts used.

For parenteral administration, the 1,4-dihydropyridine derivatives may be incorporated into a solution or suspension. These preparations should contain at least 0.1% of active compound, but the amount of active ingredient may vary between 0.5% and about 30% of the weight thereof. The amount of active compound in such compositions is such that a suitable dosage will be obtained. Preferably a parenteral dosage unit contains between 0.05 to 100 mg of active compound. The solutions or suspensions may also include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; an antibacterial agent such as benzyl alcohol or methyl parabens; an antioxidant such as ascorbic acid or sodium bisulphite; a chelating agent such as ethylenediaminetetraacetic acid; a buffer such as an acetate, a citrate or a phosphate; and an agent for the adjustment of toxicity such as sodium chloride or dextrose. Parenteral multiple dose vials may be of glass or plastics material.

Dosage forms, additional ingredients and routes of administration contemplated herein include those disclosed in US 4089969 and US 5091182, both incorporated by reference in their entirety.

The following Examples illustrate the preparation of (R,S)-lercanidipine and the (S)- and (R)-enantiomers thereof.

EXAMPLE 1

(S)-(+)-methyl 1,1-N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro--2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate [(S)-Lercanidipine] hydrochloride hemihydrate

0.13 ml of thionyl chloride was added at -10°C to a stirred suspension of 0.54 g of (R)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-5-methoxycarbonyl-pyridine-

-3-carboxylic acid in 2.9 ml of anhydrous dichloromethane and 0.75 ml of anhydrous dimethylformamide kept under nitrogen atmosphere and sheltered from direct light. After 1 hour at 0°C, a solution of 0.48 g of 2,N-dimethyl-N-(3,3-diphenyl-propyl)--1-amino-2-propanol (prepared as described in US 4705797) in 1 ml of dichloromethane was added at -5°C. After stirring for 3 hours at 0°C and standing overnight at 20-25°C, the solvent was evaporated off in vacuo and the residue was dissolved in 20 ml of ethyl acetate. The organic phase was washed sequentially with brine (4 ml), 10% aqueous sodium carbonate solution (5 x 4 ml), brine (4 ml), 1N hydrochloric acid (5 x 5 ml), brine (4 ml), 10% aqueous sodium carbonate solution (2 x 5 ml) and finally with brine (4 ml). The organic phase was dried over anhydrous sodium sulphate and evaporated to dryness in vacuo. The residue was purified by flash chromatography on a silica gel column eluting with petroleum ether:acetone 85:15. The unitary TLC fractions (petroleum ether:acetone 7:3 by volume and chloroform:5N methanolic ammonia 99:1.1 by volume) were evaporated to give a residue which was dissolved in 75 ml of diethyl ether containing 3% of acetone. After filtration the solution was acidified with 3N ethereal hydrogen chloride and the precipitate was collected by suction and dried at 78°C/15 mmHg to give 0.66 g of the title compound.

M.p. 115-125°C; $[\alpha]_D^{25}$ = + 70.56° (MeOH; c = 0.981).

Elemental analysis % for $C_{36}H_{41}N_3O_6.HCl.0.5\ H_2O$

Found: C, 65.47; H, 6.57; N, 6.29; Cl, 5.32; H_2O, 1.68

Calc.: C, 65.79; H, 6.60; N, 6.39; Cl, 5.39; H_2O, 1.37

^{1}H-NMR Spectrum of the base at 200 MHz ($CDCl_3$, δ):

8.10	(m, 1H)	nitrophenyl, 2-CH
7.97	(m,1H)	nitrophenyl, 4-CH
7.62	(m,1H)	nitrophenyl, 6-CH
7.33	(dd, 1H)	nitrophenyl, 5-CH
7.29-7.10	(m, 10H)	aromatic H atoms of $CH(Ph)_2$
5.79	(bs, 1H)	pyridine, NH
5.05	(s, 1H)	pyridine, 4-CH
3.92	(t, 1H)	$C\underline{H}(Ph)_2$
3.63	(s, 3H)	$COOCH_3$
2.57	(m, 2H)	$OC(CH_3)_2C\underline{H}_2N$
2.40-2.23	(m, 2H)	$N(CH_3)CH_2CH_2$
2.33-2.27	(2s, 6H)	pyridine, 2-CH_3 and 6-CH_3
2.19-2.09	(m, 2H)	$N(CH_3)CH_2CH_2$

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2.17	(s, 3H)	NCH_3
1.35-1.31	(2s, 6H)	$OC(CH_3)_2CH_2N$

EXAMPLE 2

(R)-(-)-methyl 1,1-N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro--2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate [(R)-Lercanidipine] hydrochloride hemihydrate

The title compound was obtained by the method described in Example 1 for its (S)-enantiomer, but using the (S)-enantiomer of 1,4-dihydro-2,6-dimethyl--4-(3-nitrophenyl)-5-methoxycarbonyl-pyridine-3-carboxylic acid instead of the (R)-enantiomer.

M.p. 115-120°C, $[\alpha]_D^{25}$ = -70.88 (MeOH, c = 0.975).

Elemental analysis % for $C_{36}H_{41}N_3O_6 .HCl .H_2O$
Found: C, 64.93; H, 6.62; N, 6.24; Cl, 5.41; H_2O, 2.50
Calc.: C, 64.90; H, 6.60; N, 6.31; Cl, 5.32; H_2O, 2.70

The ^{1}H-NMR spectrum of the base in $CDCl_3$ was exactly the same as that reported in Example 1 for the (S)-enantiomer.

EXAMPLE 3

Methyl 1,1-N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro--2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate [Lercanidipine] hydrochloride

45.8 g (0.385 mole) of thionyl chloride was dropped over a period of 15 minutes into a stirred mixture comprising 116.2 g (0.35 mole) of 2,6-dimethyl-5-methoxycarbonyl--4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (3), prepared as described in DE 2847237, 645 ml of anhydrous dichloromethane and 160 ml of anhydrous dimethylformamide, maintained in a nitrogen atmosphere at between -4°C and +1°C. The mixture was kept at the same temperature under stirring for 1 hour, and then 104.1 g (0.35 mole) of 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-propanol (4), prepared as described in US 4705797, dissolved in 105 ml of anhydrous dichloromethane was added dropwise over a period of 15 minutes at -10°C to 0°C.

After stirring for three hours at 0°C and standing overnight at room temperature, the solvent was evaporated off under vacuum and the residue was dissolved in 3500 ml of ethyl acetate. The organic phase was washed sequentially with brine (700 ml), 10% aqueous sodium carbonate solution (5 x 700 ml), brine (700 ml), 1N hydrochloric acid (5 x 700 ml) and finally with brine (700 ml). The organic phase was dried over anhydrous sodium sulphate for 30 minutes, and then filtered, shaken with 23 g of charcoal and filtered again. The volume of the solution was reduced to about 1 litre by evaporation under vacuum and then, after standing for 24 hours at from 0°C to 5°C, the crystals were collected by suction filtration and recrystallized from 99% ethanol to give 179.5 (78%) of the title compound. m.p. 186-188°C.

PHARMACOLOGICAL DATA

In the drawings:

Figure 1 is a graphical representation of the effect of lercanidipine and its enantiomers on [^{3}H]-thymidine incorporation into myocetes of rat smooth muscle cells,

Figure 2 is a graphical representation of the ability of lercanidipine and its enantiomers to interfere with the migration of arterial myocetes,

Figure 3 is a graphical representation of the ability of lercanidipine and its enantiomers to inhibit the enzyme ACAT and cholesterol esterification induced by AcLDL in mouse peritoneal macrophage,

Figure 4 is a graphical representation of the concentration-dependent effect of lercanidipine and its enantiomers on cholesterol esterification in cholesterol ester loaded macrophages,

Figures 5 and 6 are graphical representations of the effect of lercanidipine and its enantiomers on cell-mediated oxidation of LDL, and

Figure 7 is a time course plot of cell-mediated oxidation and the effects of lercanidipine after incubation.

Effects on arterial myocyte migration and proliferation

Animal models of vascular injury have shown that an arterial lesion is followed by proliferation of the medial myocytes, many of which migrate into the intima and further proliferate to form a neointimal lesion. The causes of these events are not completely understood. Recent findings have elucidated that myocytes make up approximately 90-95% of the cellular population of the atherosclerotic lesion in young adults and compose an average of 50% of the advanced atherosclerotic plaque. In addition, vascular myocytes contribute to the lesion by synthesis of extracellular matrix

and they can accumulate lipids and become foam cells. Thus, the elucidation of the factors affecting these phenomena affords new entry points for selective interference and inhibition of the process of atherogenesis.

Migration of myocytes was investigated for the present invention using rat aortic smooth muscle cells in the presence of fibrinogen as chemotactic factor, whereas for studies on their proliferation rat and human cells were used. Cell count and [^{3}H]-thymidine incorporation were used to evaluate myocytes growth. The methodology was as follows:

Myocytes were cultured from the intima-medial layer of the aortas of male Sprague-Dawley rats (200-250 g). Cells were grown in monolayers at 37°C in a humidified atmosphere of 5% CO_2 in Eagle's minimum essential medium supplemented with 10% (v/v) fetal calf serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 20 mM tricine buffer and 1% (v/v) non-essential amino acid solution. The medium was changed every third day. Cells were used between the 4th and 10th passage. Cell viability was timely assessed by tripan blue exclusion. Myocytes were identified for growth behaviour, morphology and using monoclonal antibody specific for a-actin, the actin isoform typical of myocytes. Human vascular myocytes (A 617 from human femoral artery) were grown in the same culture conditions. Cells were seeded at various densities for rat (2×10^5) and human (5×10^4) myocytes/petri dish (35 mm), and incubated with Eagle's minimum essential medium supplemented with 10% fetal calf serum. Twenty-four hours later the medium was changed to one containing 0.4% fetal calf serum to stop cell growth, and the cultures were incubated for 72 hours. At this time (time 0) the medium was replaced by one containing 10% fetal calf serum in the presence or absence of known concentrations of the tested compounds and the incubation was continued for further 72 hours at 37°C. At time 0, just before the addition of the substances to be tested, a sample from each petri dish was used for cell counting. Cell proliferation was evaluated by cell count after trypsinization of the monolayers using a Coulter Counter model ZM. Cell viability was assessed by tripan blue exclusion, and found to be higher than 95% at the drug concentrations used. The results are shown in Table 1.

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TABLE 1 - Myocytes growth inhibition measured by cell count

Myocetes from	LE $IC_{50}(\mu M)$	(R)-LE $IC_{50}(\mu M)$	(S)-LE $IC_{50}(\mu M)$	NI $IC_{50}(\mu M)$	LA $IC_{50}(\mu M)$
SD Rat	31.2	30.7	33.3	38.8	23.6
SHR Rat	14.1	9.2	16.8	n.t.	n.t.
WK Rat	16.4	15.3	20.7	n.t.	n.t.
Human	25.3	n.t.	n.t.	n.t.	n.t.

LE = Lercanidipine
NI = Nicardipine
LA = Lacidipine
SD = Sprague Dawley*
SHR = Spontaneously Hypertensive*
WK = Wistar Kyoto*
IC_{50} = concentration required to inhibit cell growth by 50%
n.t. = not tested
* = from Charles River, Calco, Italy

Lercanidipine and its enantiomers decreased the rat and human myocyte proliferation in a concentration-dependent manner, as shown in Table 1, and showed practically the same potency as the reference 1,4-dihydropyridines tested. It has to be emphasized that lercanidipine (and its enantiomers) proved active on the cells from all the species investigated, in particular in humans.

In another set of experiments, synchronization of myocytes to the G_0/G_1 interphase of the cell cycle was accomplished by incubating logarithmically growing cultures (= 3 x 10^5 cells/plate) for 96-120 hours in a medium containing 0.4% fetal calf serum. Quiescent cells were incubated for 20 hours in a fresh medium with 10% fetal calf serum in the presence of the tested drugs. Cell proliferation was then estimated by nuclear incorporation of [^{3}H]thymidine, incubated with cells (1 μCi/ml medium) for 2 hours. Radioactivity was measured with Filter-Count scintillation cocktail.

The results are shown in Figure 1, and confirm the high potency of lercanidipine and its enantiomers in inhibiting cell growth.

Migration of rat myocytes was examined using a 48-well micro chemotaxis chamber (Neuro-Probe, USA). Freshly trypsinized myocytes were suspended in a medium supplemented with 5% fetal calf serum (assay medium). The lower wells, containing

27 µl of the assay medium that included fibrinogen (600 µg/ml) as chemotactic agent, were covered with a polyvinylpyrrolidone-free polycarbonate filter (8 µM pore size). 50 µl of the cell suspension (1 x 10^6 cells/ml) were placed in the upper compartment with the tested compounds. Incubation was carried out for 5 hours at 37°C in an atmosphere of 95% air and 5% CO_2. After incubation the filter was removed from the chamber and non-migrated cells were scraped from the upper surface, and the filters washed with phosphate buffered saline three times. The filters were stained with Diff-Quik (Merz-Dade AG, Switzerland). The number of myocytes per 100 x high power field that had migrated to the lower surface of the filters was determined microscopically. Six high power fields were counted per sample and the results were averaged.

The results are shown in Figure 2, and demonstrate the ability of lercanidipine and its enantiomers to interfere with the migration of arterial myocytes. All the tested compounds were able to inhibit myocytes migration in a dose-dependent manner with the (R)-enantiomer showing the most pronounced effect.

Effects on cholesterol metabolism in mouse peritoneal macrophages

The atheroma contains two main cell types, macrophages and smooth muscle cells. Macrophages are derived from circulating monocytes and are the main lipid-loaded cells in the lesions. The mechanism by which they accumulate lipoprotein cholesterol and develop into foam cells depends mainly upon receptor-mediated processes, involving the so called "scavenger receptor" that recognizes chemically and biologically modified LDL, such as acetyl LDL (AcLDL) and oxidized LDL. The scavenger receptor, unlike the LDL receptor, is not subject to feed-back regulation and the result is a massive accumulation of cholesterol in cells. Cholesterol accumulates in macrophages in esterified form by a process involving the enzyme acyl-coenzyme A-cholesterol acyltransferase (ACAT) which catalyzes the cholesterol esterification in cytoplasm. Only free cholesterol can be removed from macrophages.

Cholesterol esterification induced by AcLDL in mouse peritoneal macrophages was investigated as follows. Mouse peritoneal macrophages were obtained by peritoneal lavage from mice (Balb/c Charles River, Calco, Italy) three days after intraperitoneal injection of thioglycolate. Cells (2-3x10^5) were plated in 35 mm wells with Dulbecco's minimum essential medium containing 10% fetal bovine serum. After 3 hours, the dishes were washed to eliminate unattached cells and maintained in Dulbecco's minimum essential medium plus 10% fetal bovine serum for 24 hours before use. After

cell plating, experiments were performed at 37°C in serum free Dulbecco's minimum essential medium containing 0.2% essentially fatty acid-free bovine serum albumin plus the indicated additions. Human LDL (d=1.019-1.063 g/ml) were isolated from plasma of healthy volunteers by sequential ultracentrifugation (Beckman L5-50, Palo Alto, CA). For acetylation, LDL were dialysed against 0.15 M NaCl, pH 7.4, diluted with an equal volume of saturated sodium acetate and treated with acetic anhydride. For [^{125}I]AcLDL, lipoproteins were labelled with sodium [^{125}I]iodide desalted by gel filtration on Sephadex G-25 eluted with phosphate buffered saline. Specific activity was 100-200 cpm/ng protein. Trichloroacetic acid non precipitable radioactivity was below 2% of total. All lipoproteins were sterile filtered. Cells were incubated for 24 hours with the tested compounds. After substitution of the medium with an identical one, incubation was continued for additional 24 hours. During this second incubation [^{125}I]AcLDL were added (50 μg/ml). Cholesterol esterification was measured after addition of [1-^{14}C] oleic acid (0.68 mCi sample) complexed with bovine serum albumin during the last 1 or 2 hours of incubation and subsequent determination of radioactivity associated with cellular cholesteryl esters. Where indicated cells were enriched with cholesterol by 24 hours incubation with 50 μg/ml of AcLDL before drug addition and experimental determinations.

Lercanidipine and its enantiomers proved able to inhibit, in a concentration dependent way, up to 90% of the formation of esterified cholesterol induced by AcLDL in mouse peritoneal macrophage (in other words the esterifying effect of enzyme ACAT). The IC_{50} values for lercanidipine and enantiomers ranged from 8 to 15 μM, as shown in Figure 3, the (R)-enantiomer being the most active compound.

Another set of experiments was performed to evaluate the effect of lercanidipine on cholesterol esterification in macrophages loaded with cholesterol ester before the addition of the compound, this condition being the same as in foam cells. Cells are loaded with cholesteryl esters by exposure for 24 hours to a medium containing 50 μg acetyl LDL. The results, in Figure 4, show that lercanidipine inhibited cholesterol esterification up to 70% in a concentration dependent manner with an IC_{50} value of about 7 μM. The (R)-enantiomer of lercanidipine proved slightly more potent than the racemate and (S)-lercanidipine was the least potent among the compounds tested.

Finally, it was proved that lercanidipine and its enantiomers at 5 μM did not impair the capability of the macrophages to hydrolyze the esterified cholesterol stored in the cytoplasm. These experiments were performed by incubating cells preloaded with [^{3}H]cholesterol in the presence of the specific ACAT inhibitor S-58035. The blockade

of intracellular re-esterification of cholesterol allowed the assessment of the ability of cells to hydrolyse the accumulated cholesterol esters. Addition of lercanidipine and its enantiomers did not influence the cellular hydrolytic activity as documented by the values of radioactivity in the esterified cholesterol fraction.

[1,2-^{3}H]Cholesterol is included in all loading media at a concentration of 0.5 μCi/ml. After 24 hours loading period, during which time the radiolabeled cholesterol is incorporated and esterified, the cell monolayers are washed and incubated an additional 24 hours in medium containing 0.1% bovine serum albumin to allow the intracellular pools of labelled cholesterol to equilibrate to the same specific activity. To quantitate cholesteryl ester hydrolysis, the loaded cells are incubated for up to 24 hours in Dulbecco's minimum essential medium containing drugs, 0.1% bovine serum albumin, and the compound S-58035, an inhibitor of acyl-coenzyme A-cholesterol acyltransferase. The inhibition of acyl-coenzyme A-cholesterol acyltransferase prevents the reesterification of any free cholesterol generated by cholesteryl ester hydrolysis and thus allows assessment of the activity of the hydrolase. The hydrolysis of the cholesteryl esters is quantitated by determining the decrease of radiolabeled cholesteryl esters [E.H. Harrison et al., *J. Lipid. Res.* 31, 2187 (1990)]. After the indicated incubations, cells were washed with phosphate buffered saline and extracted with hexane:isopropanol (3:2 v/v). Media were extracted with chloroform:methanol (2:1 v/v). After solvent removal, free and esterified cholesterol were partitioned by TLC (isooctane:diethyl ether:acetic acid, 75:25:2 by volume). Cholesterol mass or radioactivity of the spots were determined by an enzymatic method (Boehringer Mannheim, Germany) [F. Bernini et al., *Atherosclerosis* 104, 19 (1993)] or by liquid scintillation counting (Lipoluma Lumac, Landgraf, The Netherlands) respectively. The results are given in Table 2.

TABLE 2 - Effect of lercanidipine and its enantiomers on cholesteryl ester hydrolysis in macrophages

	% of cholesteryl ester
AcLDL 50 μg/ml	31 ± 0.8
AcLDL 50 μg/ml + S-58035 1 μg/ml	15 ± 0.5
AcLDL 50 μg/ml + S-58035 1 μg/ml + $5x10^{-6}$ M LE	12 ± 1.2
AcLDL 50 μg/ml + S-58035 1 μg/ml + $5x10^{-6}$ M (S)-LE	14 ± 0.4
AcLDL 50 μg/ml + S-58035 1 μg/ml + $5x10^{-6}$ M (R)-LE	16 ± 2.1

Effects on LDL oxidation

Experimental reports suggest a key role for the oxidative modification of LDL in the early stages of atherosclerosis in humans. Available data suggest that LDL undergoes

oxidative modifications in vivo and that oxidatively modified LDL (Ox-LDL) may induce atherogenesis by a number of mechanisms, including its enhanced uptake in tissue macrophages (via the scavenger receptor pathway) which leads to lipid accumulation, and chemotactic activity for monocytes, and cytotoxicity to arterial wall endothelial cells.

Lercanidipine antioxidant capacity was evaluated by incubating LDL with 20 μM Cu^{++} in the presence or absence of different concentrations of the tested compound (0.01 μM - 50 μM). LDL oxidation was followed by monitoring conjugated diene formation at 234 nm. The experimental conditions were as follows. LDL (d = 1.019-1.063) were isolated from human pooled plasma by sequential ultracentrifugation at 4°C and 40,000 rpm in a 50Ti rotor, using a L5-50 ultracentrifuge (Beckman, Palo Alto, CA). LDL were then dialyzed against 0.15 M NaCl containing 0.01% ethylenediaminetetraacetic acid pH 7.4, sterilized by filtration through a 0.2 μM millipore filter and kept at 4°C under nitrogen in the dark until use (up to 3 weeks). Before use LDL were dialysed against ethylenediaminetetraacetic acid free phosphate buffered saline pH 7.4 on Sephadex G-25 columns (PD-10, Pharmacia Fine Chemicals, Uppsala, Sweden) and then LDL were filtered through a sterile 0.22 μM filter. LDL in phosphate buffered saline (50 μg lipoprotein protein/ml) were oxidized by incubation at 25°C with 20 μM $CuSO_4$ for 3 hours. The lercanidipine solution was prepared as a 10^{-2} M stock solution in methanol and added as ethanol solution (maximum 1% v/v) prior to addition of copper. The effect of lercanidipine on the oxidation of LDL was determined by continuous monitoring the conjugated diene formation by recording the increase in the absorbance at 234 nm at 5 min intervals during a three hours period, against a phosphate buffered saline blank, using a UV spectrophotometer (Beckman DU 640) with continuous reading with an automatic 6-cell changer. The lag time of the onset of oxidation was calculated as the intercept between the line of the maximum slope of the propagation phase and the baseline where absorbance was that at time 0. The results are shown in Table 3.

TABLE 3 - Effect of lercanidipine on the lag time of LDL oxidation

Lercanidipine μM	Lag time
0	46.5 ± 4.6
0.5	45.1 ± 3.7
1.0	49.8 ± 4.7
2.5	53.6 ± 3.3*
5.0	73.2 ± 4.8†
10.0	112.7 ± 5.2†

* $P < 0.05$; † $P < 0.01$

As Table 3 shows, 10 μM lercanidipine racemate doubled the lag-phase of LDL oxidation: the effect of the compound was concentration-dependent. The activity of the enantiomers was comparable to that of the racemate.

The antioxidant capacity of lercanidipine and its enantiomers was investigated also on cell-mediated oxidation. This was evaluated by incubating ethylenediaminetetraacetic acid free LDL under sterile conditions with 5 μM Cu^{++} (100 μg Apo B/ml) in the presence of J774 cells or alternatively with EAhy-926 cells. Oxidation was blocked after 22 hours incubation by adding butylated hydroxytoluene to the medium (final concentration 40 μM) dissolved in ethanol. The extent of lipid peroxidation was measured by determining the percentage inhibition of aldehydic breakdown products using the thiobarbituric acid assay [A.N. Hanna et al., *Biochem. Pharmacol.* 45, 753 (1993)]. Briefly, to 0.250 ml of the incubated samples, 0.750 ml of trichloroacetic acid (0.20% w/v) was added followed by 0.750 ml of thiobarbituric acid (0.67% w/v). The samples were heated at 100°C for 20 min, followed by cooling and centrifugation. Malondialdehyde equivalents were calculated using 1,1,3,3-tetramethoxy-propane as standard.

The results in Figure 5, obtained with ATCC TIB 67 J774A.1 cells, show that lercanidipine and its enantiomers were effective in reducing LDL oxidation. The results in Figure 6, obtained with EAhy 926 cells which share many of the properties of endothelial cells, show that lercanidipine reduced the oxidation of LDL in a range between 10 and 100 μM.

In the above experiments of cell-mediated oxidation the effects of lercanidipine were investigated after 22 hours incubation. In order to investigate the reason for the lower potency shown by lercanidipine in these conditions, the extent of lipid peroxidation in the presence of 30 μM lercanidipine was checked at different times by withdrawing samples of the incubation medium and measuring the oxidation compounds as above. The results are shown in Figure 7, and it can clearly be seen that 30 μM lercanidipine exerted a very high inhibition of lipid oxidation after 10 hours incubation. This result supports the view that lercanidipine at appropriate time could be as potent on cell-mediated LDL oxidation as it is on Cu^{++}-mediated oxidation.

Lercanidipine proved the most potent 1,4-dihydropyridine so far tested in these assays, its potency being one order of magnitude higher than that of lacidipine, the most potent in this experimental setting among the 1,4-dihydropyridine compounds previously

known.

Effects on blood pressure in hypertensive dogs

The antihypertensive effects of oral assumption of lercanidipine and its enantiomers were tested in renal hypertensive dogs.

Male beagle dogs weighing 12-13 kg, aged 1-3 years (Nossan Allevamenti, Italy) were used. Chronic sustained hypertension was induced by bilateral renal artery constriction, according to the Goldblatt method "two-kidney, two clip hypertension". Under barbiturate anaesthesia (35 mg/kg i.v.) during two different surgical interventions one month apart from each other, both renal arteries were clipped with original renal silver clips and narrowed by about 60-70%. After two months from the last intervention, an experimental renal hypertension was produced and the animals were suitable for the implantation of a catheter. Under sodium pentobarbital anaesthesia (35 mg/kg i.v.), in sterile conditions, the dogs were catheterized by inserting an indwelling cannula (PE 200 Clay Adams) into the ascending aorta through the right common carotid artery. The catheter was subcutaneously exteriorized at the back of the neck, filled with heparinized saline solution and daily flushed to prevent clotting. After a week to recover from surgery, the animals were connected to a HP 1290A pressure transducer connected to an HP 8805B carrier amplifier of a Hewlett Packard HP 7700 multichannel polygraph, in order to monitor the arterial blood pressure. Heart rate was manually computed from the pressure trace.

All animals were alternatively treated with placebo, lercanidipine and its (R)- and (S)-enantiomers. The drugs were administered by the oral route with a straight round aseptic tip catheter (Pores Serlat - France). The drugs were suspended in aqueous 0.5% Methocel A4C plus Antifoam M10 (10%). The administered volume was 1 ml/kg. The suspending medicine was used as placebo. During experimental performance, the arterial blood pressure was continuously recorded 30 minutes before (Basal Values) and up to 8 hours after drug administration.

Lercanidipine and (S)-lercanidipine induced a dose-related decrease in arterial blood pressure. The ED_{25} values (dose inducing 25% decrease in DBP at peak effect) were evaluated by linear regression analysis and are summarized in Table 4.

TABLE 4

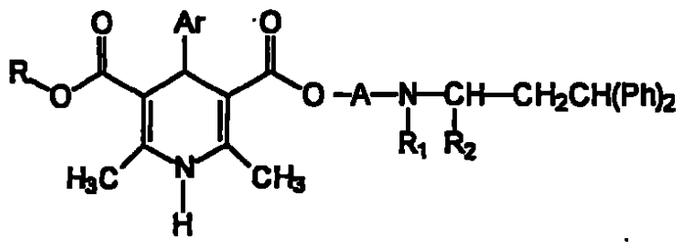
Compound	ED_{25} (mg/kg) 95% C.L.
Lercanidipine	0.9 (0.5 ÷ 1.6)
(S)-Lercanidipine	0.4 (0.3 ÷ 0.7)
(R)-Lercanidipine	>> 30

C.L. = Confidence limits

(S)-Lercanidipine exerted the most potent antihypertensive action, proving to be two fold more efficacious than the racemate, whereas the (R)-enantiomer did not affect blood pressure (less than 10% decrease in DBP) up to 30 mg/kg.

CLAIMS

1. Use of a compound of the general formula I



(I)

wherein

Ph represents a phenyl group,

Ar represents a 2-nitrophenyl, 3-nitrophenyl, 2,3-dichlorophenyl or benzofurazan-4-yl group,

A represents a branched chain alkylene group having from 3 to 6 carbon atoms,

R represents a straight or branched chain alkyl group having from 1 to 6 carbon atoms, optionally mono-substituted by an alkoxy group having from 1 to 6 carbon atoms,

R_1 represents a hydrogen atom, a hydroxy group or an alkyl group having from 1 to 4 carbon atoms, and

R_2 represents a hydrogen atom or a methyl group,

or of a salt, enantiomer, hydrate or solvate of such a compound, for the preparation of a medicament for the prevention, arrest or reversal of atherosclerotic degradation in the arterial walls of a patient.

2. Use of lercanidipine, (S)-lercanidipine or (R)-lercanidipine, or of a salt, hydrate or solvate of such a compound, for the preparation of a medicament for the prevention, arrest or reversal of atherosclerotic degradation in the arterial walls of a patient.

3. Use according to claim 1 or claim 2 for the preparation of a medicament which contains a pharmaceutically acceptable carrier.

4. Use according to any preceding claim for the preparation of a medicament in a form suitable for oral administration.

5. Use according to claim 4 for the preparation of a medicament which contains from 5% to 70% of the compound.

6. Use according to claim 4 or claim 5 for the preparation of a medicament which contains from 0.1 mg to 400 mg of the compound in single dose form.

7. Use according to claim 4 or claim 5 for the preparation of a medicament which contains from 1 mg to 200 mg of the compound in single dose form.

8. Use according to any of claims 1 to 3 for the preparation of a medicament in a form suitable for parenteral administration.

9. Use according to claim 8 for the preparation of a medicament which contains from 0.5% to 30% of the compound.

10. Use according to claim 8 or claim 9 for the preparation of a medicament which contains from 0.5 mg to 100 mg of the compound in single dose form.

Fig.1.

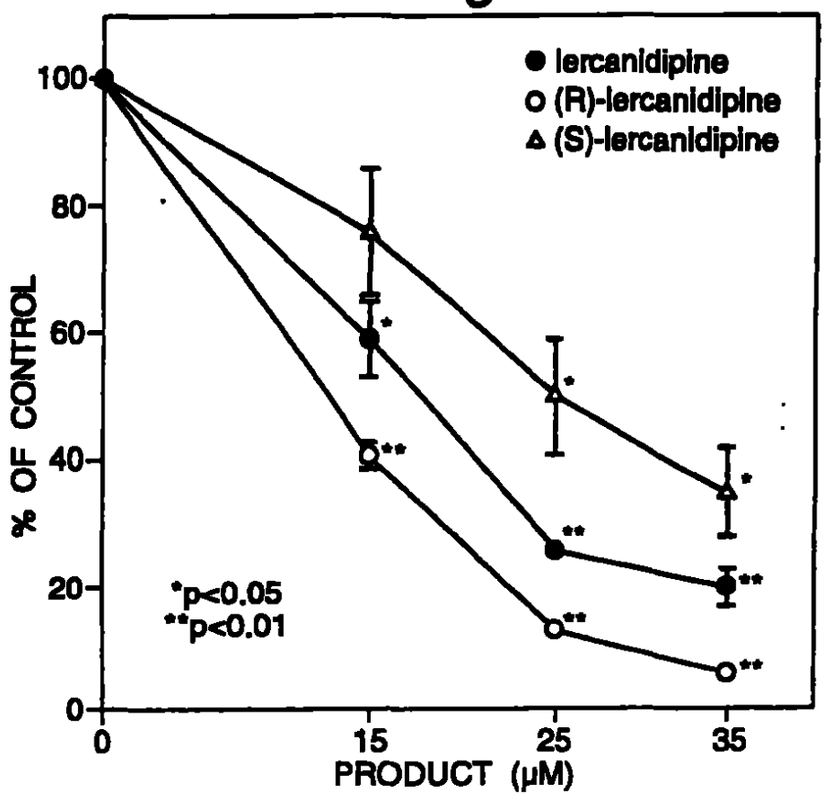

lercanidipine
(R)-lercanidipine
(S)-lercanidipine
% OF CONTROL
100
80
60
40
20
0
*p<0.05
**p<0.01
0
15
25
35
PRODUCT (μM)

Fig.2.

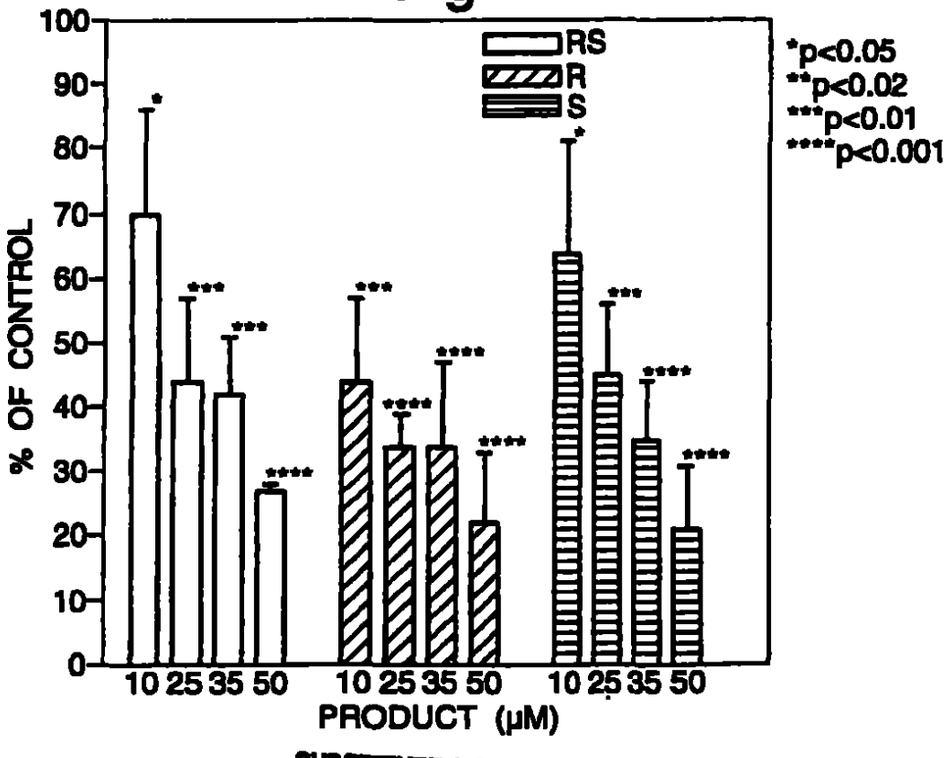

RS
R
S
*p<0.05
**p<0.02
***p<0.01
****p<0.001
% OF CONTROL
100
90
80
70
60
50
40
30
20
10
0
10 25 35 50
10 25 35 50
10 25 35 50
PRODUCT (μM)

Fig.3.

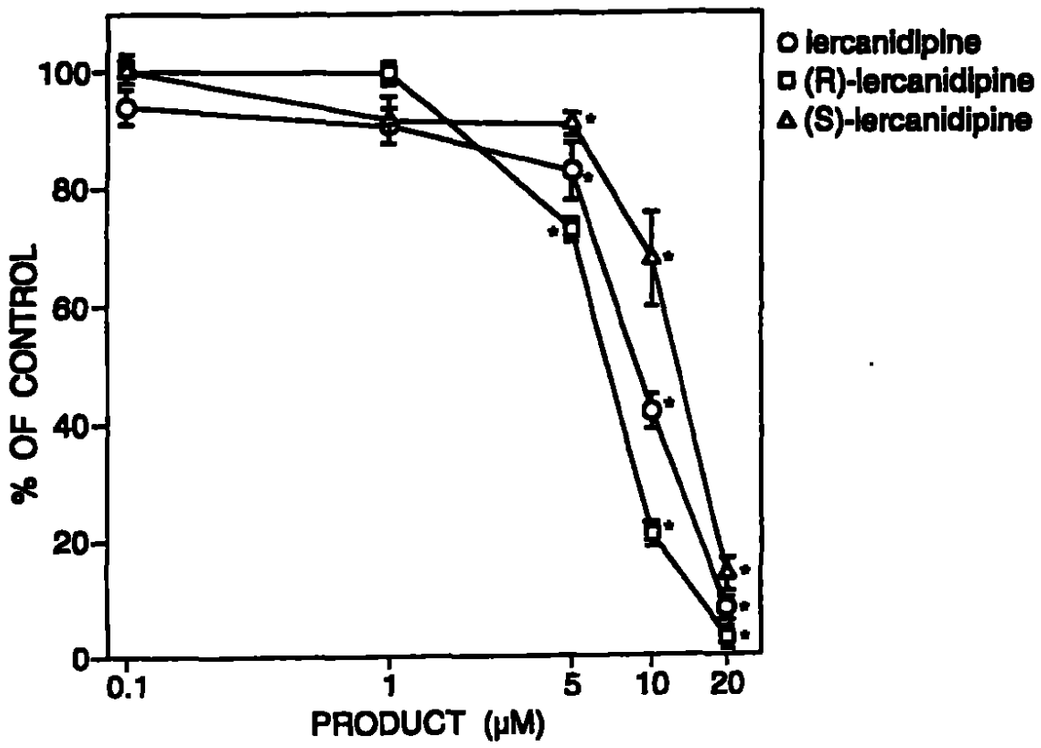

lercanidipine
(R)-lercanidipine
(S)-lercanidipine
% OF CONTROL
100
80
60
40
20
0
0.1
1
5
10
20
PRODUCT (μM)

Fig.4.

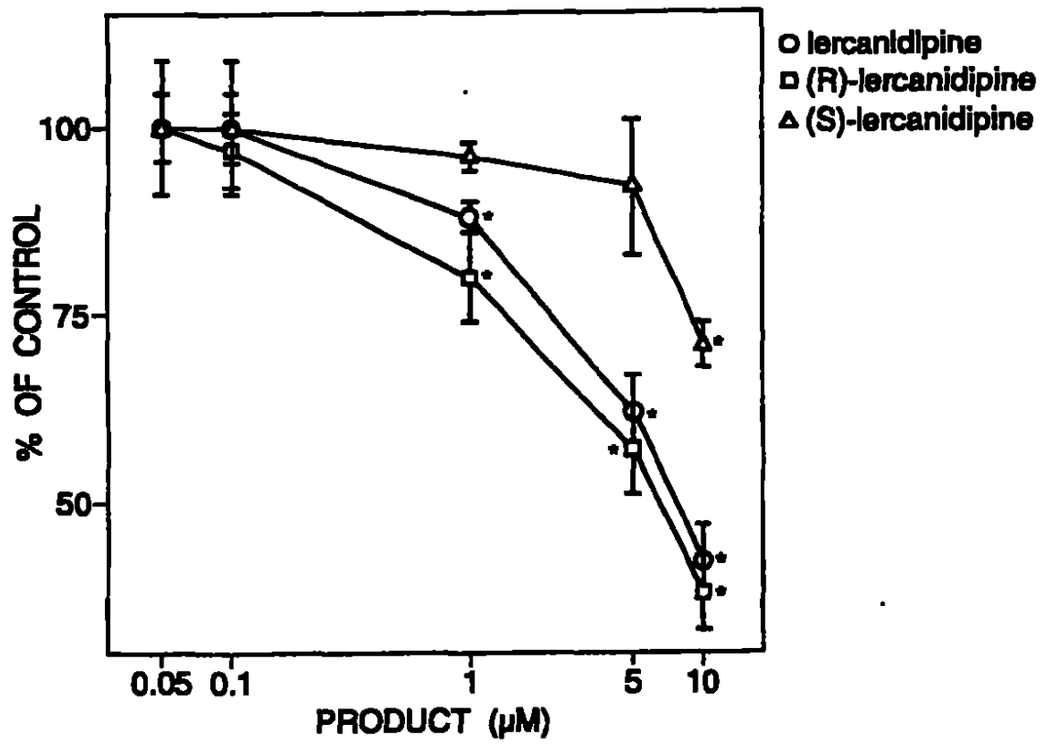

lercanidipine
(R)-lercanidipine
(S)-lercanidipine
% OF CONTROL
100
75
50
0.05
0.1
1
5
10
PRODUCT (μM)

Fig.5.

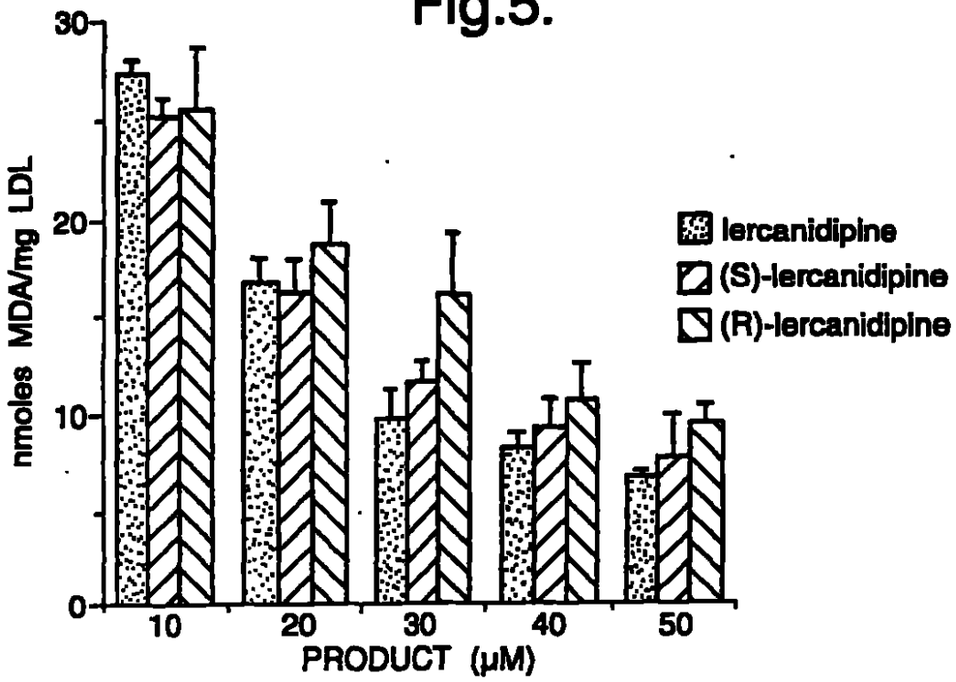

30
20
10
0
nmoles MDA/mg LDL
10
20
30
40
50
PRODUCT (μM)
lercanidipine
(S)-lercanidipine
(R)-lercanidipine

Fig.6.

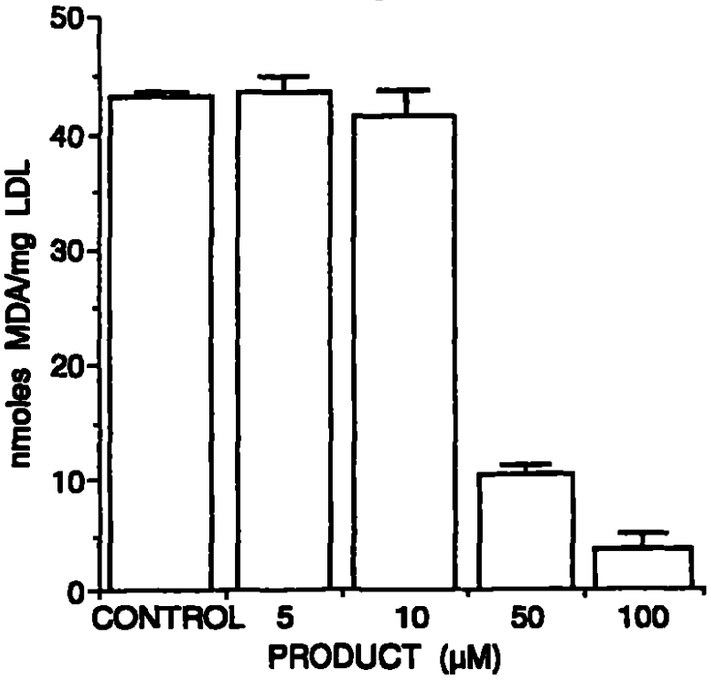
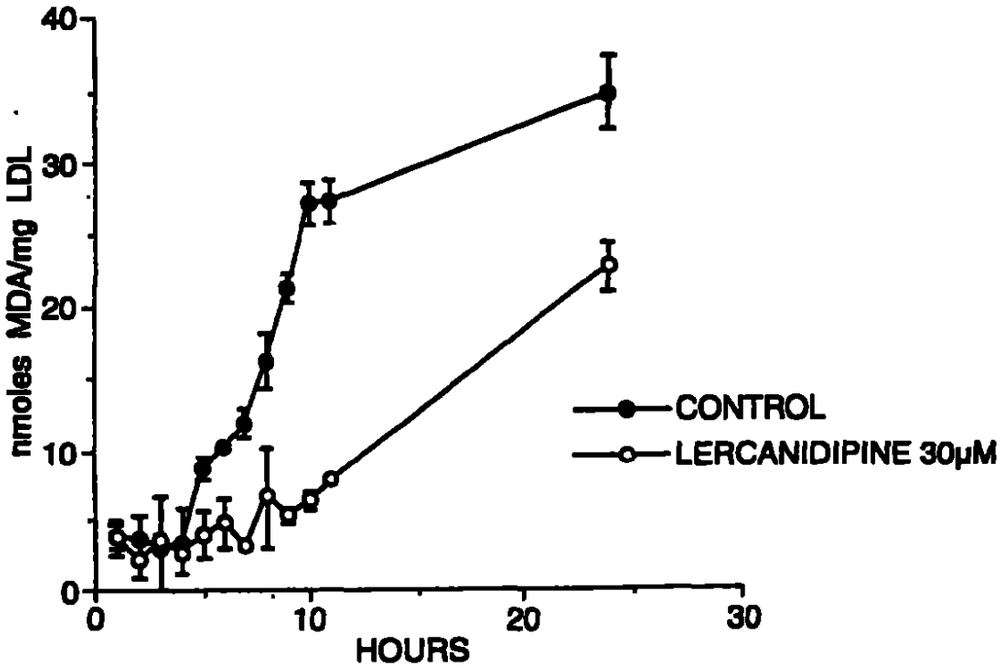

50
40
30
20
10
0
nmoles MDA/mg LDL
CONTROL
5
10
50
100
PRODUCT (μM)

Fig.7.


nmoles MDA/mg LDL
40
30
20
10
0
0
10
20
30
HOURS
CONTROL
LERCANIDIPINE 30μM

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/02872

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J.CARDIOVASC.PHARMACOL., vol. 19, no. SUPL.2, 1992, pages 8-16, XP000604014 PAULETTO P., ET AL.: "CALCIUM ANTAGONISTS AND VASCULAR SMOOTH MUSCLE CELLS IN ATHEROGENESIS" see abstract see page 11, right-hand column, paragraph 2 - page 13, right-hand column, paragraph 1 see page 14, left-hand column, line 3 - right-hand column, paragraph 1 see page 15, right-hand column, paragraph 4 --- -/--	1-10

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 October 1996	22/11/96

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/02872

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	J.CARDIOVASC.PHARMACOL., vol. 19, no. SUPL 3, 1992, pages 29-31, XP000604015 SINZINGER H., ET AL.: "ANTIATHEROSCLEROTIC ACTIONS OF ISRADIPINE" see the whole document ---	1-10
Y	CARDIOVASC.DRUGS THER., vol. 4, no. SUPL.5, August 1990, pages 1027-1031, XP000604409 KNORR A., ET AL.: "INFLUENCE OF NIFEDIPINE ON EXPERIMENTAL ARTERIOSCLEROSIS" see abstract see page 1030, left-hand column, paragraph 1 ---	1-10
Y	J.HUMAN HYPERTENS., vol. 6, no. SUPL. 1, December 1992, pages 13-18, XP000604086 FLECKENSTEIN-GRÜN G., ET AL.: "PROTECTIVE EFFECTS OF VARIOUS CALCIUM ANTAGONISTS AGAINST EXPERIMENTAL ARTERIOSCLEROSIS" see abstract see page 17, right-hand column, paragraph 3 - page 18, left-hand column, paragraph 1 ---	1-10
Y	ACTA ANAESTHESIOL. SCAND., vol. 37, no. SUPL. 99, 1993, pages 33-37, XP000604080 LESLIE J.B.: "HAEMODYNAMICS AND TISSUE SPECIFITY WITH ISRADIPINE" see abstract see page 35, left-hand column, paragraph 6 - right-hand column, paragraph 2 ---	1-10
Y	J.CARDIOVASC. PHARMACOL., vol. 24, no. SUPL. 2, 1994, pages 85-91, XP000603993 BORCHARD U.: "CALCIUM ANTAGONISTS IN COMPARISON: VIEW OF THE PHARMACOLOGIST" see page 89, left-hand column, paragraph 1 ---	1-10
Y	DRUGS, vol. 48, no. SUPL. 1, 1994, pages 1-7, XP000603980 PAULETTO P., ET AL.: "IMPACT OF NIFEDIPINE ON VASCULAR SMOOTH MUSCLE CELL DIFFERENTIATION" see abstract see page 5, left-hand column, last paragraph - page 6, right-hand column, last paragraph --- -/--	1-10

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/02872

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PHARMACOL. RES., vol. 31, no. 314, March 1995 - April 1995, pages 251-254, XP000604033 GAVIRAGHI G., ET AL.: "RECENT DEVELOPMENTS IN THE USE OF CALCIUM ANTAGONISTS IN MYOCARDIAL PROTECTION" see abstract see page 253, right-hand column, paragraph 3 ---	1-10
Y	AM. J. HYPERTENS., vol. 7, no. 10(2), October 1994, pages 126-130, XP000604034 NAYLER W.: "NEW THERAPEUTIC TRENDS IN CALCIUM ANTAGONISM. ARE THEY MEANINGFUL?" see abstract see page 129, left-hand column, paragraph 2 - last paragraph ---	1-10
Y	J. CARDIOVASC. PHARMACOL., vol. 12, no. SUPL. 6, 1988, pages 29-35, XP000604025 WEINSTEIN D.B.: "THE ANTIATHEROGENIC POTENTIAL OF CALCIUM ANTAGONISTS" see abstract see page 31, left-hand column, paragraph 3 - page 34, left-hand column, paragraph 2 ---	1-10
Y	CLIN. INVEST. MED., vol. 10, no. 6, November 1987, pages 681-685, XP000604021 HENRY P.D.: "ANTI-ATHEROSCLEROTIC EFFECTS OF CALCIUM ANTAGONISTS. A BRIEF REVIEW" see abstract see page 682, left-hand column, paragraph 3 - right-hand column, paragraph 1 see page 683, left-hand column, paragraph 3 - right-hand column, paragraph 6 ---	1-10
Y	AM. J. MED., vol. 86, no. SUPL. 4(A), 17 April 1989, pages 27-32, XP000604027 WEINSTEIN D.B., ET AL.: "ANTIATHEROGENIC PROPERTIES OF CALCIUM ANTAGONISTS" see abstract see page 28, right-hand column, last paragraph - page 29, right-hand column, paragraph 1 -----	1-10

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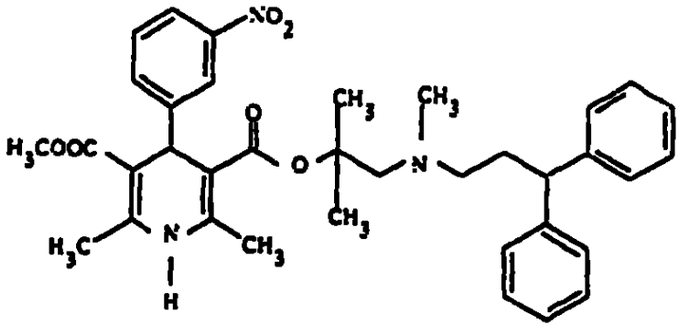
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(54) Title: A PROCESS FOR THE PREPARATION OF LERCANIDIPINE HYDROCHLORIDE

(57) Abstract

A process for the preparation of methyl 1,1,N-trimethyl-N-(3,3-diphenyl-propyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (lercanidipine) of formula I comprises reacting an acid halide of 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid with 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol in an aprotic solvent. The product can be isolated by industrially applicable crystallization techniques and is obtained in high yield as its anhydrous hydrochloride, a form which possesses high stability and low hygroscopicity.



(I)

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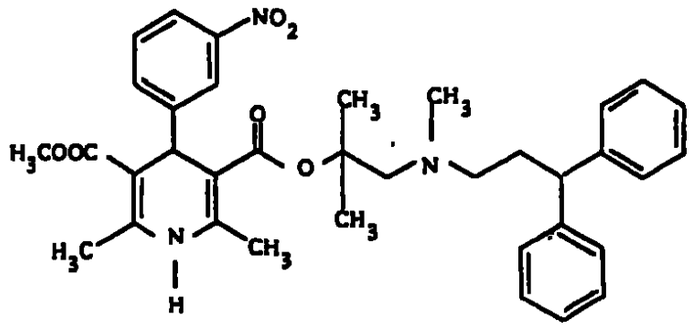
TITLE

A Process for the Preparation of Lercanidipine Hydrochloride

DESCRIPTION

The invention relates to a process for the preparation of lercanidipine hydrochloride.

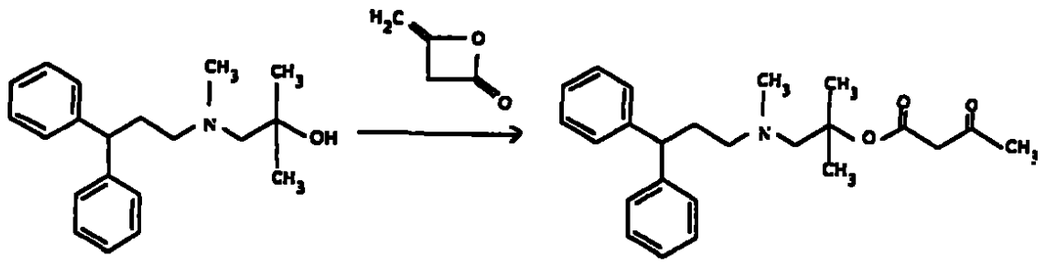
Lercanidipine is methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate. It has the formula I:



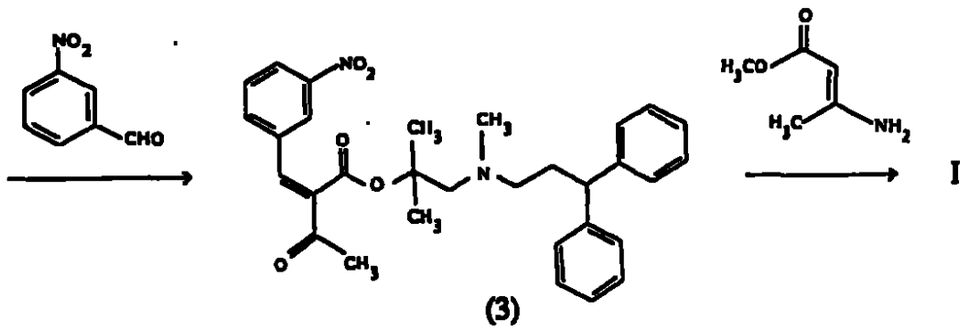
I

Lercanidipine was disclosed in United States Patent No 4705797. It is an antagonist of type-L calcium channels, and has been found to be very active as an antihypertensive and as an agent for the treatment of angina and coronary disease.

The preparation of lercanidipine, as described in the aforesaid US Patent, follows the route shown in the following reaction scheme:



(1) (2)



(3) I

According to this scheme, the aminoalcohol (1) is esterified with diketene to form the corresponding acetoacetate (2), which is then coupled with 3-nitrobenzaldehyde to give 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl α-acetyl-3-nitrocinnamate (3). This is cyclised with methyl 3-aminocrotonate in refluxing isopropanol.

This process has a number of disadvantages. By its very nature, the Hantzsch cyclisation used in the final step gives rise to several by-products. Not only does this reduce the yield of the desired product, but the removal of the by-products requires the use of purification techniques, such as column chromatography, which are difficult to apply on an industrial scale. Indeed, the yield for the final step is 35%, and the overall process yield is 23%.

The product obtained by this process is lercanidipine hydrochloride hemihydrate, melting at 119 to 123°C. This product is somewhat hygroscopic, which can lead to inconstancy of composition and difficulties in handling during the preparation of pharmaceutical formulations. Moreover, the stability of the lercanidipine hydrochloride hemihydrate is not entirely satisfactory.

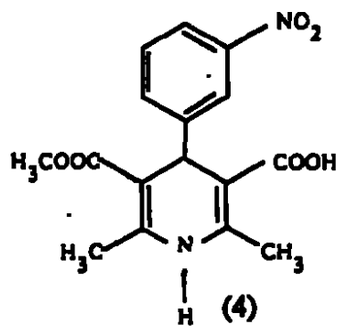
The invention provides a process for the preparation of lercanidipine hydrochloride, the process comprising:

a) halogenating 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid with a halogenating agent in an aprotic solvent;

b) adding 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol dissolved in an aprotic solvent to the resultant acid halide; and

c) isolating the resultant lercanidipine as its anhydrous hydrochloride.

The process of the invention is illustrated by the following reaction scheme.



+ (1) → I

Preferably, the halogenation is a chlorination. It may be conducted in chlorinated or non-chlorinated aprotic solvents, e.g. chloroform, dichloromethane, dichloroethane, chlorobenzene, 1,1,1-trichloroethane, ethyl acetate, methyl acetate, tetrahydrofuran, dioxane, dimethylformamide, dimethyl carbonate or any mixture thereof, using known chlorinating agents, e.g. thionyl chloride, phosphorus pentachloride, phosphorus trichloride, phosphorus oxychloride, oxalyl chloride or other commercial chlorinating agents, operating at a temperature ranging from -15°C to +40°C, optionally in an inert gas atmosphere such as nitrogen or argon. The duration of the chlorination reaction may be from 15 minutes to 3 hours.

The aminoalcohol (1) dissolved in one of the above solvents or, alternatively, in another aprotic solvent, e.g. toluene, xylene or an alkane or cycloalkane having from 5 to 7 carbon atoms, is then added while keeping the reaction temperature in the -15°C to +40°C range and the reaction is allowed to continue until completed. Completion of the reaction may be determined by testing a sample from the reaction mixture by appropriate analytical techniques such as thin layer chromatography or high performance liquid chromatography.

The process of the invention is an esterification of the corresponding dihydropyridine acid. Since the acid halide does not need to be isolated, it is in effect a one-step process. Compared to the prior art process, described above, fewer reaction by-products are formed. As a result, an improved yield is obtained and this is accompanied by simpler purification and isolation of the lercanidipine. This may be conducted by conventional methods based, for instance, on extraction with solvents from basified solutions, re-salification with hydrochloric acid and re-crystallisation. Thus the use of chromatographic columns to isolate the desired final product can be

avoided. As column chromatography always requires the use of high amounts of organic eluants, its avoidance clearly contributes to the industrial applicability of the process in terms of improved product quality, lower manufacturing costs and easier ecological disposal of process waste.

The lercanidipine hydrochloride prepared according to the present invention is in an anhydrous crystalline form and melts within two degrees Celsius in the 185 to 190°C range after re-crystallization of the crude hydrochloride first from aprotic solvents, such as ethyl acetate, methyl acetate or acetone, and then from protic solvents, such as one or more of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol and t-butanol, optionally in admixture with other solvents, including water. It has furthermore been found to be more stable and less hygroscopic than the lercanidipine hydrochloride hemihydrate obtained according to the prior process. These properties make it more suitable for pharmaceutical use, since they facilitate simpler large-scale manufacture of solid pharmaceutical preparations.

The following Examples illustrate the invention.

EXAMPLE 1
Lercanidipine hydrochloride

45.8 g (0.385 mol) of thionyl chloride were added dropwise over a period of about 15 minutes to a stirred mixture of 116.2 g (0.35 mol) of 2,6-dimethyl-5-methoxycarbonyl--4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid (4), prepared as described in German Patent 2847237, 645 ml of anhydrous dichloromethane and 160 ml of anhydrous dimethylformamide kept at a temperature of -4 to +1°C under nitrogen. The mixture was stirred for 1 hour within the same temperature range. A solution of 104.1 g (0.35 mol) of 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol (1), prepared as described in US Patent 4705797, in 105 ml of anhydrous dichloromethane was then added dropwise over a period of about 15 minutes at -10 to 0°C. After stirring for 3 hours at 0°C and standing for 18 to 20 hours at ambient temperature, the solvent was evaporated off *in vacuo* and the residue was dissolved in 3500 ml of ethyl acetate. The organic solution was washed sequentially with a saturated NaCl solution (700 ml), 10% Na_2CO_3 (700 ml x 5), saturated NaCl solution (700 ml), 1N HCl (700 ml x 5) and saturated NaCl solution (700 ml). The organic layer was separated off, dried over anhydrous Na_2SO_4 for 30 minutes, filtered, treated with 23 g of carbon and re-filtered. The resulting solution was then concentrated in vacuo to a volume of about

one litre and seeded with lercanidipine hydrochloride crystals. After standing for one day at 0 to 5°C, the solid was filtered and re-crystallized from absolute ethanol to give 179.5 g (78% of theory) of lercanidipine hydrochloride with a melting point of 186-188°C.

EXAMPLE 2

Stability at 100°C in light

Samples of anhydrous lercanidipine hydrochloride, prepared as described in Example 1, and lercanidipine hydrochloride hemihydrate, prepared as described in US Patent No 4705797, were heated at 100°C for 48 hours. The samples were checked at 0, 24 and 48 hours by HPLC analysis using the following conditions:

Column:	m-Bondapak C-18 (Waters), particle size 10 mm, 300 x 3.9 mm internal diameter.
Eluant:	CH_3CN (61%) : 0.15 M $NaClO_4$ aqueous solution brought to pH 3 by adding $HClO_4$ (39%), (v/v)
Elution:	isocratic
Flow:	1.5 ml/min
Temperature:	25°C
Detector:	UV (240nm)
Attenuation:	0.05 AUFS

Under these conditions, the retention time of lercanidipine HCl was about 7 minutes. The results are reported in Table I.

TABLE I		HPLC purity (%)		
		Initial	24 Hours	48 hours
100°C light	anhydrous	99.74	99.36	99.01
	hemihydrate	99.85	92.35	90.96

It is clear that the anhydrous form of lercanidipine hydrochloride is remarkably more stable than the hemihydrate.

EXAMPLE 3

Stability at 40°C and at 60°C under 75% Relative Humidity in the dark

Samples of the two different forms of lercanidipine hydrochloride, as identified in Example 2, were placed in open polyethylene bags inserted in open glass flasks kept at 60°C under 75% relative humidity. The samples were checked for hygroscopicity, determining water content by the Karl Fisher (K.F.) method at 0, 8 and 15 days. The experiment was repeated at 40°C under 75% relative humidity. The results are reported in Table II.

TABLE II		Water Content (%) - K.F.		
		Initial	8 Days	15 Days
60°C dark	anhydrous	0.28	0.85	0.77
	hemihydrate	1.42	4.00	4.04
40°C dark	anhydrous	0.28	0.30	0.32
	hemihydrate	1.42	3.14	3.05

The anhydrous form of lercanidipine hydrochloride is clearly less hygroscopic than the hemihydrate.

CLAIMS

1. A process for the preparation of methyl 1,1,N-trimethyl--N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)--pyridine-3,5-dicarboxylate (lercanidipine) hydrochloride, the process comprising:

a) halogenating 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid with a halogenating agent in an aprotic solvent;

b) adding 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol dissolved in an aprotic solvent to the resultant acid halide; and

c) isolating the resultant lercanidipine as its anhydrous hydrochloride.

2. A process according to claim 1 wherein the halogenating agent is thionyl chloride, phosphorus pentachloride, phosphorus trichloride, phosphorus oxychloride or oxalyl chloride.

3. A process according to claim 1 or claim 2 wherein the aprotic solvent in which the halogenation is effected is chloroform, dichloromethane, dichloroethane, chlorobenzene, 1,1,1-trichloroethane, ethyl acetate, methyl acetate, tetrahydrofuran, dioxane, dimethylformamide, dimethylcarbonate or a mixture of two or more thereof.

4. A process according to any preceding claim wherein the aprotic solvent used to dissolve the 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol is chloroform, dichloromethane, dichloroethane, chlorobenzene, 1,1,1-trichloro-ethane, ethyl acetate, methyl acetate, tetrahydrofuran, dioxane, dimethylformamide, dimethylcarbonate, toluene, xylene, an alkane having from 5 to 7 carbon atoms, a cycloalkane having from 5 to 7 carbon atoms or a mixture of two or more thereof.

5. A process according to any preceding claim wherein the lercanidipine is isolated by crystallization.

6. A process according to claim 5 wherein the crystallization is carried out in two successive steps, alternating aprotic and protic solvents.

7. A process according to claim 6 wherein the aprotic crystallization solvent is ethyl acetate, methyl acetate or acetone.

8. A process according to claim 6 or claim 7 wherein the protic crystallization solvent is one or more of methanol, ethanol, n-propanol, i-propanol, n-butanol, s-butanol and t-butanol, optionally in admixture with water.

9. Anhydrous methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate hydrochloride prepared by a process according to any preceding claim, and having a two-degree melting point within the 185 to 190°C range.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 96/02122

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IPC 6 C07D211/98

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DRUGS OF THE FUTURE, vol. 20, no. 12, 1995, pages 1284-1285, XP002010272 RECORDATI ET AL.: "Lercanidipine hydrochloride" see the whole document ---	1-9
X	EP,A,0 153 016 (RECORDATI S.A.) 28 August 1985 see page 6, line 5 - line 14 see page 5 see example 16 & US,A,4 705 797 (NARDI DANTE ET. AL.) 10 November 1987 cited in the application -----	1-9

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Information on patent family members

Inter nal Application No
PCT/EP 96/02122

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-153016	28-08-85	AR-A-	240804	28-02-91
		AU-B-	570534	17-03-88
		AU-B-	3868985	12-09-85
		CA-A-	1277666	11-12-90
		JP-C-	1811001	27-12-93
		JP-B-	5017908	10-03-93
		JP-A-	60199874	09-10-85
		US-A-	4772621	20-09-88
		US-A-	4968832	06-11-90
		US-A-	4705797	10-11-87

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Drugs 2000 Nov;60 (5): 1123-1140
0012-6667/00/0011-1123/$20.00/0

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Lercanidipine

A Review of its Use in Hypertension

P.D. 11-2000
P. 1123-1140 (18)

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Data Selection
Sources: Medical literature published in any language since 1993 on lercanidipine, identified using Adisbase (a proprietary database of Adis International, Auckland, New Zealand) and Medline. Additional references were identified from the reference lists of published articles. Bibliographical information, including confidential unpublished data, was also requested from the company developing the drug.
Search strategy: Adisbase search terms were 'lercanidipine' or 'mesnidipine' or 'R-75' or 'REC-15/2575'. Medline search terms were 'lercanidipine' or 'REC 15 2575'. Searches were last updated 9 Oct 2000.
Selection: Studies in patients with hypertension who received lercanidipine. Inclusion of studies was based mainly on the methods section of the trials. When available, large, well controlled trials with appropriate statistical methodology were preferred. Relevant pharmacodynamic and pharmacokinetic data are also included.
Index terms: pharmacodynamics, pharmacokinetics, therapeutic use.

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Summary

Abstract

Lercanidipine is a vasoselective dihydropyridine calcium antagonist which causes systemic vasodilation by blocking the influx of calcium ions through L-type calcium channels in cell membranes. It is a highly lipophilic drug and as such has a slower onset and longer duration of action than a number of other calcium antagonists. Preclinical evidence suggests that lercanidipine has antiatherogenic potential and it may also protect against end-organ damage.

In well controlled clinical studies, once daily administration of lercanidipine 10 or 20mg effectively reduced blood pressure (BP) compared with placebo in patients with mild to moderate hypertension without affecting heart rate. Response rate (percentage of patients with diastolic BP ≤90mm Hg or reduced by ≥10mm Hg from baseline) ranged from 50 to 66% with lercanidipine 10 mg/day and up to 86% with lercanidipine 20 mg/day. The drug had a long duration of action: clinical measurements for diastolic BP yielded a trough/peak ratio of >0.8 for both lercanidipine dosages in 1 study. Comparative trials, either published in full or as abstracts, found lercanidipine 10mg once daily for ≥4 weeks to be at least as effective as atenolol 50mg once daily, candesartan cilexetil 16 mg/day, captopril 25mg twice daily, enalapril 20 mg/day, hydrochlorothiazide 12.5mg once daily, irbesartan 150 mg/day and slow release nifedipine 20mg twice daily in patients with mild to moderate hypertension. In addition, lercanidipine 20 mg/day was as effective as amlodipine 10 mg/day.

Lercanidipine is effective in the treatment of elderly patients (aged 60 to 85 years) with mild to moderate essential hypertension and in those with isolated systolic hypertension. In addition, monotherapy with lercanidipine 20 or 40 mg/day has shown efficacy in patients with severe hypertension, and add-on therapy helped control BP in a large proportion of patients with severe hypertension not responding sufficiently to β-blockers, diuretics or ACE inhibitors. Unpublished data indicate that the drug reduces blood pressure in patients with type 2 (non-insulin-dependent) diabetes mellitus, without adversely affecting glucose homeostasis.

Lercanidipine was well tolerated in clinical trials, with most treatment-related adverse events typical of dihydropyridine calcium antagonists, namely headache, flushing, dizziness and ankle oedema.

Conclusions: Lercanidipine is an effective and well tolerated once daily antihypertensive agent in patients with mild to moderate hypertension. In addition, the drug may reduce BP when used as monotherapy in patients with severe hypertension or when used adjunctively in patients with resistant hypertension. Importantly, lercanidipine appears to be at least as effective and well tolerated as other commonly used antihypertensive agents. The drug therefore represents a useful therapeutic option in the management of patients with hypertension and

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will be particularly useful in patients not responding to, or intolerant of, antihypertensive agents from other drug classes.

Pharmacodynamic Properties

Lercanidipine is a third generation dihydropyridine calcium antagonist with a bulky *bis*-phenylalkylamine side chain. The drug reversibly blocks voltage-dependent Ca^{2+} influx through L-type channels in cell membranes, and the subsequent peripheral vasodilation leads to a reduction in blood pressure (BP).

Lercanidipine is highly lipophilic, and as such has a slower onset and longer duration of action than a number of other drugs in its class. Furthermore, the drug is vasoselective and has shown less *in vitro* and *in vivo* negative inotropic activity than some other dihydropyridines, including nitrendipine and felodipine.

Results from preclinical studies indicate that lercanidipine has antiatherogenic potential independent of its BP-lowering effects, and may protect against end-organ damage. Importantly, the drug does not interfere with normal cardiac excitation and conduction when used at therapeutic dosages in patients with hypertension.

Pharmacokinetic Properties

Maximum plasma concentrations (C_{max}) of *S*-lercanidipine, the enantiomer responsible for the activity of racemic lercanidipine were reached ≈2 to 3 hours after oral administration of lercanidipine 10 or 20mg in 12 patients with mild to moderate hypertension. Plasma levels and the area under the plasma concentration-time curve (AUC) were not linearly related to dose, indicating saturable metabolism.

The presence of food increases lercanidipine absorption, hence the recommendation that lercanidipine be taken before meals.

Once absorbed, the drug readily accumulates in the lipid bilayer of cell membranes in the arterial wall because of its bulky *bis*-phenyl side chain. The drug is highly (>98%) bound to plasma proteins.

After oral administration lercanidipine undergoes extensive first-pass metabolism (primarily by cytochrome P450 3A4) to largely inactive metabolites. ≈50% of an oral dose of [^{14}C]lercanidipine was eliminated in faeces and 44% in urine (mostly as metabolites). The drug appears to have a biphasic elimination profile, with terminal elimination half-lives of 2.8 to 3.7 hours (first phase) and 10.5 hours.

The bioavailability of *S*-lercanidipine after oral lercanidipine is not affected by age or cirrhosis but accumulation has been reported in patients with severe renal dysfunction. Dosage reduction is therefore recommended in this patient group.

No significant pharmacokinetic interactions have been reported between lercanidipine and midazolam, metoprolol, cimetidine or metildigoxin.

Therapeutic Efficacy

Lercanidipine 5 to 20mg once daily for 4 weeks dose-dependently and significantly reduced trough diastolic blood pressure (DBP) compared with placebo in patients with mild to moderate hypertension, without affecting heart rate. Response rates (percentage of patients with DBP ≤90mm Hg or reduced from baseline by ≥10mm Hg) ranged from 50 to 66% with lercanidipine 10 mg/day and up to 86% with lercanidipine 20 mg/day. The effects of lercanidipine 10 and 20mg were sustained throughout the 24-hour dosage interval; 1 study found that both dosages yielded a DBP trough/peak ratio >0.8.

Published studies (or abstracts) have shown that lercanidipine 10mg once daily for ≥4 weeks is at least as effective in patients with mild to moderate essential hypertension as a number of other antihypertensive drugs, including atenolol 50mg once daily, candesartan cilexetil 16 mg/day, captopril 25mg twice daily, enalapril 20 mg/day, hydrochlorothiazide 12.5mg once daily, irbesartan 150 mg/day

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and slow release nifedipine 20mg twice daily. A higher dosage (lercanidipine 20 mg/day) had antihypertensive efficacy similar to that of amlodipine 10 mg/day. Studies of lercanidipine 10mg once daily which permitted dosage titration to lercanidipine 20mg once daily after 4 weeks in nonresponders found that normalisation rates were higher at week 8 than week 4.

Monotherapy with lercanidipine 20 or 40 mg/day effectively reduced BP in the majority of patients with severe hypertension in 1 study. Furthermore, lercanidipine 10 to 30 mg/day was effective add-on therapy in patients with hypertension not responding sufficiently to β-blockers, diuretics or ACE inhibitors.

Lercanidipine is effective in the treatment of mild to moderate essential hypertension or isolated systolic hypertension in elderly patients (aged 60 to 85 years). The drug also demonstrated antihypertensive efficacy in patients with type 2 (non–insulin-dependent) diabetes mellitus but these data have yet to be published.

Tolerability

Lercanidipine was well tolerated in clinical trials, with most treatment-emergent adverse events related to vasodilation. A pooled analysis of data from 20 clinical trials involving almost 1800 patients with hypertension found that 11.8% of lercanidipine 10 or 20mg once daily recipients compared with 7.0% of placebo recipients reported adverse events, the most common being headache, flushing, asthenia, vertigo, palpitations and ankle oedema. Approximately 5 and 3% of patients in the respective groups withdrew because of poor tolerability.

Two postmarketing surveillance studies involving a total of 9605 patients with hypertension have confirmed that lercanidipine is well tolerated in clinical practice.

Evidence suggests that the drug is well tolerated in the elderly, and when administered long term (1 year). In addition, the drug was well tolerated with regard to ECG parameters in clinical trials.

Comparative trials have shown that lercanidipine 10 or 20 mg/day is as well tolerated as captopril 50 to 100 mg/day and atenolol 50 or 100 mg/day, although the tolerability profiles of these agents differed slightly.

An abstract report of a study in patients who had reported adverse vasodilatory effects while taking amlodipine, nifedipine GITS (gastrointestinal therapeutic system), felodipine or nitrendipine showed that, after switching to lercanidipine 10 to 20 mg/day for 1 month, the prevalence of calf oedema (from 94.8 to 51.4%), headache (from 38 to 18%), flushing (from 35 to 17%) and skin rash (from 38 to 18%) decreased significantly.

Dosage and Administration

Oral lercanidipine is indicated in Europe for the treatment of mild to moderate hypertension for which the recommended initial dosage is 10mg once daily at least 15 minutes before meals. In patients with an initial unsatisfactory response, dosage titration to 20 mg/day should be done gradually.

Special care should be exercised when initiating treatment with lercanidipine in the elderly or patients with mild to moderate renal or hepatic dysfunction, although dosage adjustments are not required in such patients. Use of lercanidipine is contraindicated in patients with severe hepatic or renal (creatinine clearance <10 ml/min).

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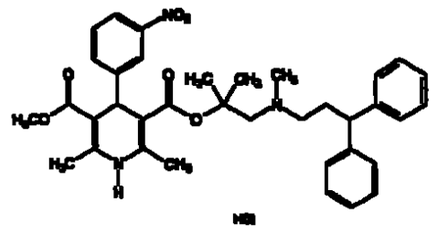


Fig. 1. Lercanidipine.

1. Introduction

Since their introduction, calcium antagonists have been widely used for the treatment of cardiovascular disease, particularly angina pectoris, arrhythmias and hypertension.[1] Their beneficial effects are related to systemic vasodilation, caused by inhibition of the inward-flow of calcium ions through the L-type calcium channel.[2] Three main types of calcium antagonists, classified according to chemical structure, are used in the treatment of hypertension: dihydropyridines, benzothiazepines and phenylalkylamines.[3] Lercanidipine (fig. 1) is a third generation dihydropyridine calcium antagonist with a bulky *bis*-phenylalkylamine side chain which makes it more lipophilic than most other drugs in its class, including amlodipine, nitrendipine, isradipine and nimodipine (but similar to lacidipine).[4] This review evaluates the pharmacological properties of lercanidipine and its efficacy and tolerability in the treatment of patients with hypertension.

2. Pharmacodynamic Profile

Lercanidipine is a racemic mixture of *R*- and *S*-lercanidipine. An *in vitro* binding displacement study showed that the *S*-enantiomer of lercanidipine had 100- to 200-fold greater affinity than the *R*-enantiomer for the L-type calcium channel.[5] Consistent with this finding, the pharmacodynamic effects of lercanidipine are primarily produced by the *S*-enantiomer.[6,7] The main pharmacodynamic properties of the drug are listed in table I.

2.1 Receptor-Binding and *In Vitro* Properties

Lercanidipine reversibly blocks voltage-dependent Ca^{2+} influx through L-type channels in the cell membrane.[5,8,9] *In vitro* studies in isolated rat aortic strips[5,9] have shown that, consistent with its high lipophilicity (and hence ready accumulation in and slow diffusion from cell membranes; section 3.2), the drug has a slower onset and longer duration of action than most other drugs in its class (table II). Similar effects were observed in K^+-contracted isolated rabbit aorta: lercanidipine had an onset of relaxant activity comparable to that of amlodipine and lacidipine but slower than that of nitrendipine and felodipine.[10]

The drug has only weak *in vitro* affinity for sodium binding sites, poor affinity for α_1-adrenoceptors, dopamine D_2 receptors and serotonin 5-HT_2 receptors, and negligible affinity for α_2- and β-adrenoceptors, muscarinic receptors and serotonin 5-HT_{1a} receptors.[9] Furthermore, lercanidipine does not appear to affect cardiac potassium currents in human ventricular myocytes.[20]

2.2 Haemodynamic Effects

Preclinical evidence[6,7] has shown that the blood pressure (BP)-lowering effects of lercanidipine occur via peripheral vasodilation. In anaesthetised open-chest dogs, the hypotensive effects of intravenous lercanidipine 3 to 30 μg/kg were associated with a reduction in total peripheral and femoral vascular resistance but no significant change in heart rate (HR).[6] Peak increases in femoral

Table I. Pharmacodynamic properties of lercanidipine

Reversibly blocks voltage-dependent Ca^{2+} influx through L-type channels in cell membranes[5,8,9]
Slow onset and long duration of *in vitro* activity compared with nifedipine, nitrendipine and felodipine[5,8,10]
Reduces blood pressure via peripheral vasodilation[6,7]
More vasoselective than amlodipine, felodipine, nitrendipine and lacidipine in isolated rabbit tissue[10]
Weaker negative inotropic activity than felodipine,[11] nitrendipine[11] and nifedipine[11,12]
Antiatherogenic potential independent of blood pressure-lowering activity[13,14]
Possible end-organ protection[15-19]

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Table II. Functional calcium antagonist activity of lercanidipine and various reference compounds in rat aortic strips[6,9]

Agent	IC_{50} ($\times 10^{-9}$ mol/L)[a]		Washout period[b]
	0.5h	1.5h	(h)
Lercanidipine	7.0	≤1.8	>8
Nifedipine	8.7	4.8	
Nitrendipine	1.8	1.3	2
Amlodipine	48	28	1-8

a Concentration producing 50% inhibition of 80 mmol/L KCl-induced contractions after 0.5 and 1.5 hours of incubation.

b Approximate washout period required for recovery of tissue after 0.5h incubation with lercanidipine 1×10^{-9} mol/L, nitrendipine 3×10^{-9} mol/L or amlodipine 3×10^{-9} mol/L.

blood flow induced by intravenous lercanidipine 30 μg/kg (134%) were greater than those induced by the same dosages of nifedipine (20%) and nitrendipine (24%).[6]

In conscious instrumented dogs,[7] intravenous lercanidipine 10 μg/kg significantly reduced mean BP, total peripheral resistance and coronary resistance by 11, 34 and 41%, respectively (all $p<0.001$ *vs* baseline). Corresponding increases in both coronary blood flow (+64%; $p < 0.001$) and left ventricular contractility index (dP/dt, +21%; $p < 0.05$) were observed.[7]

2.3 Vascular Selectivity

An *in vitro* study[10] has shown lercanidipine to be more vasoselective than various other calcium antagonists: the IC_{50} (concentration causing 50% inhibition of contractility) ratio for lercanidipine in isolated rabbit cardiac *versus* vascular tissue incubated for 4 hours was 730. This was higher than that reported for amlodipine (95), felodipine (6), nitrendipine (3) and lacidipine (193) in this model.[10]

In accordance with its vasoselectivity, lercanidipine has demonstrated weak negative inotropic activity *in vitro* and *in vivo* compared with other calcium antagonists. In electrically stimulated rabbit ventricular tissue, the negative inotropic activity of lercanidipine was 857-fold weaker than that of felodipine and 667-fold weaker than that of nitrendipine.[11] In anaesthetised dogs, intravenous lercanidipine dose-dependently reduced diastolic BP (DBP) without impairing cardiac contractility.[11,21] The effects of both nitrendipine and nifedipine on BP in this model were similar to those of lercanidipine, but their effects on cardiac contractility were less favourable.[11]

In conscious rabbits, the BP-lowering effects of intravenous lercanidipine were associated with a significant improvement in cardiac contractility ($p < 0.01$ *vs* pretreatment values).[12]

2.4 Antiatherogenic Effects

Results from preclinical studies indicate that lercanidipine has antiatherogenic potential independent of its BP-lowering effects. Lercanidipine 10 to 50 μmol/L concentration-dependently inhibited replication and migration of arterial myocytes *in vitro* and inhibited fibrinogen-induced myocyte migration.[14,22] The antiproliferative potency of lercanidipine was similar to that of nifedipine and lacidipine.[14,22]

Compared with untreated controls, subcutaneous lercanidipine 0.3 to 3 mg/kg/week for 10 weeks significantly delayed the development of fatty streaks (induced by cholesterol feeding) and hyperplastic lesions (induced by positioning a hollow silastic collar around an artery) in the carotid arteries of hypercholesterolaemic rabbits.[13] The effects of lercanidipine appeared to be independent of its calcium antagonistic activity.

Although the antiatherogenic properties of lercanidipine have not been investigated clinically, the drug demonstrated neutral effects on lipoprotein and apolipoprotein profiles in patients with essential hypertension in a 24-week clinical trial (section 4.1.2).[23]

2.5 End-Organ Protection

Preclinical data indicate that the drug may protect the kidneys[15,24] and brain[16] in spontaneously hypertensive rats (SHR) and may reduce the incidence of cerebral stroke and left ventricular (LV) hypertrophy compared with controls in salt-fed SHR.[17] In addition, abstract reports of 2 clinical studies suggest that the drug can reverse LV hypertrophy in patients with hypertension and LV hyper-

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trophy. Administration of lercanidipine 10 or 20 mg/day for 1 year reduced LV mass by a larger amount than losartan potassium 50 or 100 mg/day (−18.6 *vs* −9.9 g/m²; p = 0.037) in 47 evaluable patients with hypertension, type 2 (non-insulin-dependent) diabetes mellitus and LV hypertrophy.[16] Secondly, 6 months' treatment with lercanidipine caused a reduction in LV mass similar to that seen with enalapril (neither dosage reported) in 42 patients with hypertension and LV hypertrophy.[17] Most of these end-organ data (particularly the clinical findings) must be interpreted with caution because they have yet to be published in full.

2.6 Other Effects

Lercanidipine does not interfere with normal cardiac excitation and conduction when used at therapeutic dosages in patients with mild to moderate essential hypertension. A randomised, double-blind pilot study in patients with mild to moderate hypertension demonstrated that 2 weeks' treatment with lercanidipine 20mg once daily (n = 18) had no significant effects on QRS duration, or PR, QT and QT_c intervals.[25] In addition, lercanidipine had no effects on the types of cardiac arrhythmias present in these patients. The effects of lercanidipine were generally similar to those of sustained release (SR) verapamil 240mg once daily (n = 19), although verapamil tended to prolong the PR interval.[25]

A study (presented as an abstract)[26] has reported that lercanidipine has less oedematous potential than nifedipine GITS (gastrointestinal therapeutic system). 60 patients with mild to moderate hypertension were randomised to receive either lercanidipine 10 to 20mg once daily or nifedipine GITS 30 to 60mg once daily for 12 weeks. Both drugs increased ankle-foot volume from baseline after 12 weeks ($p < 0.05$ and $p < 0.01$ for lercanidipine and nifedipine, respectively) and pretibial subcutaneous tissue pressure (both $p < 0.01$). However, values for both parameters at week 12 were lower in lercanidipine than nifedipine GITS recipients ($p < 0.05$). Increases in both groups were independent of the clinical presence or absence of oedema.

3. Pharmacokinetic Profile

The pharmacokinetics of lercanidipine have been examined in healthy volunteers, in elderly and nonelderly patients with hypertension, and in patients with renal or hepatic impairment (table III). Most of the human data reviewed in this section were reported by Barchielli et al.[27] from data

Table III. Pharmacokinetic profile of *S*-lercanidipine after a single oral dose of racemic lercanidipine 10 or 20mg. Data reported by Barchielli et al. from data on file (Recordati)[27,28]

	C_{max} (μg/L)		t_{max} (h)		AUC (μg/L · h)		$t_{1/2}$ (h)	
	10mg	20mg	10mg	20mg	10mg	20mg	10mg	20mg
Patients with mild to moderate hypertension								
Nonelderly (mean age 40 years; n = 12)	1.78	4.09	2.5	2.5	4.66	16.38	NC	2.88
Elderly (mean age 68 years; n = 12)		5.22		2.1		17.53		3.06
Mild to moderate renal impairment[a] (n = 7)		3.97		1.8		12.27		
Severe renal impairment[b] (n = 6)		6.86		2.5		18.40		
Other individuals								
Male volunteers (mean age 26 years; n = 12)	1.23	3.20	1.3	1.8	1.87	9.05	NR	3.73
Patients with cirrhosis[c] (n = 12)	1.98		1.4		4.16			
Patients with angina pectoris (n = 46)[d]							8.5	10.5

a Creatinine clearance 0.6-3.5 L/h (10-58 ml/min) but not requiring dialysis.

b Patients undergoing regular dialysis.

c Child-Pugh class A.

d Data on file, Recordati.[28] The limit of detection was 0.1 ng/ml in this study vs 0.5 ng/ml in other studies.

AUC = area under the plasma concentration-time curve; C_{max} = maximum plasma concentration; NC = not calculable; NR = not reported; $t_{1/2}$ = elimination half-life; t_{max} = time taken to reach C_{max}.

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on file (Recordati). Because the antihypertensive effects of lercanidipine are primarily ascribed to the *S*-enantiomer (section 2), this section focuses on the pharmacokinetics of *S*-lercanidipine. Interconversion of the enantiomers has not been observed *in vivo*.[28]

3.1 Absorption

After oral administration of lercanidipine 10 or 20mg in 12 patients with mild to moderate hypertension, maximum plasma concentrations (C_{max}) of *S*-lercanidipine (1.75 and 4.09 μg/L, respectively) were reached in ≈2 to 3 hours (table III). Plasma levels and the area under the plasma concentration-time curve were not linearly related to dose, indicating a saturable first-pass metabolism.

The absorption of lercanidipine increases in the presence of food: C_{max} for *S*-lercanidipine after a single dose of lercanidipine 20mg increased from 3.20 μg/L when fasting to 10.21 μg/L after a high-fat meal in 12 healthy volunteers.[27] Hence the recommendation that lercanidipine be taken before meals (section 6).

3.2 Distribution

Lercanidipine 2mg had a volume of distribution of 2 to 2.5 L/kg in healthy volunteers after a 15-minute intravenous infusion.[27] Because of its bulky *bis*-phenylalkylamine side chain, lercanidipine readily accumulates in the lipid bilayer of cell membranes in the arterial tissue wall compartment.[4] The drug appears to undergo a high degree of plasma protein binding (>98%) in humans.[27]

3.3 Metabolism and Elimination

Orally administered lercanidipine undergoes extensive first-pass metabolism, primarily by cytochrome P450 (CYP) 3A4,[28] to pyridine derivatives which are largely inactive.[27] After administration of a single oral dose of [^{14}C]lercanidipine 20mg in 4 healthy volunteers, ≈50% of the dose was eliminated in faeces and 44% in urine, mostly as metabolites.[27] Terminal elimination half-lives ($t_{1/\beta}$) of 2.8 to 3.7 hours have been reported in healthy volunteers and patients with hypertension.[27] In a study in patients with angina pectoris that used a more sensitive assay, the $t_{1/\beta}$ of lercanidipine 10 and 20mg was 8.5 and 10.5 hours (table III).[29] The discrepancy between the 2 ranges may be due to a biphasic elimination profile.[29]

3.4 Special Patient Populations

The bioavailability of *S*-lercanidipine after single oral administration of lercanidipine was not affected by age or cirrhosis (table III).[27] However, accumulation of *S*-lercanidipine was reported in patients with severe renal dysfunction undergoing regular dialysis: after receiving lercanidipine 20mg once daily for 7 days, C_{max} values for *S*-lercanidipine increased from 5.95 μg/L on day 1 (table III) to 9.17 μg/L on day 7.[27] The drug is contraindicated in patients with severe renal or hepatic dysfunction (section 6).

3.5 Potential for Drug-Drug Interactions

In vitro evidence suggested that clinically relevant concentrations of lercanidipine cause minimal inhibition of CYP3A4 and CYP2D6 isozymes in hepatic microsomes.[30,31] This was confirmed *in vivo*, where the drug had no significant effects on the metabolism of coadministered midazolam (a substrate of CYP3A4) or metoprolol (CYP2D6) in 12 healthy volunteers.[30] In addition, no significant pharmacokinetic interactions were reported between lercanidipine 20mg and steady-state cimetidine 400mg twice daily (an inhibitor of CYP2C9/10) in 12 healthy volunteers or between lercanidipine 20mg and steady-state metildigoxin (β-methyldigoxin) or digoxin in 10 women with cardiovascular disease.[27]

In spite of the lack of evidence of clinically significant effects on CYP-mediated metabolism, the manufacturer advises that caution be exercised when lercanidipine is administered concomitantly with inhibitors, inducers and other substrates of CYP3A4 (section 6).[28]

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Table IV. Randomised, double-blind, parallel-group dose-ranging studies of lercanidipine (LER) in patients with mild to moderate hypertension.[a] All studies were preceded by a 3-week placebo (PL) run-in period

Reference	Regimen	No. of pts	Efficacy variables at week 4		
			mean reduction in trough DBP from baseline (mm Hg)	response[b] rate (% pts)	normalisation[c] rate (% pts)
Cinco[30]	PL od 16wk	40	2.6[d]	23	18
	LER 10mg od 16wk	41	11.5[d]	66**	54**
	LER 20mg od 16wk	35	12.8[d]	66**	63**
Mindi et al.[31]	PL od 4wk	45	0.1	36	29
	LER 10mg od 4wk[e]	88	10.3**	59*	52*
Cimbert et al.[27]	PL od 4wk	50	3.4[d]	28	
	LER 2.5mg od 4wk	58	4.9[d]	31	
	LER 5mg od 4wk	54	6.8**[d]	56**†	
	LER 10mg od 4wk	49	7.7**[d]	57**†	
Rimoldi et al.[28]	PL od 4wk	10	3.8	20	20
	LER 10mg od 4wk	10	10.2*	60*	30
	LER 20mg od 4wk	9	13.6*	78*	44

a Defined as sitting DBP 95-115mm Hg,[30] supine DBP 95-115mm Hg,[31] supine DBP 90-109mm Hg[27] and supine DBP 95-114mm Hg[28] at the end of the run-in period.

b Defined as DBP ≤90mm Hg or reduced by ≥10mm Hg from baseline,[30,31] DBP reduced by ≥10mm Hg,[28] and DBP ≤89mm Hg or reduced by ≥10mm Hg from baseline.[27]

c DBP ≤90mm Hg.

d Value estimated from graph.

e The dosage was increased after 4 weeks in nonresponders but these results are not reported here.

DBP = diastolic blood pressure; od = once daily; pts = patients; trough = ~24h after drug administration; * $p < 0.05$, ** $p < 0.01$ vs PL; † $p < 0.01$ vs LER 2.5mg.

4. Therapeutic Efficacy

The therapeutic efficacy of lercanidipine has been evaluated in a number of well designed dose-ranging and comparative studies in patients with varying degrees of hypertension. All studies reported in this review were randomised and double-blind unless stated otherwise.

The efficacy of the drug has primarily been investigated in patients with mild to moderate hypertension, generally defined as DBP between 95 and 115mm Hg at the end of a 3-week placebo run-in period (BP limits varied slightly in some studies). In addition, 1 nonblind study assessed the efficacy of lercanidipine in patients with severe hypertension (DBP ≥110mm Hg),[32] 1 assessed its use as add-on therapy in patients with resistant hypertension (DBP between 100 and 119mm Hg despite monotherapy with atenolol, enalapril or hydrochlorothiazide/amiloride)[33] and 1 study evaluated its use in patients with isolated systolic hypertension (systolic BP ≥160mm Hg and DBP <95mm Hg).[34]

All studies included patients aged between 18 and 70 years, with the exception of 2 studies which evaluated the use of lercanidipine in the elderly (aged 60 to 85 years).[34,35]

In patients with mild to moderate hypertension, any previous antihypertensive therapy was usually withdrawn at least 2 weeks prior to the placebo run-in period, but studies of severe or resistant hypertension employed a reduced washout period (≤1 week) for ethical reasons.

Lercanidipine was administered orally once daily, usually as a 10 or 20 mg/day dosage. In studies where the efficacy of lercanidipine was compared with that of agents which were administered twice daily, a double-dummy approach was employed.

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BP reduction after 4 weeks was the primary endpoint in most studies of mild to moderate hypertension, despite study durations generally ranging from 4 to 16 weeks. Secondary end-points included the number of responders (usually defined as patients with DBP ≤90mm Hg or reduced by ≥10mm Hg from baseline) and normalisation rates (DBP ≤90mm Hg). Where stated, BP was measured in the supine or sitting position ≈24 hours after the last dose (trough). Exclusion criteria included conditions such as secondary hypertension and major heart disease.

4.1 Mild to Moderate Hypertension

4.1.1 Dose-Ranging Studies

Once daily lercanidipine 5 to 20mg significantly reduced BP compared with placebo in patients with mild to moderate hypertension, without affecting HR.

In dose-ranging studies[35-38] reported in table IV, lercanidipine 5 to 20mg once daily dose-dependently and significantly ($p < 0.05$) reduced trough DBP compared with placebo in patients with mild to moderate hypertension treated for 4 weeks; lercanidipine 2.5 mg/day had no significant effects. Normalisation and response rates after 4 weeks were also dose-dependent, with response rates ranging from 50 to 66% with lercanidipine 10 mg/day and up to 86% with lercanidipine 20 mg/day. Lercanidipine had no significant effects on HR.[35-38]

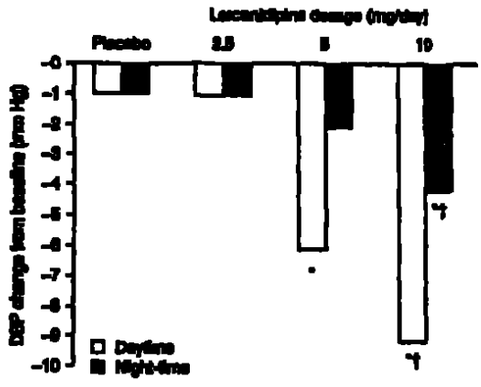


Fig. 2. Effects of lercanidipine (LER) on daytime and night-time DBP. 105 evaluable patients with mild to moderate hypertension received placebo (PL) or lercanidipine 2.5, 5 or 10mg once daily for 4 weeks in a randomised, double-blind study.[37] DBP was assessed by ambulatory blood pressure monitoring. daytime = 0700-2200h; DBP = diastolic blood pressure; night-time = 2200-0700h; * $p < 0.01$ vs baseline; † $p < 0.05$ vs PL.

Ambulatory BP monitoring of 105 evaluable patients participating in a study by Omboni et al.[37] showed that the effects of lercanidipine 10 mg/day, but not those of lercanidipine 2.5 or 5 mg/day, were sustained throughout the 24-hour dosing interval (fig. 2). This long duration of action was confirmed by another study[36] which found the drug to have a DBP trough/peak ratio >0.8 after a 10mg or 20mg once daily dosage.

In the only long term study[39] of lercanidipine published to date (n = 295), the antihypertensive effect of lercanidipine 10 to 30 mg/day was sustained for 12 months. However, this study was nonblind and noncomparative and its findings therefore require confirmation.

4.1.2 Comparisons with Other Antihypertensives

Four weeks' treatment with lercanidipine 10mg once daily was at least as effective as atenolol 50mg once daily,[40] captopril 25mg twice daily,[41] hydrochlorothiazide 12.5mg once daily[33] and nifedipine SR 20mg twice daily,[42] and a 20 mg/day dosage was as effective as amlodipine 10 mg/day,[43] in patients with mild to moderate hypertension. All agents reduced mean BP significantly from baseline (table V). Studies which permitted dosage titration (within the therapeutic dosage range) after 4 weeks in nonresponders found that normalisation rates improved after dosage titration in all groups, but no significant between-group differences emerged (table V).

Although requiring confirmation, evidence reported in abstracts indicates that lercanidipine 10mg once daily may be as effective as enalapril 20 mg/day[39] and candesartan cilexetil 16 mg/day,[44] and more effective than irbesartan 150 mg/day,[45] in patients with mild to moderate essential hypertension.

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Table V. Studies comparing the efficacy of lercanidipine (LER) with that of other antihypertensive agents in the treatment of patients with mild to moderate hypertension (DBP 95-115mm Hg).[a] BP reduction at week 4 was a primary end-point in all these studies

Reference	Study design	Regimen	No. of pts	Mean SBP/DBP (mm Hg)		Normalisation[b] rate (% pts)	
				baseline	4 weeks	4 weeks	8 weeks
Comparison with amlodipine (AML)							
De Giorgio et al.[42]	r, db, co[c]	AML 10mg od 4wk	18	158/99[d]	149**/95*[d]	33 (1st period); 66 (2nd period)	
		LER 20mg od 4wk	18	161/99[d]	143**/89**[d]	39 (1st period); 56 (2nd period)	
Comparison with atenolol (ATE)							
Morisco et al.[43]	mc, r, db, pg	ATE 50 or 100mg od 16wk[e]	98	167/100	142**/86**	76	86
		LER 10 or 20mg od 16wk[e]	101	167/100	145**/90**	68	83
Comparison with captopril (CAP)							
Barbagallo Sangiorgi et al.[44]	mc, r, db, dd, pg	CAP 25-50mg bid 16wk[f]	57	159/100	148**/91**	74	89
		LER 10-20mg od 16wk[f]	58	161/100	147**/89**	81	90
Comparison with hydrochlorothiazide (HCTZ)							
Notarbartolo et al.[45]	mc, r, db, pg	HCTZ 12.5 or 25mg od 24wk[g]	86	159/103	148**/93**	54	78
		LER 10 or 20mg od 24wk[g]	86	159/105	143**/92**	64	84
Comparison with slow release nifedipine (NIF)							
Policicchio et al.[46]	mc, r, db, dd, pg	NIF 20 or 40mg bid 16wk[h]	59	163/101	151***/91***	53	86[i]
		LER 10 or 20mg od 16wk[h]	57	163/101	151***/91***	51	75[i]

a Confirmed during a 3-week placebo run-in period[40-46] or during a 2-week washout period.[39]

b DBP ≤90mm Hg.

c Seven pts received LER for 4 weeks then switched to AML for 4 weeks; 9 pts received AML then LER. A 2-week washout period separated the 2 phases.

d The values reported are a mean of both treatment periods.

e Pts received ATE 50mg or LER 10mg od for the first 4 weeks. These dosages were doubled after 4 weeks in 18 atenolol and 29 LER recipients who were considered nonresponders (their DBP remained ≥90mm Hg or had not reduced by ≥10mm Hg from baseline). Pts who were nonresponders after 8 weeks had the alternate agent added but these data are not presented here.

f Pts received CAP 25mg bid or LER 10mg od for 4 weeks. These dosages were increased to 75 mg/day and 20 mg/day, respectively, in nonresponders after 4 weeks and to 100 and 30 mg/day after 8 weeks (a total of 14 CAP and 13 LER recipients required dosage titration).

g Pts received HCTZ 12.5mg or LER 10mg od for 4 weeks. These dosages were doubled after 4 weeks in nonresponders. Only 1 patient in each group required a higher dosage (37.5 and 20mg, respectively) at week 8 for BP control.

h Pts received NIF 20mg bid or LER 10mg od for the first 4 weeks. These dosages were doubled after 4 weeks in 24 nonresponders.

i Value estimated from graph.

bid = twice daily; BP = blood pressure; co = crossover; db = double-blind; DBP = diastolic blood pressure; dd = double-dummy; mc = multicentre; od = once daily; pg = parallel group; pts = patients; r = randomised; SBP = systolic blood pressure; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs baseline.

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4.2 Severe Hypertension

Monotherapy with lercanidipine 20 or 40 mg/day effectively reduced BP in the majority of patients with severe essential hypertension (DBP ≥110mm Hg at the end of a 3- to 5-day washout period; n = 50) participating in an nonblind study.[32] The drug significantly reduced mean DBP from 115mm Hg at baseline to 93mm Hg after 30 days ($p < 0.001$) and to 86mm Hg after 60 days ($p < 0.001$) in both groups. Twice daily administration of lercanidipine tended to be slightly more effective than once daily administration: BP was normalised (DBP ≤90mm Hg) at 60 days in 84 *vs* 73% of patients in the respective groups, but this between-group difference failed to achieve statistical significance. When patients whose DBP was normalised at 60 days were followed for a further 30 days, the BP-lowering effect of lercanidipine remained stable.

4.3 Resistant Hypertension

Lercanidipine 10 to 30 mg/day is effective add-on therapy in patients with hypertension not responding sufficiently to antihypertensive agents from other drug classes.

An unpublished study[46] in 241 patients with mild to moderate hypertension poorly controlled (seated DBP 95 to 114mm Hg) after 4 weeks' treatment with hydrochlorothiazide 25 mg/day, showed that the addition of lercanidipine 10mg once daily for 8 weeks caused significant reductions in seated DBP compared with placebo (–8.0 *vs* –5.9mm Hg; $p = 0.017$). However, a lower dosage of lercanidipine (5mg once daily) did not cause a significant reduction in BP.

The effects of lercanidipine 10 to 30 mg/day were similar to those of nitrendipine 10 to 30 mg/day when used as add-on therapy for 12 weeks in 80 patients with resistant hypertension (DBP remained between 100 and 119mm Hg despite treatment with atenolol 50 to 100 mg/day, enalapril 10 to 20 mg/day or hydrochlorothiazide/amiloride 25/2.5 or 50/5 mg/day).[33] In this study, 76% of lercanidipine recipients and 65% of nitrendipine recipients had DBP ≤90mm Hg after 4 weeks' treatment with a 10 mg/day dosage. Titration to a 20 mg/day dosage in nonresponders at week 4, or to 30 mg/day at week 8, resulted in further improvements in normalisation rates (fig. 3).

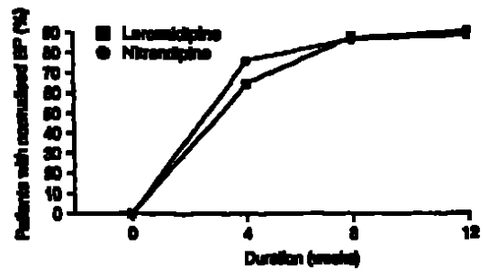


Fig. 3. Antihypertensive efficacy of lercanidipine (LER) compared with nitrendipine (NIT) in patients with resistant hypertension. LER or NIT 10mg once daily was added to ongoing therapy with atenolol, enalapril or hydrochlorothiazide plus amiloride in 80 patients with DBP 100-118mm Hg. Dosages of LER and NIT were titrated in 10mg increments every 4 weeks if DBP remained >89mm Hg.[33] DBP = diastolic blood pressure; normalised = DBP ≤90mm Hg.

4.4 Special Patient Populations

Lercanidipine is effective in the treatment of elderly patients with mild to moderate essential hypertension[35] and those with isolated systolic hypertension.[34]

When administered at a dosage of 10mg once daily for 4 weeks to 133 elderly patients aged 60 to 85 years with mild to moderate hypertension, lercanidipine significantly reduced mean DBP compared with placebo (response rate 59%; table IV).[35] Titration to 20 mg/day after 4 weeks in nonresponders increased the response rate to 87.5% at week 8; only 11 of 88 lercanidipine recipients required titration to 30 mg/day for BP control.

In a study[34] involving 70 elderly patients aged (mean age 67 years) with isolated systolic hypertension, lercanidipine 10 or 20mg once daily for 8 weeks reduced systolic BP significantly compared with placebo (–32.4 *vs* –9.6mm Hg; $p < 0.001$).

The drug has also demonstrated antihypertensive efficacy in a randomised double-blind trial in

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40 patients with type 2 diabetes mellitus and mild to moderate essential hypertension.[47] Patients in this study were randomised to lercanidipine 10mg (n = 20) or 20mg (n = 20) for 4 weeks; the dosages were increased by 10mg at week 4 in non-responders. Active treatment significantly reduced BP from baseline at week 8, and glucose homeostasis improved overall.[34] However, the findings of this study must be interpreted with caution because it was not placebo-controlled and has yet to be published.

5. Tolerability

Lercanidipine was well tolerated in clinical trials, with most treatment-emergent adverse events related to vasodilation. A pooled analysis of data from 20 clinical trials involving almost 1800 patients with hypertension found that 11.5% of lercanidipine 10 or 20mg once daily recipients (n = 1317) compared with 7.0% of placebo recipients (n = 227) reported adverse events; ≈5 and 3%, respectively, withdrew because of poor tolerability. The most commonly reported events were headache, flushing, asthenia, vertigo, palpitations and ankle oedema (fig. 4).[48]

Two postmarketing surveillance studies[49,50] involving a total of 9605 patients with hypertension have confirmed that lercanidipine is well tolerated in clinical practice. A preliminary report from the larger study (presented as an abstract)[49] showed that adverse events were reported in 7.6% of 7469 patients with mild to moderate hypertension who received lercanidipine 10 or 20mg once daily for 3 months. The most frequently reported events were headache (2.7%) and ankle oedema (1.1%). In the smaller study (n = 2136), also reported as an abstract,[50] 122 patients (5.7%) reported adverse events with lercanidipine 10 mg/day, the most common being headache (1.2%), ankle oedema (1.2%) and skin rash (0.9%).

Lercanidipine appears to be well tolerated in the elderly. In a study[39] that enrolled 144 elderly patients, between 8 and 11% of patients receiving placebo or lercanidipine 10 or 20 mg/day for 4 weeks reported adverse events (necessitating withdrawal in ≈4% of patients in each group). Most adverse events in this study were mild to moderate and were related to the vasodilatory action of the drug.

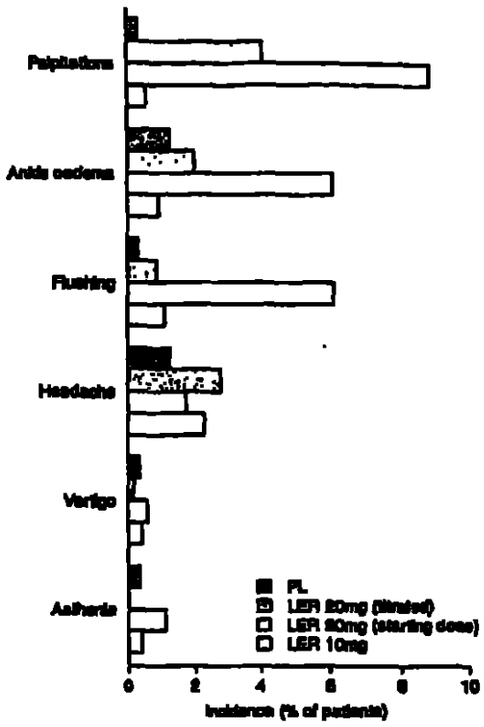


Fig. 4. Adverse events reported with lercanidipine (LER) in 20 clinical trials (pooled data). Patients in these randomised, double-blind trials received LER 10mg once daily (n = 1128), LER 20mg once daily as a starting dose (n = 180), LER 20mg once daily as a titrated dose (n = 460) or placebo (PL; n = 227) for between 1 and 52 weeks.[48]

The tolerability profile of lercanidipine 10 to 30 mg/day has also been reported in a long term (1 year) study.[39] Again, most adverse events were related to vasodilation but generally resolved during treatment or after drug withdrawal.[39]

Consistent with data published in section 2.6, lercanidipine was well tolerated with regard to

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ECG parameters in clinical trials; few clinically significant events were reported.[23,33,39-42,45]

5.1 Comparisons With Other Antihypertensive Agents

Well controlled clinical trials have shown that lercanidipine 10 or 20 mg/day is as well tolerated as captopril 50 to 100 mg/day[41] and atenolol 50 or 100 mg/day,[40] although the tolerability profiles of these agents were consistent with their drug class and therefore differed slightly.

When compared with the calcium antagonists nitrendipine (10 or 20mg once daily)[33] and nifedipine SR (20 or 40mg twice daily),[42] once daily administration of lercanidipine 10 or 20mg for 12[33] or 16[42] weeks was associated with a lower incidence of adverse events and fewer withdrawals, although the between-group differences did not achieve statistical significance. All 3 calcium antagonists caused adverse vasodilation, but 1 study found that lercanidipine (n = 64) was better tolerated than nifedipine (n = 66): 4 vr 14 vasodilatory events were reported in the respective groups.[42]

In line with these comparative data, an abstract report[51] of a study in 115 patients who had reported adverse vasodilation while taking a dihydropyridine calcium antagonist (amlodipine, nifedipine GITS, felodipine or nitrendipine) showed that, after switching to lercanidipine 10 to 20 mg/day for 1 month, the prevalence of these adverse events was significantly reduced: calf oedema from 94.8 to 51.4%, headache from 38 to 18%, flushing from 35 to 17% and skin rash from 38 to 18%; all $p < 0.001$.

6. Dosage and Administration

Oral lercanidipine is used in Europe for the treatment of hypertension. In the UK, the drug is indicated for the treatment of mild to moderate hypertension for which the recommended initial dosage is 10mg once daily at least 15 minutes before meals (to enhance absorption, section 3.1).[28] It may take 2 weeks before the maximal antihypertensive effect is apparent, thus dosage titration to 20 mg/day in patients with an initial unsatisfactory response should be done gradually.[28]

Dosage adjustments are not required in the elderly or patients with mild to moderate renal or hepatic dysfunction, although the manufacturer recommends that special care be exercised when initiating treatment with lercanidipine in such patients. Use of lercanidipine is contraindicated in patients with severe hepatic or renal dysfunction (creatinine clearance <10 ml/min, section 3.4).[28]

Use of lercanidipine is also contraindicated during pregnancy and lactation, in women of childbearing potential unless effective contraception is used, patients with LV outflow tract obstruction, untreated congestive heart failure, unstable angina pectoris or within 1 month of a myocardial infarction.[28]

The manufacturer advises that until further evidence is available, great caution should be exercised when lercanidipine is administered concomitantly with inhibitors (e.g. ketoconazole, itraconazole, erythromycin and fluoxetine), inducers [e.g. phenytoin, carbamazepine and rifampicin (rifampin)] and other substrates (e.g. terfenadine, astemizole, cyclosporine, amiodarone, quinidine, diazepam, midazolam, propranolol and metoprolol) of CYP3A4.[28] Use of grapefruit juice should also be avoided as this has been shown to inhibit the metabolism of some dihydropyridine calcium channel blockers.[28,52]

7. Place of Lercanidipine in the Management of Hypertension

The prevalence of hypertension in Western populations appears to be approximately 20% based on epidemiological data from England[53] and the USA[54] which used BP ≥160/95 mm Hg as the cut-off point. The disease is a major risk factor for heart disease and stroke,[55] 2 complications which impose an enormous financial burden on healthcare systems and which also have major social implications.[56] The effective management of hypertension is therefore a primary healthcare objective in most countries.

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Current guidelines differ slightly in their recommended BP goals: JNC-VI (the sixth report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure)[56] recommends reducing BP below 140/90mm Hg if tolerated (or even lower in high risk populations such as those with diabetes mellitus or renal disease). The WHO-ISH (World Health Organisation-International Society of Hypertension)[55] recommends BP levels below 130/85mm Hg, and the BHS (British Hypertension Society) recommends BP <140/85mm Hg.[57] Despite these slight variations, all 3 guidelines agree that the primary goal in the treatment of hypertension is to reduce morbidity and mortality by lowering BP and modifying other cardiovascular risk factors.

Nonpharmacological approaches to the treatment of hypertension include lifestyle modifications such as weight reduction, reduced salt intake, limited alcohol consumption and regular physical exercise.[57] These measures alone may reduce the need for drug treatment, particularly in patients with mild to moderate hypertension. However, pharmacological treatment may be required in a large proportion of patients.

Once the decision has been made to initiate therapy, JNC-VI[56] guidelines suggest starting with either a diuretic or a β-blocker unless there are strong indications for another drug class (or β-blockers or diuretics are contraindicated). This is because numerous randomised controlled clinical trials have shown a reduction in morbidity and mortality with diuretics and β-blockers in patients with hypertension (recent evidence[58,59] indicates this may also be the case with some calcium antagonists though). There are certain patient groups in whom calcium antagonists are favoured as first-line therapy by JNC-VI: patients with isolated systolic hypertension and patients with coexisting angina pectoris. Unlike JNC-VI,[56] the WHO-ISH guidelines state that all drug classes (diuretics, β-blockers, calcium antagonists, ACE inhibitors, angiotensin II antagonists and α-blockers) are suitable for the initiation and maintenance of antihypertensive therapy. Most importantly, antihypertensive treatment needs to be tailored to the individual needs and preferences of the patient.

Regardless of which drug is used first, treatment must commence with a low dosage. If BP control is not achieved, dosage titration or the addition of a second drug is advocated. Interestingly, evidence suggests that fewer than 50% of patients have their hypertension controlled by monotherapy and as many as 66% require ≥3 drugs.[57]

Lercanidipine is a vasoselective dihydropyridine calcium antagonist. Because of its lipophilicity, the drug accumulates into the phospholipid bilayer of the cell membranes from where it gradually interacts with L-type calcium channels. This results in a slow onset and long duration of action. Well controlled clinical trials have shown that lercanidipine 10 or 20mg provides 24-hour efficacy after once daily administration in patients with mild to moderate essential hypertension and is as effective as recommended dosages of amlodipine, atenolol, hydrochlorothiazide, captopril and nifedipine SR. In addition, evidence reported in abstracts suggests that lercanidipine 10mg once daily may also be as effective as enalapril 20 mg/day and candesartan cilexetil 16 mg/day and more effective than irbesartan 150 mg/day in patients with mild to moderate essential hypertension.

Lercanidipine has shown efficacy in elderly patients with mild to moderate hypertension or isolated systolic hypertension. Furthermore, it effectively reduced BP in patients with severe hypertension when used alone in 1 study, and in patients with hypertension not responding to other agents when used adjunctively (in these patients the drug appears to be as effective as nitrendipine). Its antihypertensive effects in patients with type 2 diabetes mellitus have been demonstrated but have yet to be published.

Importantly, the antihypertensive effects of the drug are not associated with reflex tachycardia. Other potential benefits of lercanidipine include its vasoselectivity (the drug lowers BP without reducing cardiac contractile force) and its antiatherogenic activity and end-organ protection as shown

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in preclinical trials. These effects suggest that the drug might have a role in the treatment of hypertensive patients with coexisting ischaemic heart disease, but this has yet to be confirmed in clinical trials.

Lercanidipine is a well tolerated drug, with vasodilatory effects such as headache, flushing, dizziness and ankle oedema being the most commonly reported adverse events in clinical trials. The drug was as well tolerated as captopril and atenolol, and possibly better tolerated than amlodipine, nifedipine (SR or GITS), felodipine and nitrendipine in patients with mild to moderate hypertension.

In conclusion, clinical trials have shown that lercanidipine is an effective and well tolerated antihypertensive agent in patients with mild to moderate hypertension after once daily administration. In addition, the drug may reduce BP when used as monotherapy in patients with severe hypertension or when used adjunctively in patients with resistant hypertension. Importantly, lercanidipine appears to be at least as effective and well tolerated as other commonly used antihypertensive agents. It therefore represents a useful therapeutic option in the management of patients with hypertension and will be particularly useful in patients not responding to, or intolerant of, antihypertensive agents from other drug classes.

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United States Patent [19]

Sartani et al.

[11] Patent Number: 5,767,136

[45] Date of Patent: Jun. 16, 1998

[54] 1,4-DIHYDROPYRIDINES USEFUL FOR PREVENTION OR REDUCTION OF ATHEROSCLEROTIC LESIONS ON ARTERIAL WALLS

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[21] Appl. No.: 645,964

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[52] U.S. Cl. 514/356

[58] Field of Search 514/356

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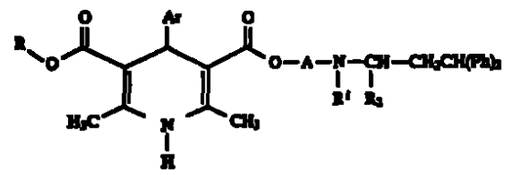
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Primary Examiner—Theodore J. Criares
Attorney, Agent, or Firm—Darby & Darby

[57] ABSTRACT

The subject invention provides methods for preventing, retarding, or reducing atherosclerotic lesions or atherosclerotic degradation of arterial walls or treating atherosclerosis comprising administering to a mammal in need of such prevention, retardation, reduction or treatment at least one compound having the formula I:



I

wherein:

Ph is phenyl.

Ar is: 2-nitrophenyl, 3-nitrophenyl, 2,3-dichlorophenyl or benzofurazan-4-yl.

A is a branched chain alkylene radical having from 2 to 6 carbon atoms.

R is a straight or branched chain alkyl radical having from 1 to 6 carbon atoms, optionally mono-substituted by an alkoxy substituent having from 1 to 6 carbon atoms.

R_1 is hydrogen, hydroxy, or an alkyl radical having from 1 to 4 carbon atoms.

R_2 is hydrogen, or methyl;

or an enantiomer of a compound having formula I; a hydrated compound having formula I; a solvated compound having formula I; a pharmaceutically acceptable acid addition salt, or any of the foregoing.

25 Claims, 6 Drawing Sheets

FIG. 1

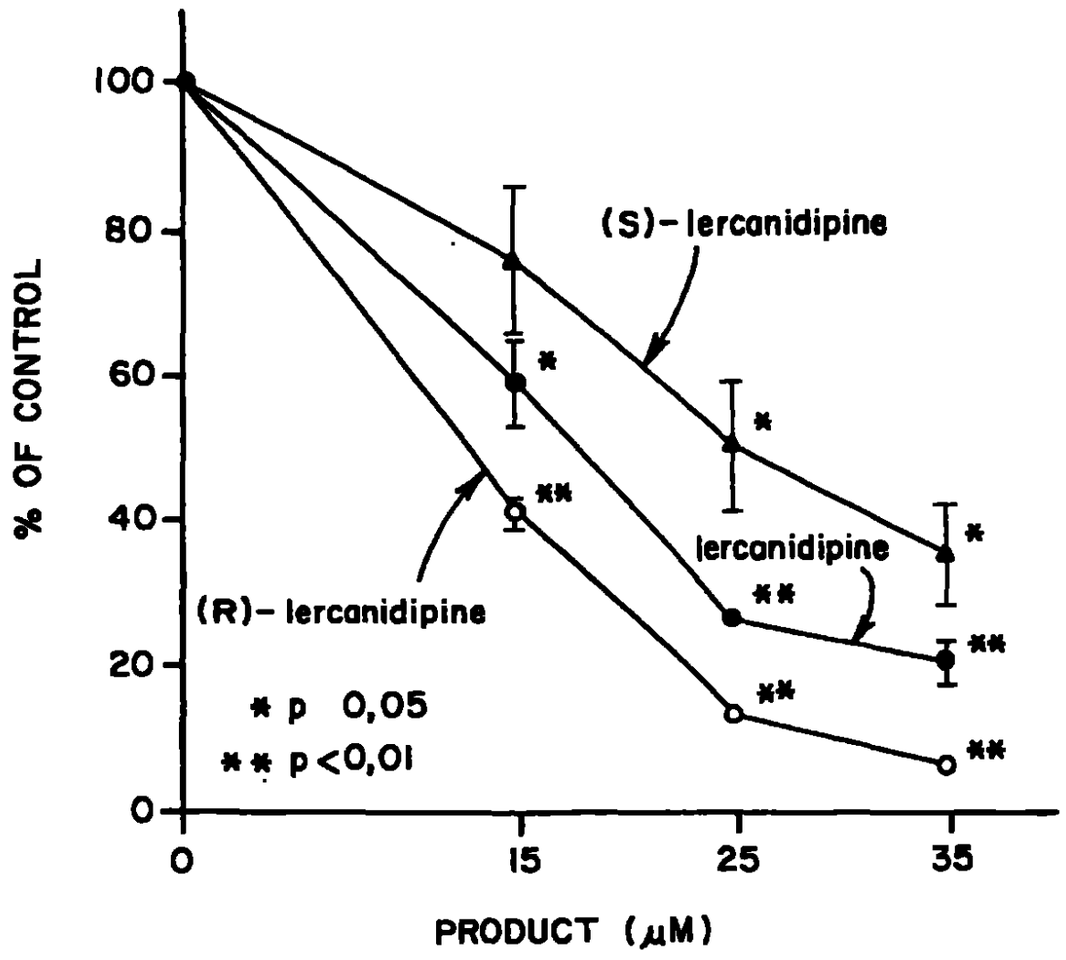
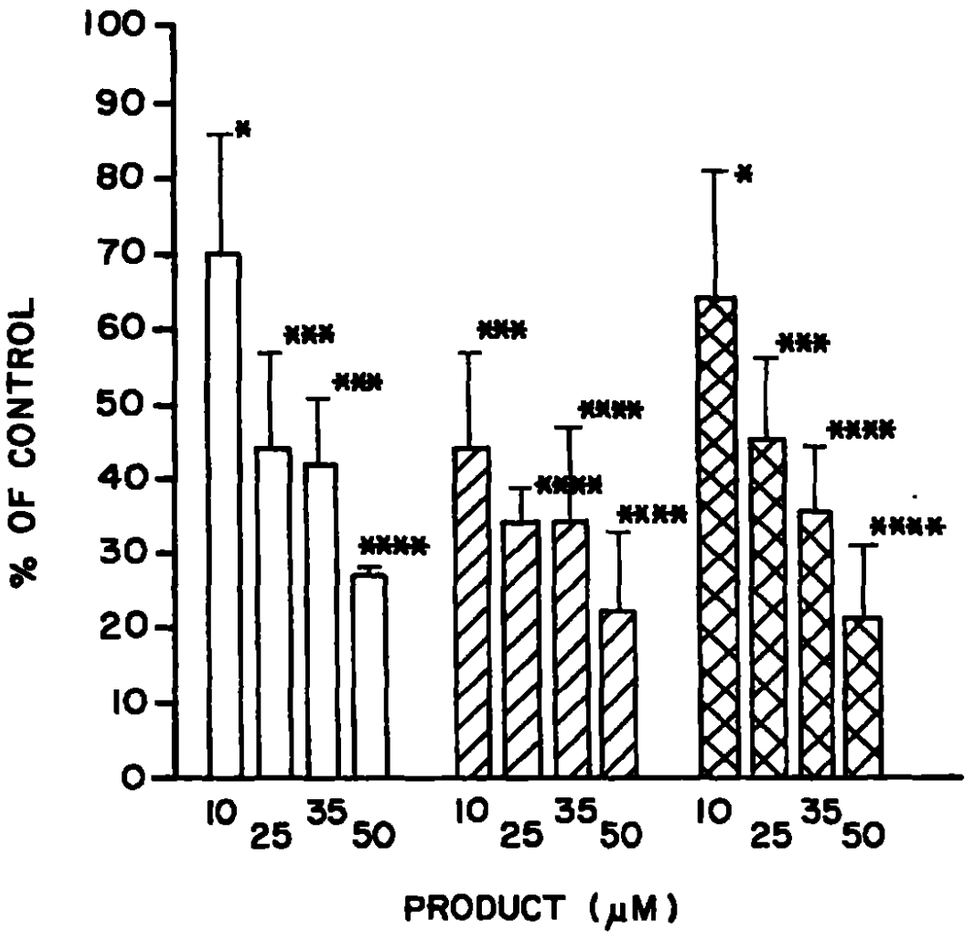

100
80
60
40
20
0
% OF CONTROL
(S)- lercanidipine
lercanidipine
(R)- lercanidipine
* p 0,05
** p <0,01
0
15
25
35
PRODUCT (μM)

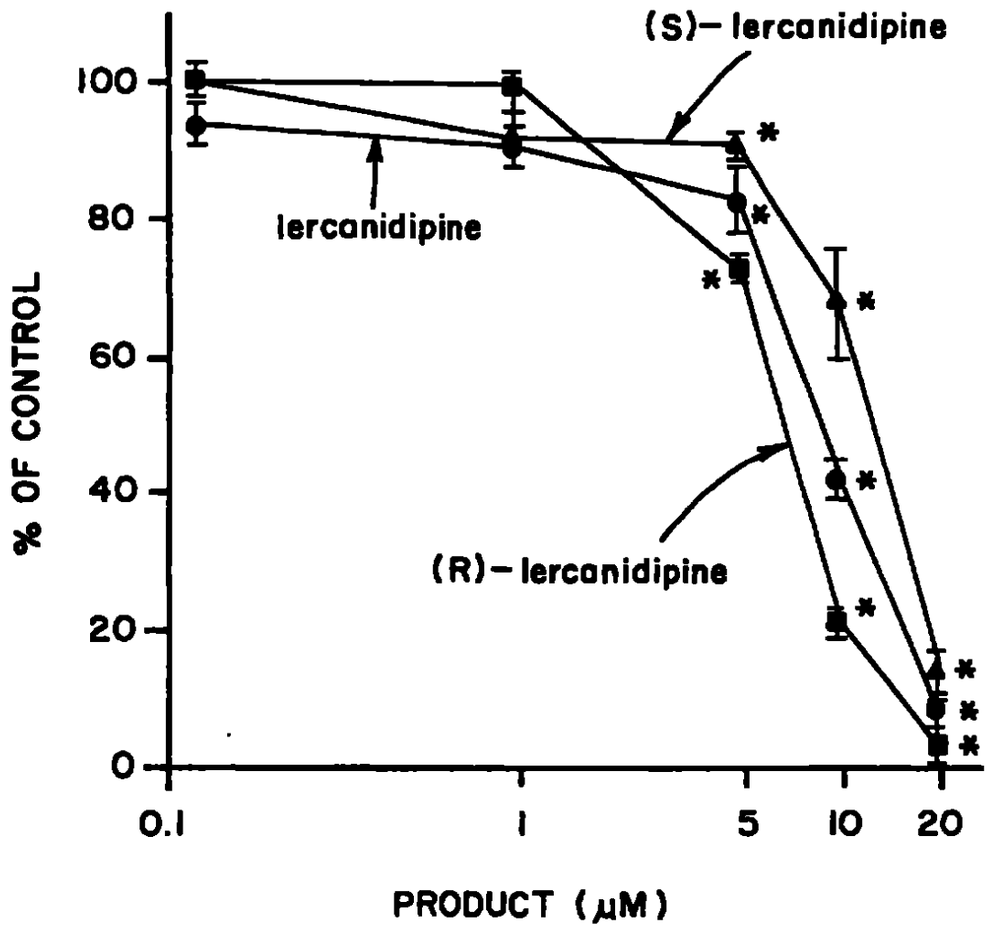
FIG. 2



x $p<0.05$
xx $p<0.02$
xxx $p<0.01$
xxxx $p<0.001$

RS
R
S

FIG. 3



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FIG. 4

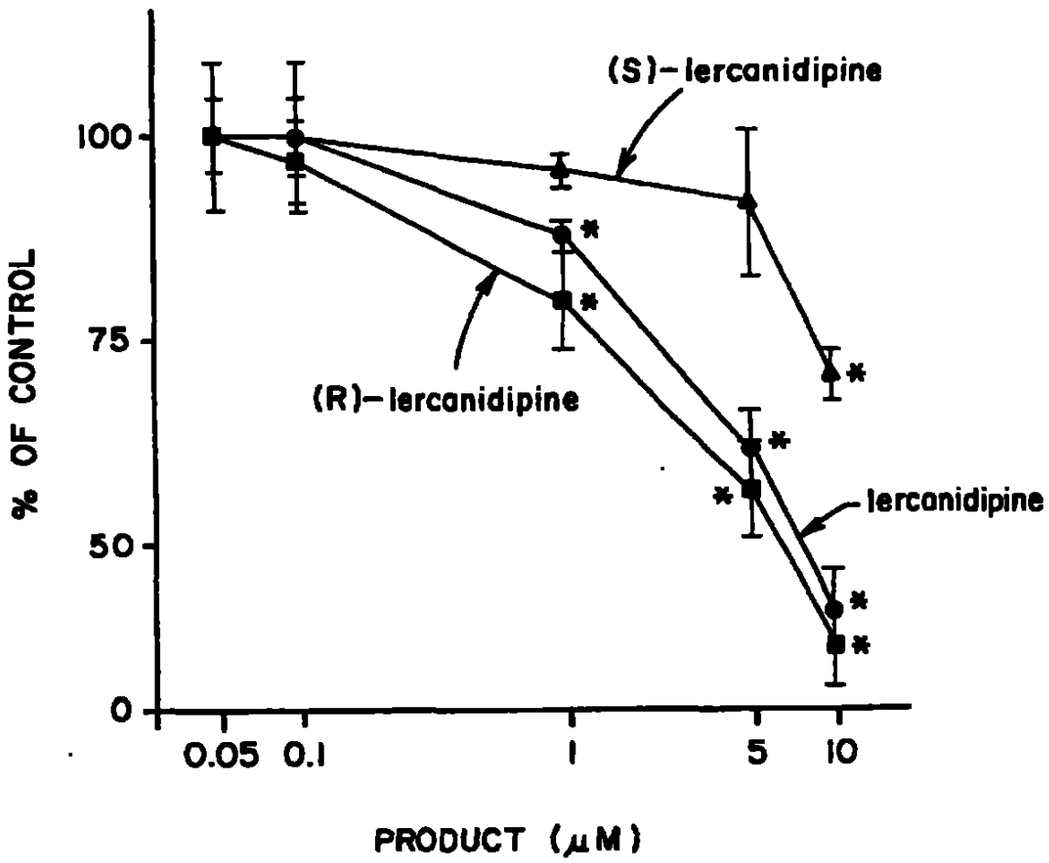

(S)-lercanidipine
(R)-lercanidipine
lercanidipine
100
75
50
0
% OF CONTROL
0.05
0.1
1
5
10
PRODUCT (μM)

FIG. 5

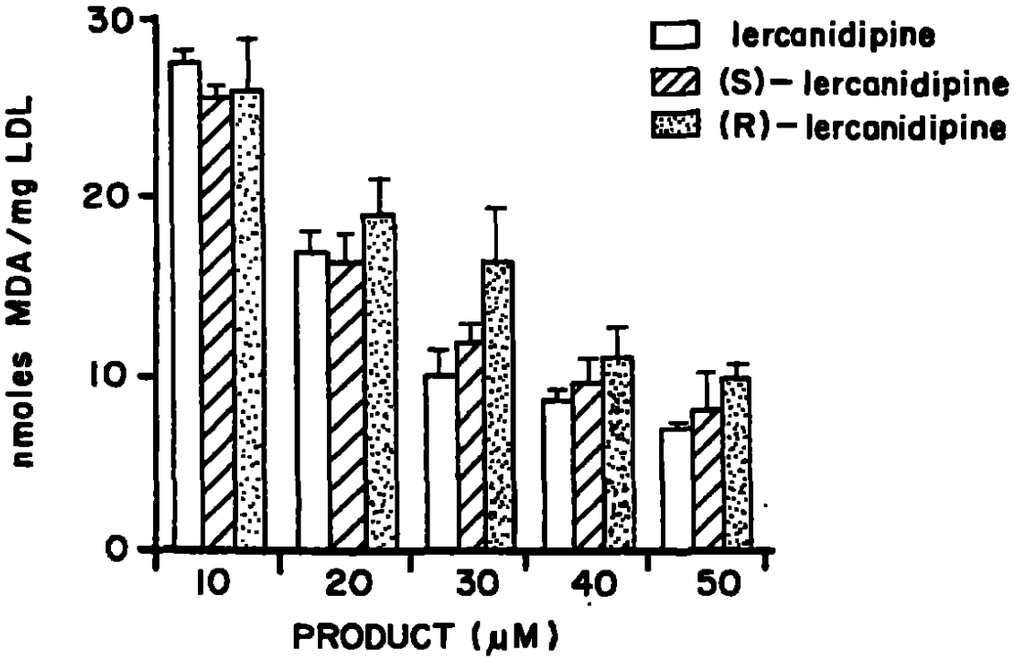

lercanidipine
(S)– lercanidipine
(R)– lercanidipine
nmoles MDA/mg LDL
30
20
10
0
10
20
30
40
50
PRODUCT (μM)

FIG. 6

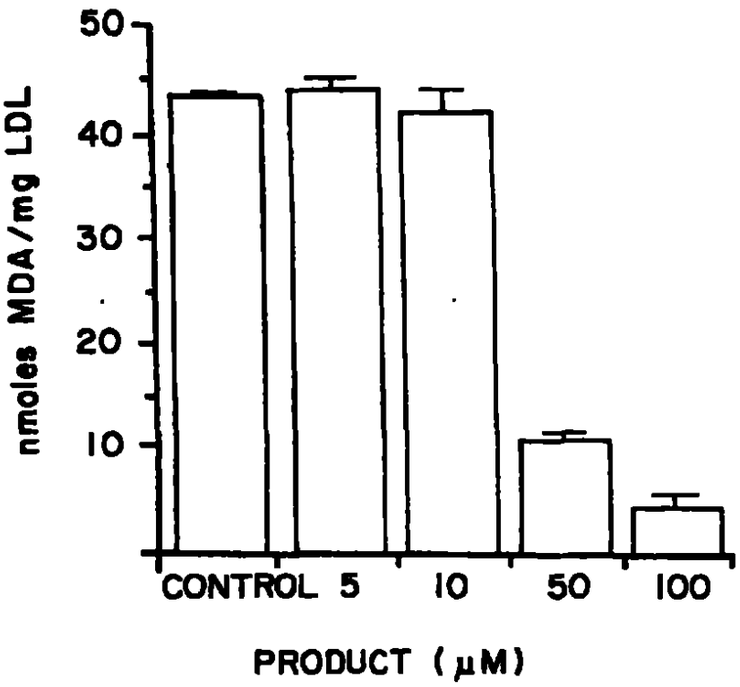
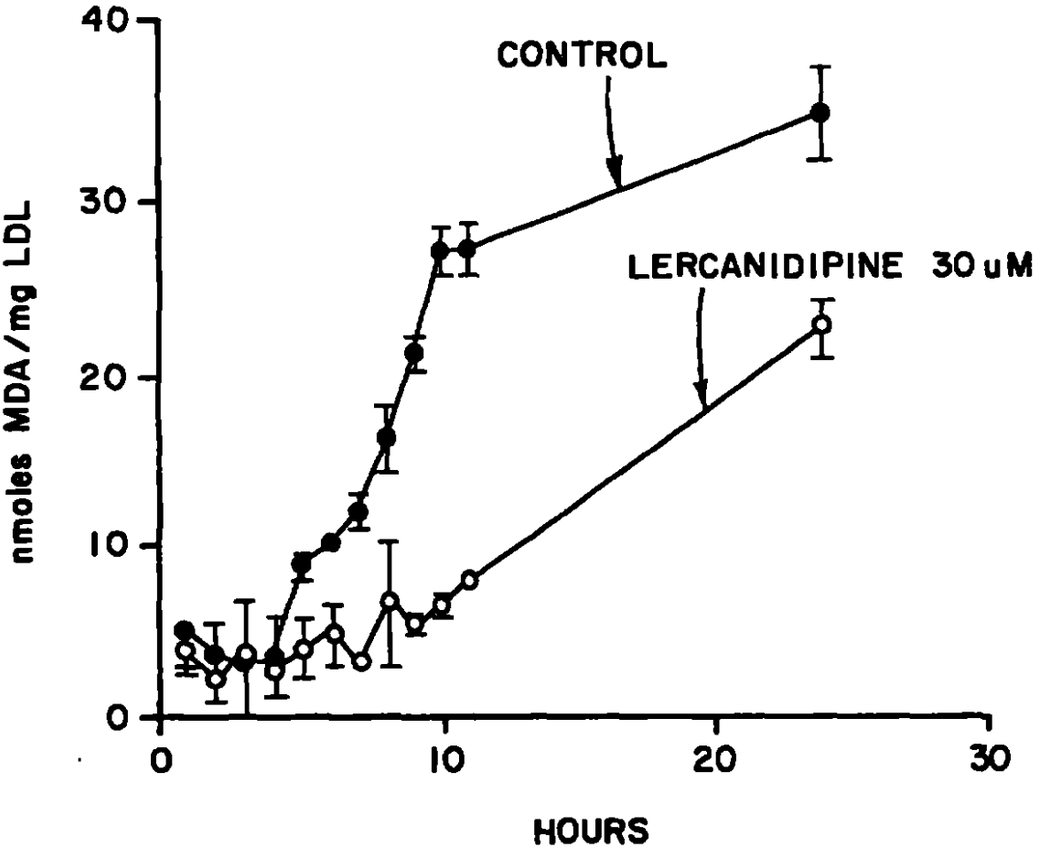

nmoles MDA/mg LDL
50
40
30
20
10
CONTROL
5
10
50
100
PRODUCT (μM)

FIG. 7


40
30
20
10
0
nmoles MDA/mg LDL
CONTROL
LERCANIDIPINE 30 uM
0
10
20
30
HOURS

1,4-DIHYDROPYRIDINES USEFUL FOR PREVENTION OR REDUCTION OF ATHEROSCLEROTIC LESIONS ON ARTERIAL WALLS

FIELD OF THE INVENTION

The present invention relates to the use of 1,4-dihydropyridines to counter several processes which affect the development of atherosclerotic vascular lesions, such as, for example, myocyte proliferation and migration, cholesterol metabolism in macrophages and oxidative modification of low-density lipoproteins (LDL). Beneficial effects on the above biological processes are the basis for prevention of atherosclerotic degradation in arterial walls of humans. Another aspect of the invention relates to particular forms of, and compositions containing, such compounds and adapted for this therapeutic use.

BACKGROUND OF THE INVENTION

The compounds of the invention are known for their coronary dilating and antihypertensive activity from U.S. Pat. No. 4,705,797. However, the anhydrous form of such compounds is novel and forms part of the present invention.

A particularly preferred compound is methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (lercanidipine), and pharmaceutically acceptable salts thereof. These compounds can be prepared according to the methods cited in U.S. Pat. No. 4,705,797 or the methods disclosed herein.

Also, particularly preferred are the resolved enantiomers of lercanidipine. The preparation of the individual enantiomers is described in the present specification.

Arteriosclerosis, a generic term for thickening and hardening of the arterial wall, is responsible for many deaths in the United States and other westernized societies. One type of arteriosclerosis is atherosclerosis, a disorder of the larger arteries that underlies most coronary artery disease, aortic aneurysm, and arterial disease of the lower extremities. Atherosclerosis plays a major role in cerebrovascular disease and is a leading cause of death in the United States, both above and below age 65 (E. L. Bierman in *Harrison's Principles of Internal Medicine XII* Ed., page 992 (1991)).

It is now recognized that atherosclerosis is a multifactorial process that, when leading to clinical sequelae, is based on extensive proliferation of migrated smooth muscle cells (myocytes) within the intima of the affected artery. The atherosclerotic plaque formation is believed to be the result of three fundamental biological processes. These are:

1) migration and proliferation of intimal smooth muscle cells, together with variable numbers of accumulated macrophages and T-lymphocytes;

2) formation by the proliferated smooth muscle cells of large amounts of connective tissue matrix, including collagen, elastic fibers, and proteoglycans; and

3) accumulation of lipid, principally in the form of cholesterol esters and free cholesterol within the cells as well as in the surrounding connective tissues.

(R. Ross et al., *Science*, 180:1332 (1973); R. Ross et al., *N. Eng. J. Med.*, 295:369 (1976); R. W. Wissler et al. *Prog. Cardiovasc. Dis.*, 18:341 (1976)).

In addition, a number of experimental reports ascribe a key role to the oxidative modification of low density lipoproteins (LDL) in the early stages of atherosclerosis in humans, where hypercholesterolemia represents the major

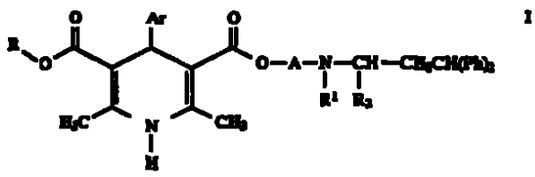
risk factor associated with the increased incidence of the disease. Available data has lead to the belief that LDL can undergo oxidative modification (D. Steinberg et al., *N. Eng. J. Med.*, 320:915 (1989); J. L. Witztum, *Lancet*, 344:793 (1994)) and that oxidized LDL has been shown to promote atherogenesis by a number of mechanisms, including enhanced uptake of LDL in tissue macrophages which leads to lipid accumulation and chemotactic activity for monocytes (M. S. Brown et al. *Ann. Rev. Biochem.*, 52:223 (1983)), and cytotoxicity to arterial wall endothelial cells (S. Parthasarathy et al., *Prog. Lipid Res.*, 31:127 (1992)).

SUMMARY OF THE INVENTION

It has now been found that 1,4-dihydropyridines, such as lercanidipine and each enantiomer of lercanidipine are able to counteract many of the biological processes leading to atherosclerotic lesions. Therefore, these compounds are useful in a mammal, including a human in need of such treatment to prevent, retard, or reduce the atherosclerotic degradation of the arterial wall, hypercholesterolemia and the various diseases caused by these, such as, for example, ischemic heart diseases such as myocardial infarction and cerebrovascular diseases such as cerebral infarction and cerebral apoplexy.

The compounds of the invention are also useful for the inhibition of restenosis following percutaneous transluminal coronary angioplasty (PTCA) and for suppressing the progression of vascular hypertrophy associated with hypertension.

The subject invention provides a method for preventing, retarding, and/or reducing atherosclerotic lesions and preventing atherosclerotic degradation of arterial walls. The method comprises administration of an effective amount of a compound having Formula I:



I

wherein:

Ph is phenyl.

Ar is: 2-nitrophenyl, 3-nitrophenyl, 2,3-dichlorophenyl or benzofurazan-4-yl.

A is a branched chain alkylene radical having from 2 to 6 carbon atoms.

R is a straight or branched chain alkyl radical having from 1 to 6 carbon atoms, optionally mono-substituted by an alkoxy substituent having from 1 to 6 carbon atoms.

R_1 is hydrogen, hydroxy, or an alkyl radical having from 1 to 4 carbon atoms, and

R_2 is hydrogen, or methyl.

Also useful in the present invention are resolved enantiomers of the compounds having formula I, pharmaceutically acceptable acid addition salts, and hydrated or solvated forms of any of these compounds.

The present invention also contemplates unit dosage forms that include an effective amount of at least one of the (R)-enantiomer, the (S)-enantiomer, or the racemate of the compounds of the invention.

It has been discovered that the compounds of the invention can be prepared in anhydrous (unsolvated) form by recrystallizing the compound in an aprotic solvent and a protic solvent. In the recrystallization either aprotic or protic

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solvent can be used prior to the other. The anhydrous compounds formed have improved stability when compared to the hydrated or solvated compounds. Particularly preferred among these 1,4-dihydropyridine derivatives are lercanidipine, its enantiomers, and pharmaceutically acceptable salts thereof. Lercanidipine is methyl 1,1-N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate.

The (S)-enantiomer and the racemate of lercanidipine, both possess antihypertensive activity, and can be used for patients in need of treatment for both hypertension and diseases related to atherosclerotic phenomena.

On the other hand, (R)-lercanidipine, having only minimal antihypertensive activity, has substantial activity in, and can be used for treating conditions involving, smooth muscle cell migration and proliferation and diseases related to atherosclerotic phenomena without any concomitant cardiovascular effect. This enantiomer is useful for treatment of patients for whom reduction of blood pressure would be undesirable.

The invention also provides for the preparation of a composition useful for the treatment, i.e., the prevention, arrest, retardation, or at least partial reversal of atherosclerotic degradation in the arterial walls of a patient in need of said treatment using a compound of the general formula I.

DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graphical representation of the effect of lercanidipine and its enantiomers on [^{3}H]-thymidine incorporation into myocytes of rat smooth muscle cells.

FIG. 2 is a graphical representation of the ability of lercanidipine and its enantiomers to interfere with the migration of arterial myocytes.

FIG. 3 is a graphical representation of the ability of lercanidipine and its enantiomers to inhibit the enzyme ACAT and cholesterol esterification induced by AcLDL in mouse peritoneal macrophage.

FIG. 4 is a graphical representation of the concentration-dependent effect of lercanidipine and its enantiomers on cholesterol esterification in cholesterol ester loaded macrophages.

FIGS. 5 and 6 are graphical representations of the effect of lercanidipine and its enantiomers on cell-mediated oxidation of LDL.

FIG. 7 is a time course plot of cell-mediated oxidation and the effects of lercanidipine after incubation.

DETAILED DESCRIPTION OF THE INVENTION

All patents, patent applications, and literature references cited in the specification are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure, including definitions, will prevail.

"Treatment" of atherosclerosis is intended to include preventing, retarding, and/or reducing atherosclerotic lesions and preventing, retarding, and/or reducing atherosclerotic degradation of arterial walls, in mammals, particularly humans. In particular, the inhibition or counteracting of the following processes, which lead to atherosclerotic lesions, can be considered very useful for the treatment of atherosclerosis. They are:

1) migration and proliferation of intimal smooth muscle cells, together with variable numbers of accumulated macrophages and T-lymphocytes;

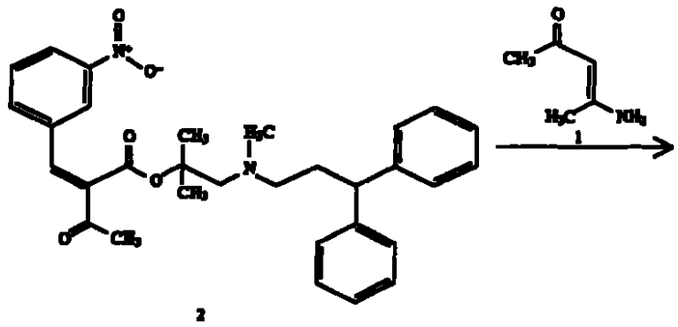
2) formation by the proliferated smooth muscle cells of large amounts of connective tissue matrix, including collagen, elastic fibers, and proteoglycans; and

3) accumulation of lipid, principally in the form of cholesteryl esters and free cholesterol within the cells as well as in the surrounding connective tissues.

According to the present invention, treatment constitutes any improvement in one or more clinical or histological symptoms or diagnostic markers observed by the attending physician or determined by quantitative or semiquantitative techniques. Non-limiting examples include techniques such as, analysis of blood, ultrasound and other imaging techniques such as angiography. Several of these techniques have been developed and are well-known. Patients who are likely to require this treatment are those suffering from or at risk for myocardial infarct, stroke, hyperlipidemia, or hypertension.

Lercanidipine can be prepared by Hantzsch cyclization of methyl 3-aminocrotonate (1) with 1,1-N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl α-acetyl-3-nitrocinnamate (2) (Scheme 1), according to the method described in U.S. Pat. No. 4,705,797.

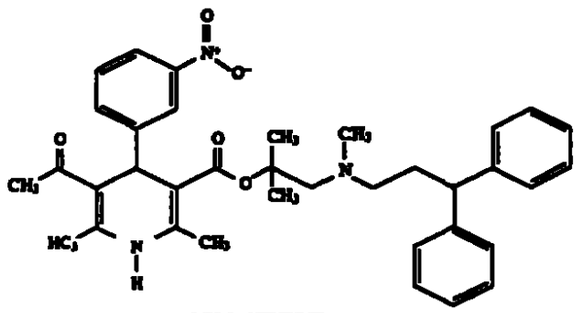
SCHEME 1



2

-continued

SCHEME 1



LERCANIDIPINE

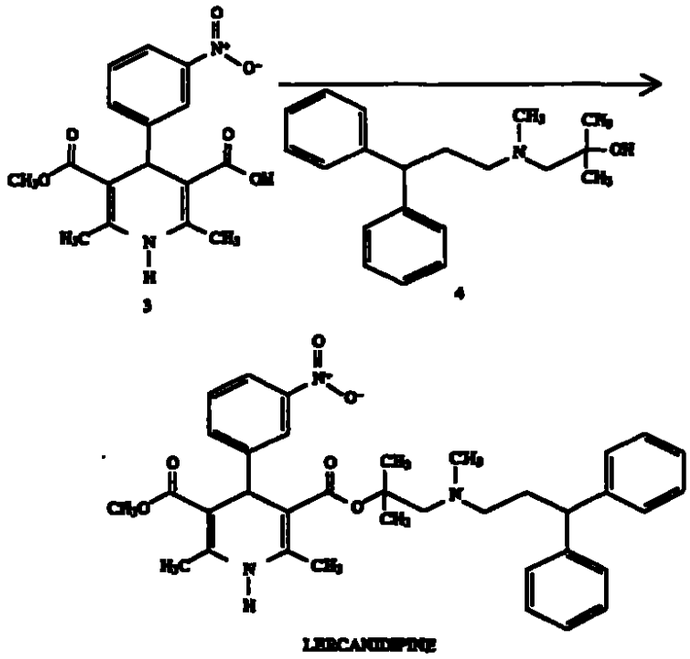
However, the cyclisation method described in the U.S. Pat. No. 4,705,797 leads to several impurities and consequently a low yield of the product is obtained. The removal of the reaction byproducts requires the use of purification techniques, e.g., column chromatography, which are difficult to apply on an industrial scale.

Alternatively, lercanidipine can be prepared by esterification of 1,4-dihydro-2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl) pyridine-3-carboxylic acid (3) with 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol (4) (Scheme 2) according to the method described in Example 3.

The homochiral acids, hereinbelow called for convenience, acid 5 (the (R)-enantiomer) and acid 6 (the (S)-enantiomer), can be conveniently prepared by resolution of racemic acid 3 according to the method reported by A. Ashimori et al., *Chem. Pharm. Bull.* 39:108 (1991).

The esterification reaction can be performed in the presence of a coupling agent (e.g.: dicyclohexylcarbodiimide, N,N'-carbonyldiimidazole or diethyl cyanophosphonate), optionally in the presence of a promoting agent (e.g.: N-hydroxysuccinimide or 4-dimethylaminopyridine) in aprotic or chlorinated solvents (e.g.: N,N-dimethylformamide or chloroform) at temperatures ranging

SCHEME 2



3 4

LERCANIDIPINE

Resolved lercanidipine enantiomers can be obtained by the esterification method illustrated in Scheme 2 using the corresponding homochiral acids which, when present in 1:1 ratio, constitute the above cited racemic acid 3.

from −10° to 140° C. according to well-known synthetic methods: Albertson, *Org. React.* Vol. 12, 205-212 (1962); Doherty et al. *J. Med. Chem.* 35:2 (1992); Ishihara, *Chem. Pharm. Bull.* 39:3238 (1991).

Alternatively, lercanidipine enantiomers can be prepared by reacting acid 5 (or acid 6) with alkyl chloroformate in presence of a tertiary amine (e.g., triethylamine), then adding the intermediate alcohol (4) at about 0°–80° C. Optionally, a promoting agent (e.g., 1-hydroxypiperidine) may be added before the addition of the intermediate (4) (Albertson, *Org. React.* Vol. 12. 157 (1962)).

The compounds and enantiomers of the invention can also be prepared by conversion of acid 5 (or acid 6) into the corresponding acyl halide using inorganic acid halides (e.g.: phosphorous pentachloride, oxalyl chloride, phosphorous trichloride, phosphorous oxychloride, or thionyl chloride) in a chlorinated solvent (e.g.: chloroform, dichloroethane, dichloromethane, 1,1,1-trichloroethane and the like), optionally in the presence of promoting agents (e.g.: N,N-dimethylformamide) at temperature ranging from about −10° to about 85° C. The resolved acyl halides can optionally, but need not, be isolated before the addition of the intermediate alcohol (4).

The lercanidipine enantiomers thus obtained are purified according to methods known in the art, either as a free base (e.g.: by column chromatography) or as a salt (e.g.: by reprecipitation or recrystallization). The above methods can also be used for all compounds having formula I.

It has been discovered that racemic mixtures of the compounds of the invention purified by recrystallization can be prepared in anhydrous or unsolvated form by recrystallizing the compound from a solution of the compound in: (I) an aprotic solvent; and (II) a protic solvent. The anhydrous compounds have improved stability and lower hygroscopicity when compared to the hydrated/solvated compounds.

The lercanidipine can be obtained by esterification from the corresponding dihydropyridine acid in accordance with a one-step reaction process starting with the acid chloride of compound 3, prepared *in situ*. This route provides improved yields and the formation of fewer reaction byproducts. The improved purity of the compounds of the invention avoids the use of chromatographic columns to isolate the desired final product. The compounds can be obtained directly by crystallisation as the hydrochloride salt with a high degree of purity.

The lercanidipine hydrochloride can be prepared according to the present invention in the anhydrous crystalline form, melting within a two degree range from about 185° to 190° C. range after recrystallisation of the crude hydrochloride compound from a solution of the compound in: (I) an aprotic solvent; and (II) a protic solvent.

Examples of aprotic solvents include chlorinated solvents, such as, for example, chloroform, dichloromethane, dichloroethane, chlorobenzene, 1,1,1-trichloroethane, and the like; non-chlorinated solvents, such as, for example, ethyl acetate, methyl acetate, tetrahydrofuran, dioxane, N,N-dimethyl-formamide, dimethylcarbonate, toluene, xylene, an (C_5–C_7) alkane, a (C_5–C_7) cycloalkane and the like.

Examples of protic solvents include solvents, such as, for example, methanol, ethanol, propanol, isopropanol, n-butanol, sec-butanol, tert-butanol and the like. All solvents can be used alone or optionally in a mixture with other solvents. All water miscible solvents can be used alone or in a mixture which may comprise at least one solvent and optionally include water.

Pharmaceutically acceptable salts are prepared from the free bases in a conventional manner.

Preferred pharmaceutically acceptable acid addition salts are those such as, for example, hydrochloric, sulfuric, maleic, succinic, citric, methanesulfonic and toluenesulfonic acids, and the like.

The hydrated and solvated forms of the compounds of the invention include compounds which have water or solvent molecules associated with them. There can be as little as about 0.25 molecules of water or solvent or there can be several water or solvent molecules for each lercanidipine molecule.

Typical solvents which associate or solvate the lercanidipines are solvents which can be used for recrystallization. Examples of these solvents include water, alcohols such as, for example, methanol, ethanol, propanol, isopropanol, n-butanol, sec-butanol, tert-butanol and the like.

According to the invention, the 1,4-dihydropyridine derivative having formula I may be administered to the patient as such, or in the form of any of its pharmaceutically acceptable salts, hydrates or solvates. It has been determined that the bioavailibility and efficacy of the compounds having formula I is comparable whether administered in the anhydrous form or in a hydrated or solvated form.

Preferred pharmaceutically acceptable acid addition salts include those formed with hydrochloric, sulfuric, maleic, succinic, citric, methanesulphonic and toluenesulphonic acids; they may be prepared from the free bases in conventional manner. Whatever the form (base, salt, hydrate or solvate), the active ingredient will usually be administered in admixture with a pharmaceutically acceptable carrier.

For the purpose of oral therapeutic administration, the active compounds of the invention can be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gum, and the like.

These preparations will typically contain at least about 0.5% of active compounds, but the amount of active ingredient can be varied depending upon the particular form and may conveniently contain from about 5% to about 70% of the weight of the unit. The amount of active compound in such compositions is such that a suitable dosage will be obtained. However, the desired dosage can be obtained by administering a plurality of dosage forms.

The compounds of the invention can be administered as an oral dosage of from about 0.1 to about 400 mg, preferably from about 1 to about 200 mg, and most preferably from about 5 to about 100 mg, per subject treated, per day. Therapy can continue for several months or years or indefinitely.

Preferred compositions and preparations according to the invention are prepared so that an oral dosage unit form contains from about 0.1 to about 400 mg of active compound.

The tablets, pills, capsules, troches, and the like can also contain, for example, the following optional ingredients: a binder such as, for example, microcrystalline cellulose, tragacanth gum, gelatin, and the like; an excipient such as, for example, starch or lactose, a disintegrating agent such as, for example, alginic acid, sodium starch glycolate, corn starch, and the like; a lubricant such as, for example, magnesium stearate or hydrogenated castor oil, a glidant such as, for example, colloidal silicon dioxide. Sweetening agents such as, for example, sucrose or saccharin or flavoring agents such as, for example, peppermint, methyl salicylate, or orange flavoring can also be added.

The active compounds of the invention can be orally administered, for example, with an inert diluent or with an edible carrier. They can, for example, be enclosed in gelatin capsules, or they can be compressed into tablets.

When the dosage unit form is a capsule, it can contain, in addition to materials described above, a liquid carrier such as, for example, a fatty oil.

For the purpose of parenteral administration, the 1,4-dihydropyridine derivatives may be incorporated into a solution or suspension.

These preparations typically contain at least 0.1% of active compound, but the amount of active ingredient may vary between 0.5% and about 30% of the weight thereof. The amount of active compound in such compositions is such that a suitable dosage will be obtained.

Preferably a parenteral dosage unit contains between 0.5 to 100 mg of active compound. The solutions or suspensions may also include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; an antibacterial agent such as benzyl alcohol or methyl parabens; an antioxidant such as ascorbic acid or sodium bisulfite; a chelating agents such as ethylenediaminetetraacetic acid; a buffer such as an acetate, a citrate or a phosphate and an agent for the adjustment of tonicity such as sodium chloride or dextrose. Parenteral multiple dose vials may be of glass or plastics material.

The parenteral multiple dose vials can be of glass or plastic materials.

Other dosage unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings. Thus, tablets or pills can be coated with a sugar, shellac, or another enteric coating agent.

A syrup can contain, in addition to the active compounds, sucrose as a sweetening agent, preservatives, dyes, colorings, and flavorants.

For the purpose of parenteral therapeutic administration, the active compounds of the invention can be incorporated into a physiologically acceptable solution or suspension.

These preparations preferably contain at least about 0.1% of active compound, but may be varied to from about 0.5% to about 30% by weight thereof. The amount of active compound in these compositions is such that a suitable dosage of from about 5 to about 100 mg will be obtained upon administration of the solution or suspension.

Preferred compositions and preparations according to the present inventions are prepared so that a parenteral dosage unit contains from about 5 to about 100 mg of active compound.

The solutions or suspensions can also include the following components: a sterile diluent such as, for example, water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol, other synthetic solvents, and the like; antibacterial agents such as, for example, benzyl alcohol, methyl parabens, and the like; antioxidants such as, for example, ascorbic acid, sodium bisulfite, and the like; chelating agents such as, for example, ethylenediaminetetraacetic acid and the like; buffers such as, for example, acetates, citrates, phosphates, and the like, and agents for the adjustment of tonicity such as, for example, sodium chloride, dextrose, and the like.

Materials used in preparing these various compositions for practicing the invention should be pharmaceutically pure and non-toxic at the level employed.

Additional compositions suitable for administration by various routes and containing compounds according to the present invention are also within the scope of the invention.

Dosage forms, additional ingredients and routes of administration contemplated herein include those disclosed in U.S. Pat. No. 4,089,969 and U.S. Pat. No. 5,091,182, both incorporated by reference in their entirety.

EXAMPLES

The following examples illustrate the invention without limitation.

Example 1

(S)-(+)-Methyl 1,1-N-Trimethyl-N-(3,3-Diphenylpropyl)-2-Aminoethyl 1,4-Dihydro-2,6-Dimethyl-4-(3-Nitrophenyl)pyridine-3,5-Dicarboxylate ((S)-Lercanidipine) Hydrochloride.0.5 H_2O

Thionyl chloride, 0.13 ml, was added, at −10° C., to a stirred suspension of 0.54 g of (R)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-5-methoxycarbonylpyridine-3-carboxylic acid in 2.9 ml of anhydrous dichloromethane and 0.75 ml of anhydrous N,N-dimethylformamide under a nitrogen atmosphere, and sheltered from the direct light.

After 1 hour at 0° C., a solution of 0.48 g of 2,N-dimethyl-N-(3,3-diphenyl-propyl)-1-amino-2-propanol, (prepared as described in U.S. Pat. No. 4,705,797), in 1 ml of dichloromethane, cooled to −5° C., was added. After stirring for 3 hours at 0° C. and standing overnight at 20°–25° C., the solvent was evaporated in vacuo and the residue was dissolved in 20 ml of ethyl acetate. The organic phase was washed sequentially, with brine (4 ml), 10% aqueous sodium carbonate solution (5×4 ml), brine (4 ml), 1N hydrochloric acid (5×5 ml), brine (4 ml), 10% aqueous sodium carbonate solution (2×5 ml) and with brine (4 ml). The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness in vacuo. The product was purified by flash chromatography on silica gel column eluting with petroleum ether-acetone 85:15. The individual, pure, TLC fractions (petroleum ether-acetone 7:3 by volume and chloroform-5N methanolic ammonia 99:1.1 by volume) were combined and evaporated to provide a residue that was dissolved in 75 ml of diethyl ether containing 3% of acetone. After filtration the solution was acidified with 3N ethereal hydrogen chloride and the precipitate was collected by suction filtration and dried at 78° C./15 mmHg to provide 0.66 g of the title compound.

M.P. 115°–125° C.; $[\alpha]_D^{25}$=+70.56° (MeOH; c=0.981).

Elemental analysis % for $C_{36}H_{41}N_3O_6$.HCl.0.5 H_2O Found: C. 65.47; H. 6.57; N. 6.29; Cl. 5.32; H_2O. 1.68 Calc.: C. 65.79; H. 6.60; N. 6.39; Cl. 5.39; H_2O. 1.37

^{1}H-NMR Spectrum of the base at 200 MHz ($CDCl_3$, δ): 8.10 (m,1H) nitrophenyl. CH in 2 7.97 (m,1H) nitrophenyl. CH in 4 7.62 (m, 1H) nitrophenyl. CH in 6 7.33 (dd,1H) nitrophenyl. CH in 5 7.29–7.10 (m, 10H) H benzhydryl aromatics 5.79 (bs,1H) pyridine. NH 5.05 (s, 1H) pyridine. CH in 4 3.92 (t,1H) benzhydryl. CH 3.63 (s, 3H) $COOCH_3$2.57 (m, 2H) $OC(CH_3)_2CH_2N$ 2.40–2.23 (m, 2H) $N(CH_3)CH_2CH_2$2.33/2.27 (2s, 6H) pyridine. CH_3 in pos. 2 and 6 2.19–2.09 (m, 2H) $N(CH_3)CH_2CH$2.17 (s, 3H) $NCH_3$1.35/1.31 (2s 6H) $OC(CH_3)_2CH_2N$

Example 2

(R)-(−)Methyl 1,1-N-Trimethyl-N-(3,3-Diphenylpropyl)-2-Aminoethyl 1,4-Dihydro-2,6-Dimethyl-4-(3-Nitrophenyl)pyridine-3,5-Dicarboxylate ((R)-Lercanidipine) Hydrochloride.H_2O

The title compound was obtained by the method described in Example 1 for the (S)-enantiomer, starting with (S)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-5-methoxycarbonylpyridine-3-carboxylic acid.

M.p. 115°–120° C.. $[\alpha]_D^{25}$=−70.88° (MeOH. c=0.975).

Elemental analysis % for $C_{36}H_{41}N_3O_6.HCl.H_2O$ Found: C. 64.93; H. 6.62; N. 6.24: Cl. 5.41; H_2O. 2.50 Calc.: C. 64.90; H. 6.60; N. 6.31: Cl. 5.32: H_2O. 2.70

The ^{1}H-NMR spectrum of the base in $CDCl_3$ was identical to the ^{1}H-NMR spectrum for the (S)-enantiomer from Example 1.

Example 3

Methyl 1.1-N-Trimethyl-N-(3.3-Diphenylpropyl)-2-Aminoethyl 1.4-Dihydro-2.6-Dimethyl-4-(3-Nitrophenyl) Pyridine-3.5-Dicarboxylate (*Lercanidipine*) Hydrochloride

Thionyl chloride. 45.8 g (0.385 mole). was added dropwise over 15 minutes into a stirred mixture comprising 11 6.2 g (0.35 mole) of 2.6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1.4-dihydropyridine-3-carboxylic acid (3) (prepared as described in German patent DE 2.847.237). 645 mL of anhydrous dichloromethane and 150 mL of anhydrous dimethylformamide. maintained under a nitrogen atmosphere at a temperature of −4° to +1° C. The mixture was stirred and maintained at this temperature for 1 hour. This was followed by dropwise addition of 104.1 g (0.35 mole) of 2.N-dimethyl-N-(3.3-diphenyl-propyl)-1-amino-2-propanol (4) (prepared as described in U.S. Pat. No. 4.705. 797). dissolved in 105 mL of anhydrous dichloromethane. over 15 minutes at a temerature of −10° to 0° C. After stirring for 3 hours at 0° C. and standing overnight at room temperature. the solvent was evaporated under vacuum and the residue dissolved in 3500 mL of ethyl acetate. The organic solution was washed sequentially with: brine (700 mL). 10% aqueous sodium carbonate solution (5×700 mL). brine (700 mL). 1N hydrochloric acid (5×700 mL) and finally with brine (700 mL). The organic layer was dried over anhydrous sodium sulphate for 30 minutes. filtered. shaken with 23 g of charcoal and filtered again. The solution volume was first reduced to about 1 liter by evaporation under vacuum. and the product allowed to crystallize. After standing 24 hours at 0° to 5° C.. the crystalline product was collected by vacuum filtration and recrystallized from EtOH 99% to provide 179.5 g (78% yield) of the title compound. M.P. 186°–188° C.

Example 4A

Effects on Arterial Myocyte Proliferation

Animal models of vascular injury have shown that an arterial lesion is followed by proliferation of medial myocytes. many of which migrate into the intima and further proliferate to form a neointimal lesion. The causes of these events are not completely understood. Recent findings have concluded that myocytes make up approximately 90–95% of the cellular population of the atherosclerotic lesion in young adults and compose an average of 50% of the advanced atherosclerotic plaque. In addition. vascular myocytes contribute to lesion formation and growth by synthesis of an extracellular matrix or they can accumulate lipids and become foam cells.

Thus. the elucidation of the factors affecting these phenomena affords new entry points for selective interference and inhibition of the process of atherogenesis (R. W. Wissler et al:. *Am. J. Med.* 91:(S1B). 3S (1991); S. M. Schwartz et al.. *Circ. Res.* 38:427 (1986); A. W. Cloves et al., *J. Cardiov. Pharmacol.* 14:512 (1989); R. W. Wissler et al., *Ann. NY Acad. Sci.* 598:418 (1990).

Migration of myocytes was investigated for the present invention using rat aortic smooth muscle cells in the presence of fibrinogen as chemotactic factor. whereas for studies on their proliferation rat and human cells were used. Cell count and [3H]-thymidine incorporation were used to evaluate myocytes growth. The methodology was as follows:

Myocytes were cultured from the intima-medial layer of the aortas of male Sprague-Dawley rats (200–250 g). Cells were grown in monolayers at 37° C. in a humidified atmosphere of 5% CO_2 in Eagle's minimum essential medium supplemented with 10% (v/v) fetal calf serum. 100 U/ml penicillin. 0.1 mg/ml streptomycin. 20 mM tricine buffer and 1% (v/v) non-essential amino acid solution. The medium was changed every third day. Cells were used between the 4th and 10th passage. Cell viability was timely assessed by trypan blue exclusion. Myocytes were identified for growth behavior. morphology and using monoclonal antibody specific for α-actin. the actin isoform typical of myocytes. Human vascular myocytes (A 617 from human femoral artery) kindly provided by Prof. G. Gabbiani of The General Pathology Institute (Geneva University—Switzerland) were grown in the same culture conditions. (These or other human vascular myocytes can be obtained and cultured as described by Corsini et al. *Atherosclerosis*, 101:117–125. (1993); Corsini et al. *Pharm. Res*, 23:173 (1991); Ross. R. *J. Cell Bio.*, 50:172. (1971); and Skalli. O. et al. *J. Cell Bio.*, 103:2787 (1986)).

Cells were seeded at various densities for rat (2×10^3) and human (5×10^4) myocytes/petri dish (35 mm). and incubated with Eagle's minimum essential medium supplemented with 10% fetal calf serum. Twenty-four hours later the medium was changed to one containing 0.4% fetal calf serum to stop cell growth. and the cultures were incubated for 72 hours. At this time (time 0) the medium was replaced by one containing 10% fetal calf serum in the presence or absence of known concentrations of the 1.4-dihydropyridine compounds being tested (shown in Table 1). The incubation was continued for an additional 72 hours at 37° C. Just before the addition of the test substances. an aliquot from each petri dish was used for cell counting. Cell proliferation was evaluated by cell count after trypsinization of the monolayers using a Coulter Counter model ZM. Cell viability was assessed by trypan blue exclusion. and found to be greater than 95% at the drug concentrations used.

TABLE 1

MYOCYTES GROWTH INHIBITION MEASURED BY CELL COUNT.

Myocytes from	LE IC_{50}(nM)	(R)-LE IC_{50}(nM)	(S)-LE IC_{50}(nM)	NI IC_{50}(nM)	LA IC_{50}(nM)
SD Rat	31.2	30.7	33.5	38.8	23.6
SHR Rat	14.1	9.2	16.8	n.t.	n.t.
WK Rat	16.6	15.3	20.7	n.t.	n.t.
Human	25.3	n.t.	n.t.	n.t.	n.t.

LE = Lercanidipine SD = Sprague Dawley
NI = Nicardipine SHR = Spontaneously Hypertensive
LA = Lacidipine WK = Wistar Kyoto
IC_{50} = concentration required to inhibit cell growth by 50%
n.t. = not tested

The data from Table 1 show that compounds of the invention. particularly. racemic lercanidipine and its resolved enantiomers inhibited the rat and human myocyte proliferation in a concentration-dependent manner and showed practically the same activity as the reference 1.4-dihydropyridines tested (NI and LA).

It was found that lercanidipine (and its enantiomers) proved active on the cells from all the species investigated. in particular. humans.

Example 4B

Effects on Arterial Myocyte Proliferation

In another experiment, synchronization of myocytes to the G_o/G_1 interphase of the cell cycle was accomplished by incubating logarithmically growing cultures (3×10^5 cells/plate) for 96–120 hours in a medium containing 0.4% fetal calf serum. Quiescent cells were incubated for 20 hours in a fresh medium with 10% fetal calf serum in the presence of the tested drugs. Cell proliferation was then estimated by nuclear incorporation of [^{3}H]thymidine, incubated with cells (1 μCi/ml medium) for 2 hours. Radioactivity was measured with Filter-Count scintillation cocktail (AQUASOL® Scintillation cocktail available from Packard-Groningen, Netherlands).

The results, expressed as cell proliferation (as % of control) against amount of the compound of the invention (Lercanidipine and its enantiomers) tested, are shown in FIG. 1. The decrease in [^{3}H]-thymidine uptake of myocytes confirmed the high potency of the compounds of the invention in inhibiting cell proliferation.

Example 5

Effects on Arterial Myocyte Migration

Migration of rat myocytes was examined using a 48-well micro chemotaxis chamber (Neuro-Probe, U.S.A.). Freshly trypsinized myocytes prepared according to the procedure in Example 4A were suspended in a medium supplemented with 5% fetal calf serum (assay medium). The lower wells, containing 27 μl of the assay medium that included fibrinogen (600 μg/ml) as chemotactic agent, were covered with a polyvinylpyrrolidone-free polycarbonate filter (8 μM pore size). Fifty μl of the cell suspension (1×10^6 cells/ml) were placed in the upper compartment with the tested compounds. Incubation was carried out for 5 hours at 37° C. in an atmosphere of 95% air and 5% CO_2.

After incubation the filter was removed from the chamber and non-migrated cells were scraped from the upper surface, and the filters washed with phosphate buffered saline three times. The filters were stained with Diff-Quik (Merz-Dade AG, Switzerland). The number of myocytes per 100×high power field that had migrated to the lower surface of the filters was determined microscopically. Six high power fields were counted per sample and the results were averaged.

The results, expressed as migration of cells (as % of control) against amount of the compound of the invention (Lercanidipine and its enantiomers) tested, are shown in FIG. 2. These data demonstrate the ability of lercanidipine, and its enantiomers to interfere with the migration of arterial myocytes. All the tested compounds were able to inhibit myocytes migration in a dose-dependent manner with the (R)-enantiomer showing the most pronounced effect.

Example 6A

Effects on Cholesterol Metabolism in Mouse Peritoneal Macrophages

Atheromas contain two main cell types, macrophages and smooth muscle cells (R. Ross, *Nature* 362:801 (1993)). Macrophages are derived from circulating monocytes and are the main lipid-loaded cells in the lesions. The mechanism by which they accumulate lipoprotein cholesterol and develop into foam cells depends mainly upon receptor-mediated processes, involving the so called "scavenger receptor" that recognizes chemically and biologically modified LDL, such as acetyl LDL (AcLDL) and oxidized LDL (Y. Kurihara et al., *Curr. Opin. Lipidol* 2:295 (1991)). The scavenger receptor, unlike the LDL receptor, is not subject to feed-back regulation and the result is a massive accumulation of cholesterol in foam cells. Cholesterol accumulates in macrophages in esterified form by a process involving the enzyme acylcoenzyme A-cholesterol acyltransferase (ACAT) which catalyzes the cholesterol esterification in cytoplasm (M. S. Brown et al. *J. Biol. Chem.* 255:9344 (1980)). Only free cholesterol, i.e., unesterified cholesterol can be removed from macrophages.

Cholesterol esterification induced by AcLDL in mouse peritoneal macrophages was investigated as follows. Mouse peritoneal macrophages were obtained by peritoneal lavage from mice (Balb/c Charles River, Calco, Italy) three days after intraperitoneal injection of thioglycolate. Cells ($2–3\times10^6$) were plated in 35 mm wells with Dulbecco's minimum essential medium containing 10% fetal bovine serum. After 3 hours, the dishes were washed to eliminate unattached cells and maintained in Dulbecco's minimum essential medium plus 10% fetal bovine serum for 24 hours before use. After cell plating, experiments were performed at 37° C. in serum free Dulbecco's minimum essential medium containing 0.2% essentially fatty acid-free bovine serum albumin, AcLDL, a specific ACAT inhibitor, S-58035 (See C. A. Ross et al., *J. Biol. Chem.* 259:815–819 (1984)), and lercanidipine, or the lercanidipine enantiomers indicated. Human LDL (d=1.019–1.063 g/ml) were isolated from plasma of healthy volunteers by sequential ultracentrifugation (Beckman L5-50, Palo Alto, Calif.). For acetylation, LDL were dialyzed against 0.15M NaCl, pH 7.4, diluted with an equal volume of saturated sodium acetate and treated with acetic anhydride. For [^{125}I]AcLDL, lipoproteins were labelled with sodium [^{125}I]iodide desalted by gel filtration on Sephadex G-25 eluted with phosphate buffered saline. Specific activity was 100–200 cpm/ng of protein. Trichloroacetic acid non-precipitable radioactivity was below 2% of total. All lipoproteins were sterile filtered. The cells were incubated for 24 hours with the tested compounds. After substitution of the medium with fresh solution, incubation was continued for additional 24 hours. During this second incubation [^{125}I]AcLDL was added (50 μg/ml). Cholesterol esterification was measured after addition of [1-^{14}C] oleic acid (0.68 mCi sample) complexed with bovine serum albumin during the last 1 or 2 hours of incubation by subsequent determination of radioactivity associated with cellular cholesteryl esters.

The results, expressed as level of inhibition (as % of control) against amount of the compound of the invention (Lercanidipine and its enantiomers) tested, are shown in FIG. 3. The compounds of the invention, particularly, lercanidipine and its enantiomers inhibited, in a concentration dependent manner, up to 90% of the formation of esterified cholesterol (in other words the compounds of the invention inhibited the esterifying effect of enzyme ACAT) induced by AcLDL in mouse perihoneal macrophage. The IC_{50} values for lercanidipine and the enantiomers ranged from 8 to 15 μM, as shown in FIG. 3. The (R)-enantiomer was the most potent compound.

Example 6B

Effects on Cholesterol Metabolism in Mouse Peritoneal Macrophages

Another set of experiments was performed to evaluate the effect of lercanidipine on cholesterol esterification in mac-

rophages loaded with cholesterol ester before the addition of the compounds of the invention, this condition being the same as in foam cells.

Cells, prepared according to the procedure in Example 6A, are loaded with cholesteryl esters by exposure for 24 hours to a medium containing 50 μg/ml acetyl LDL. ACAT inhibition was then determined as in Example 6A.

The results, expressed as level of inhibition (as % of control) against amount of the compound of the invention (Lercanidipine and its enantiomers) tested, are shown in FIG. 4. The compounds of the invention, particularly, lercanidipine and its enantiomers inhibited, in a concentration dependent manner, up to 70% of the formation of esterified cholesterol induced by AcLDL in mouse peritoneal macrophage. The IC_{50} value for the (R)-enantiomer was about 7 μM. The (R)-enantiomer of lercanidipine was slightly more potent than the racemate and (S)-lercanidipine was the least potent among the compounds tested.

Example 7

Cholesteryl Ester Hydrolysis

It was shown that lercanidipine and its enantiomers at a 5 μM concentration did not impair the capability of the macrophages to hydrolyze the esterified cholesterol stored in the cytoplasm. These experiments were performed by incubating cells preloaded with [^{3}H]cholesterol in the presence of the specific ACAT inhibitor S-58035. The blockage of intracellular reesterification of cholesterol allowed the assessment of the ability of cells to hydrolyze the accumulated cholesterol esters.

For studies involving the quantitation of the hydrolysis of cholesteryl esters in cytoplasmic lipid droplets, cells, prepared according to the procedure in Example 6A, are loaded with cholesteryl esters by exposure for 24 hours to the medium containing 50 μg/ml acetyl LDL. [1,2-^{3}H] Cholesterol is included in all loading media at a concentration of 0.5 μCi/ml. After 24 hours loading period, during which time the radiolabeled cholesterol is incorporated and esterified, the cell monolayers are washed and incubated an additional 24 hours in medium containing 0.1% bovine serum albumin to allow the intracellular pools of labelled cholesterol to equilibrate to the same specific activity. To quantitate cholesteryl ester hydrolysis, the loaded cells are incubated for up to 24 hours in Dulbecco's minimum essential medium containing, 0.1% bovine serum albumin, lercanidipines, and the compound S-58035, an inhibitor of acyl-coenzyme A-cholesterol acyltransferase. The inhibition of acyl-coenzyme A-cholesterol acyltransferase prevents the reesterification of any free cholesterol generated by cholesteryl ester hydrolysis and thus allows assessment of the activity of the hydrolase. The hydrolysis of the cholesteryl esters is quantified by determining the decrease of radiolabeled cholesteryl esters (R. H. Harrison et al., *J. Lipid. Res.* 31:2187 were washed with phosphate buffered saline and extracted with hexane:isopropanol (3:2 v/v). The media were extracted with chloroform:methanol (2:1 v/v). After solvent removal, free and esterified cholesterol were separated by TLC (isooctane:diethyl ether:acetic acid, 75:25:2 by volume). Cholesterol mass or radioactivity of the spots were determined by an enzymatic method (Boehringer Mannheim, Germany) (F. Bernini et al., *Atherosclerosis* 104:19 (1993)) or by liquid scintillation counting (Lipoluma Lumac, Landgraf, The Netherlands) respectively.

The results are given in Table 2. These data show that the addition of lercanidipine and its enantiomers did not influence the cellular hydrolytic activity, documented by the values of radioactivity in the esterified cholesterol fraction. The compounds of the invention do not impair the capability of the macrophages to hydrolyze the esterified cholesterol stored in the cytoplasm.

TABLE 2

EFFECT OF LERCANIDIPINE AND ITS ENANTIOMERS ON CHOLESTERYL ESTER HYDROLYSIS IN MACROPHAGES

	% of cholesteryl ester
AcLDL 50 μg/ml	31 ± 0.8
AcLDL 50 μg/ml + S-58035 1 μg/ml	15 ± 0.5
AcLDL 50 μg/ml + S-58035 1 μg/ml + 5×10^{-6}M LE*	12 ± 1.2
AcLDL 50 μg/ml + S-58035 1 μg/ml + 5×10^{-6}M (S)-LE*	14 ± 0.4
AcLDL 50 μg/ml + S-58035 1 μg/ml + 5×10^{-6}M (R)-LE*	16 ± 2.1

*LE = lercanidipine

Example 8

Effects of Lercanidipine on Chemical Oxidation of LDL

Experimental reports ascribe a key role for the oxidative modification of LDL in the early stages of atherosclerosis in humans. These reports suggest that LDL undergoes oxidative modifications in vivo (D. Steinberg et al., *N. Eng. J. Med.* 320:915 (1989); D. Steinberg etal., *JAMA* 264:3047 (1990); D. Steinberg, *Circulation* 84:1420 (1991); S. Yla-Herttuala, *Ann. Med.* 23:561 (1991); U.P. Steinbrecher, *Curr. Opin. Lipidol.* 1:411 (1990); J. L. Witztum, *Lancet* 344:793 (1994)) and that oxidatively modified LDL (OX-LDL) may induce atherogenesis by a number of mechanisms, including its enhanced uptake in tissue macrophages (via the scavenger receptor pathway) which leads to lipid accumulation, and chemotactic activity for monocytes, and cytotoxicity to arterial wall endothelial cells (S. Parthasarathy et al. *Prog. Lipid. Res.* 31:127 (1992).

The ability of Lercanidipine to function as an antioxidant was evaluated by incubating LDL, isolated from human plasma, for 22 hours with an oxidizing agent (20 μM Cu^{++}) in the presence of different concentrations of the test compounds (0.01 μM–50 μM). The LDL oxidation was followed by monitoring conjugated diene formation with a ultraviolet (UV) spectrometer at 234 nm.

The experimental conditions were as follows. LDL (d=1.019–1.063) were isolated from human pooled plasma by sequential ultracentrifugation at 4° C. and 40.000 rpm in a 50 Ti rotor, using a L5-50 ultracentrifuge (Beckman, Palo Alto, Calif.). The LDL were then dialyzed against 0.15M NaCl containing 0.01% ethylenediaminetetraacetic acid pH 7.4, sterilized by filtration through a 0.2 μM millipore filter and stored at 4° C. under nitrogen in the dark until use (up to 3 weeks). Before use, LDL were dialyzed against ethylenediaminetetraacetic acid free phosphate buffered saline, pH 7.4, on Sephadex G-25 columns (PD-10, Pharmacia Fine Chemicals, Uppsala, Sweden). Then the LDL were filtered through a sterile 0.22 μM filter. In a phosphate buffered saline (50 μg lipoprotein protein/ml), the LDL were oxidized by incubation at 25° C. with 20 μM $CuSO_4$ for 3 hours. The lercanidipine solution was prepared as a 10^{-3}M stock solution in methanol or ethanol and added as a methanol or ethanol solution (maximum 1% v/v) prior to addition of the copper solution. The effect of lercanidipine on the oxidation of LDL was determined by continuous monitoring of the formation of conjugated dienes by recording the increase in

the absorbance at 234 nm at 5 min intervals dunl a three hour period, against a phosphate buffered saline blank, using a UV spectrophotometer (Beckman DU 640) equipped with a continuous reading and an automatic 6-cell changer. The time prior to the onset of oxidation (lag time) was calculated as the intercept between the line of the maximum slope of the propagation phase and the x-axis. The results are shown in Table 3.

TABLE 3

EFFECT OF LERCANIDIPINE ON THE LAG TIME OF LDL OXIDATION

Lercanidipine μM	Lag time
0.0	46.5 ± 4.6
0.5	45.2 ± 3.7
1.0	49.8 ± 4.7
2.5	59.6 ± 2.3*
5.0	78.2 ± 4.8**
10.0	112.7 ± 5.2**

*P < 0.05
**P < 0.01

As shown in Table 3, lercanidipine, at a concentration of 10 μM clearly increased the lag time for LDL oxidation, the time required before oxidation begins, more than 2 times. The data show that the effect of the racemate was concentration-dependent, with concentrations greater than or equal to 2.5 μM significantly suppressing the onset of LDL oxidation. The activity of the resolved enantiomers was comparable to that of the racemate.

Example 9A

Effects of Lercanidipine on Cell-mediated Oxidation

The antioxidant capacity of the compounds of the invention, on cell-mediated oxidation, was evaluated by incubating LDL and mouse monocyte-macrophage cells (ATCC TIB 67 J774A.1) with $CuSO_4$ solution in the presence of apolipoprotein B (Apo B), lercanidipine or one of its enantiomers at several concentrations. The inhibition of formation of aldehydic breakdown products, i.e., secondary metabolites of lipid peroxidation, malonic dialdehyde (MDA), was assessed by detection of dialdehyde formation using the thiobarbituric acid assay (A. N. Hanna et al., *Biochem. Pharmacol.* 45:753 (1993)).

The antioxidant capacity was evaluated by incubating ethylene-diaminetetraacetic acid free-LDL under sterile conditions with 5 μM Cu^{++} (100 μg Apo B/ml) in the presence of J774A.1 cells. Oxidation was stopped after 22 hours incubation by addition of butylated hydroxytoluene to the medium (final concentration 40 μM) dissolved in ethanol. To the incubated sample (0.250 ml), was added trichloroacetic acid (0.750 ml, 0.20% w/v) followed by of thiobarbituric acid (0.750 ml, 0.67% w/v). The samples were heated at 100° C. for 20 min, followed by cooling and centrifugation. Malonic dialdehyde equivalents were calculated using 1,1,3,3-tetramethoxypropane as the standard.

The results, expressed as millimoles of MDA produced against amount of the compound of the invention (Lercanidipine and its enantiomers) tested, are shown in FIG. 5. The results show that lercanidipine and its enantiomers were effective in reducing LDL oxidation.

Example 9B

Effects of Lercanidipine on Cell-mediated Oxidation

Following the procedure in Example 9A, the effects of lercanidipine on cell-mediated oxidation were studied with a different cell line which shares many of the properties of endothelial cells (EAhy-926). (The cells were provided by: Prof. A. Catapano, Pharmacology Dept., Universita Statale—Milano.) (These or other endothelial cells can be obtained as described by C-J. S. Edgell et al. *Proc. Nat. AcAd. Sci.*, 80:3734-3737, (1983); and Gimbrone, M., *Prog. Hemostasis Thromb.*, 3:1–28, (1976)). The cells were treated with lercanidipine at a concentration of from about 10 to about 100 μM.

The results, expressed as millimoles of MDA produced against amount of Lercanidipine, are shown in FIG. 6. The results show that lercanidipine reduced the oxidation of LDL at a concentration of 10 to 100 μM.

Example 9C

Effects of Lercanidipine on Cell-mediated Oxidation

In experiments 9A and 9B, the cell-mediated oxidation effects of lercanidipine were investigated after incubation for 22 hours. In order to investigate the lower potency shown by lercanidipine in these conditions, the extent of lipid peroxidation in its presence was studied over time. A reaction mixture was prepared according to the procedure in Example 9A using lercanidipine at a concentration of 30 μM. Samples were withdrawn from the incubation medium and the oxidation product, MDA, measured as described above.

The results, expressed as millimoles of MDA produced against time are shown in FIG. 7. It can be seen that at a concentration of 30 μM, lercanidipine exerted a very high inhibition of lipid oxidation after 10 hours incubation. This shows that lercanidipine, administered at an appropriate time is as effective on cell mediated LDL oxidation as it is on Cu^{++}-mediated oxidation.

Lercanidipine proved the most potent 1,4-dihydropyridine tested in the assays herein, its potency being one order of magnitude higher than that of lacidipine, the most potent of the 1,4-dihydropyridine compounds previously known to have this activity.

Example 10

Effects on Blood Pressure in Hypertensive Dogs

The antihypertensive effects of oral administration of lercanidipine and its enantiomers were tested in renal hypertensive dogs.

Male Beagle dogs weighing 12–13 kg, aging 1–3 years (Nossan Allevamenti, Italy) were used. Chronic sustained hypertension was induced by bilateral renal artery constriction, according to the Goldblatt method "two-kidney, two clip hypertension". Under barbiturate anaesthesia (35 mg/kg i.v.), during two different surgical interventions one month apart from each other, both renal arteries were clipped with original renal silver clips and narrowed by about 60–70%. After two months from the last intervention, an experimental renal hypertension was produced and the animals were suitable for the implantation of a catheter. Under sodium pentobarbital anaesthesia (35 mg/kg i.v.), in sterile conditions, the dogs were catheterized by inserting an indwelling cannula (PH 200 Clay Adams) into the ascending aorta through the right common carotid artery. The catheter was subcutaneously exteriorized at the back of the neck, filled with heparinized saline solution and flushed daily to prevent clotting. After a week recovery time from surgery, the animals were connected to a HP 1290A pressure trans-

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ducer connected to an HP 8805B carrier amplifier of a Hewlett Packard HP 7700 multichannel polygraph, to monitor the arterial blood pressure. Heart rate was manually computed from the pressure trace.

All animals were alternatively treated with placebo, lercanidipine and its (R)- and (S)- enantiomers. The drugs were administered orally with a straight round aseptic tip catheter (Pores Serist—France). The drugs were suspended in aqueous 0.5% Methocel A4C plus Antifoam M10 (10%). The amount administered was 1 ml/kg.

The suspending medicine was used as placebo. During experimental performance, the arterial blood pressure was continuously recorded 30 min. before (basal values) and up to 8 hours after drug administration.

Lercanidipine and (S)-lercanidipine induced a dose-related decrease in arterial blood pressure. The ED_{25} values (dose inducing 25% decrease in DBP at peak effect) were evaluated by linear regression analysis and summarized in the Table 4.

TABLE 4

Compound	ED_{25} (mg/kg) 95% C.L.
Lercanidipine	0.9 (0.5 ÷ 1.6)
(S)-Lercanidipine	0.4 (0.3 ÷ 0.7)
(R)-Lercanidipine	>>30

C.L. = Confidence limits

The (S)-Lercanidipine enantiomer exerted the most potent antihypertensive action. This compound was two fold more efficacious at lowering blood pressure than the racemate. The (R)-enantiomer did not affect blood pressure up to 30 mg/kg (<10% decrease in DBP).

Example 11A

High Temperature Stress Stability

Lercanidipine hydrochloride hemihydrate, prepared according to the procedure described in U.S. Pat. No. 4,705,797, was compared with anhydrous lercanidipine prepared according to Example 3, to determine the stress stability of the compounds. The two samples, lercanidipine hydrochloride hemihydrate, and anhydrous lercanidipine hydrochloride were heated, in the light, at 100° C. for 48 hours. The samples were tested at the following times; 0 hours, 24 hours, and 48 hours. The purity of the lercanidipine was checked by HPLC analysis under the following conditions:

Column: m-Bondapak C-18 (Waters), particle size 10 mm. 300×3.9 mm i.d.

Eluant: CH_3CN (61%):0.15M $NaClO_4$ aqueous solution at pH 3 (adjusted with $HClO_4$) (39%; v/v)

Elution: isocratic

Flow: 1.5 mL/min

Temp. 25° C.

Detector: UV (240 nm)

Attenuation: 0.05 AUFS

The results, expressed as assay % of the compound present, based on an HPLC analysis, are shown in Table 5 below:

TABLE 5

Stress Stability at 100° C.

	Assay % by HPLC		
Compound	0 hr	24 hrs	48 hrs
Anhydrous	99.74	99.36	99.01
Hemihydrate	99.85	92.35	90.96

From Table 5, it can be seen that the anhydrous form of lercanidipine hydrochloride exhibited superior stability over the hydrated form.

Example 11B

Water Content at 75% Relative Humidity

Samples of anhydrous lercanidipine hydrochloride and lercanidipine hydrochloride hemihydrate were placed in open polyethylene bags in flasks and heated, in the dark, at 60° C. and 75% relative humidity. The samples were checked for hygroscopicity by determination of the water content using the Karl-Fisher method. The samples were tested at 8 and 15 days.

Two additional samples of the anhydrous lercanidipine hydrochloride and lercanidipine hydrochloride hemihydrate were placed in open polyethylene bags in flasks and heated, in the dark, at 40° C. and 75% relative humidity. The samples were tested as described above at 8 and 15 days. The results of these tests are shown in Table 6.

TABLE 6

Water Content at 75% Relative Humidity

		Water Content		
		0 days	8 days	15 days
60° C.	Anhydrous	0.28	0.85	0.77
	Hemihydrate	1.42	4.00	4.04
40° C.	Anhydrous	0.28	0.30	0.32
	Hemihydrate	1.42	3.14	3.05

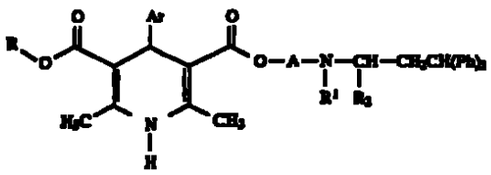
From Table 6, it can be seen that the anhydrous lercanidipine has a much lower tendency to absorb water than the hemihydrate form. The lack of hygroscopicity is advantageous because the anhydrous compound will remain unchanged. Thus, it will be easier to handle during the preparation and dispensing of pharmaceutical formulations.

The invention has been described above by reference to preferred embodiments but, as those skilled in the art will appreciate, many additions, omissions and modifications are possible all within the scope of the claims below.

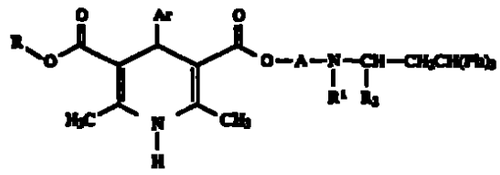
We claim:

1. A method for preventing, retarding, or reducing atherosclerotic lesions or atherosclerotic degradation of arterial walls in a patient in need thereof;

said method comprising, administering to a mammal in need of said prevention, retardation, or reduction, at least one compound having the formula I:



I



I

wherein:

Ph is phenyl.

Ar is: 2-nitrophenyl. 3-nitrophenyl. 2.3-dichlorophenyl or benzofurazan-4-yl

A is a branched chain alkylene radical having from 2 to 6 carbon atoms.

R is a straight or branched chain alkyl radical having from 1 to 6 carbon atoms. optionally mono-substituted by an alkoxy substituent having from 1 to 6 carbon atoms.

R_1 is hydrogen. hydroxy. or an alkyl radical having from 1 to 4 carbon atoms.

R_2 is hydrogen. or methyl;

or an enantiomer of a compound having formula I: a hydrated compound having formula I; a solvated compound having formula I; a pharmaceutically acceptable acid addition salt. or any of the foregoing.

2. The method according to claim 1. wherein the compound having formula I is an R-enantiomer.

3. The method according to claim 1. wherein the compound having formula I is an S-enantiomer.

4. The method according to claim 1. wherein R is methyl. Ar is 3-nitrophenyl. R_1 is methyl and R_2 is hydrogen.

5. The method according to claim 1. wherein A is $—C(CH_3)_2—CH_2—$ or $—CH_2—C(CH_3)_2—CH_2—$.

6. The method according to claim 1. wherein the compound having formula I is methyl 1.1.N-trimethyl-N-(3.3-diphenylpropyl)-2-aminoethyl 1.4-dihydro-2.6-dimethyl-4-(3-nitrophenyl)pyridine-3.5-dicarboxylate or a pharmaceutically acceptable acid addition salt thereof.

7. The method according to claim 6. wherein the compound having formula I is:

R-(−)-methyl 1.1.N-trimethyl-N-(3.3-diphenylpropyl)-2-aminoethyl 1.4-dihydro-2.6-dimethyl-4-(3-nitrophenyl)pyridine-3.5-dicarboxylate.

8. The method according to claim 6. wherein the compound having formula I is:

S-(+)-methyl 1.1.N-trimethyl-N-(3.3-diphenylpropyl)-2-aminoethyl 1.4-dihydro-2.6-dimethyl-4-(3-nitrophenyl)pyridine-3.5-dicarboxylate.

9. The method according to claim 1. wherein the dosage of said compound is from 0.1 to about 400 mg. per day.

10. The method according to claim 9. wherein the dosage of said compound is from about 1 to about 200 mg. per day.

11. The method according to claim 10. wherein the dosage of said compound is from about 0.5 to about 100 mg. per day.

12. The method according to claim 1. wherein said mammal in need of said treatment suffers from hypertension.

13. The method according to claim 1 wherein said mammal is need of said treatment does not suffer from hypertension.

14. A method for the treatment of atherosclerosis, said method comprising. administering to a patient. in need of such treatment. a compound having the formula I:

wherein:

Ph is phenyl.

Ar is: 2-nitrophenyl. 3-nitrophenyl. 2.3-dichlorophenyl or benzofurazan-4-yl.

A is a branched chain alkylene radical having from 2 to 6 carbon atoms.

R is a straight or branched chain alkyl radical having from 1 to 6 carbon atoms. optionally mono-substituted by an alkoxy substituent having from 1 to 6 carbon atoms.

R_1 is hydrogen. hydroxy. or an alkyl radical having from 1 to 4 carbon atoms.

R_2 is hydrogen. or methyl;

or an enantiomer of a compound having formula I; a hydrated compound having formula I; a solvated compound having formula I; a pharmaceutically acceptable acid addition salt. or any of the foregoing.

15. The method according to claim 14. wherein the compound having formula I is an R-enantiomer.

16. The method according to claim 14. wherein the compound having formula I is an S-enantiomer.

17. The The method according to claim 14. wherein R is methyl. Ar is 3-nitrophenyl. R_1 is methyl and R_2 is hydrogen.

18. The method according to claim 14. wherein A is $—C(CH_3)_2—CH_2—$ or $—CH_2—C(CH_3)_2—CH_2—$.

19. The method according to claim 14. wherein the compound having formula I is methyl 1.1.N-trimethyl-N-(3.3-diphenylpropyl)-2-aminoethyl 1.4-dihydro-2.6-dimethyl-4-(3-nitrophenyl) pyridine-3.5-dicarboxylate or a pharmaceutically acceptable acid addition salt thereof.

20. The method according to claim 17. wherein the compound having formula I is:

R-(−)-methyl 1.1.N-trimethyl-N-(3.3-diphenylpropyl)-2-aminoethyl 1.4-dihydro-2.6-dimethyl-4-(3-nitrophenyl)pyridine-3.5-dicarboxylate.

21. The method according to claim 17. wherein the compound having formula I is:

S-(+)-methyl 1.1.N-trimethyl-N-(3.3-diphenylpropyl)-2-aminoethyl 1.4-dihydro-2.6-dimethyl-4-(3-nitrophenyl)pyridine-3.5-dicarboxylate.

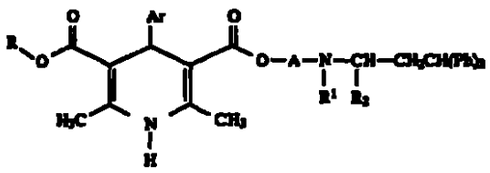
22. The method according to claim 14. wherein the dosage of said compound is from 0.1 to about 400 mg. per day.

23. The method according to claim 22. wherein the dosage of said compound is from about 1 to about 200 mg. per day.

24. The method according to claim 23. wherein the dosage of said compound is from about 0.5 to about 100 mg. per day.

25. A method for reducing the migration of cells. reducing build up of connective tissues. or preventing lipid accumulation in the blood vessels of a mammal in need of said treatment comprising administering to said mammal from 0.1 to about 400 mg. per day. of a compound having Formula I:

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I

wherein:

Ph is phenyl.

Ar is: 2-nitrophenyl, 3-nitrophenyl, 2,3-dichlorophenyl or benzofurazan-4-yl.

A is a branched chain alkylene radical having from 2 to 6 carbon atoms.

R is a straight or branched chain alkyl radical having from 1 to 6 carbon atoms, optionally mono-substituted by an alkoxy substituent having from 1 to 6 carbon atoms.

R_1 is hydrogen, hydroxy, or an alkyl radical having from 1 to 4 carbon atoms, and

R_2 is hydrogen, or methyl.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,767,136
DATED : Jun. 16, 1998
INVENTOR(S) : Abraham Sartani, Amedeo Leonardi, Rodolfo Testa

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page, column 1, in section "[30] Foreign Application Priority Data", change "MI94 A 001513" to --MI95 A 001513--.

Signed and Sealed this
Fifth Day of January, 1999

Attest:

Attesting Officer

Acting Commissioner of Patents and Trademarks

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,767,136
DATED : June 16, 1998
INVENTOR(S) : Abraham Sartani, Amedeo Leonardi, Rodolfo Testa

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item [30], **Foreign Application Priority Data**, change "MI94 A 001513" to – MI95 A 001513 –.

Signed and Sealed this

Fifteenth Day of January, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,767,136 Page 1 of 1
DATED : June 16, 1998
INVENTOR(S) : Abraham Sartani, Amedeo Leonardi and Rodolfo Testa

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page,
Item [56], References Cited, OTHER PUBLICATIONS, please insert:

-- Pauletto et al., *J. Cardiov. Pharmacol.*, 19:2 (1992), S8-S16
Sinzinger et al., *J. Cardiov. Pharmacol.*, 19:3 (1992), S29-S31
Knorr et al., *Cardiovasc. Drugs Ther.*, 4:S5 (1990), 1027-1031,
Fleckenstein-Grün et al., *J. Human Hypertens.*, 6:S1 (1992), S13-S18
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Gaviraghi et al., *Pharmacol. Res.*, 31:314 (1995), 251-254
Nayler, *Am. J. Hypertens.*, 7:10(2) (1994), 126S-130S
Weinstein, *J. Cardiov. Pharmacol.*, 12:6 (1988) S29-S35
Henry, *Clin. Invest. Med.*, 10:6 (1987) 601-605
Weinstein, *Am. J. Med.*, 86:S4(A) (1989) 27-32 --

Signed and Sealed this

Twenty-second Day of October, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

D8.)

UNITED STATES PATENT OFFICE.

WERNER BOLTON, OF LEIPSIC, GERMANY, ASSIGNOR, BY MESNE ASSIGNMENTS, TO JAMES A. DEUTHER, OF BOSTON, MASSACHUSETTS.

PROCESS OF GENERATING ACETYLENE GAS.

SPECIFICATION forming part of Letters Patent No. 596,139, dated December 28, 1897.

Application filed October 16, 1895. Serial No. 566,096. (No specimens.)

To all whom it may concern:

Be it known that I, WERNER BOLTON, a subject of the Emperor of Russia, residing at Leipsic, in the Kingdom of Saxony, German Empire, have invented certain new and useful Improvements in the Generation of Acetylene Gas; and I do hereby declare the following to be a full, clear, and exact description of the invention, such as will enable others skilled in the art to which it appertains to make and use the same.

My invention relates to improvements in the generation of acetylene gas.

Acetylene gas has not been hitherto made suitable for use in portable lamps, the rate at which it is generated by the action of the water upon the carbid of calcium being so much in excess of the rate at which it is consumed as to establish a degree of pressure that would be dangerous in an ordinary lamp-reservoir. Hence the generation of the gas at a regulated rate becomes a condition precedent to the use of it in the class of lamps indicated above.

According to the present invention the carbid of calcium is impregnated in a vacuum with any substance which will not mix with water—such as stearin, paraffin-oil, and the like in that respect. The rate at which the generation from the impregnated carbid proceeds varies according to the degree of impregnation and can therefore be provided for accordingly. The impregnation has the further effects of decreasing the weight of the carbid and of preventing its decomposition by air which might otherwise enter it.

What I claim is—

1. The process of generating acetylene gas at a regulated rate, which consists in first impregnating carbid of calcium with a substance substantially insoluble in water, and then subjecting the prepared carbid to the action of water, substantially as set forth.

2. A step in the process of generating acetylene gas at a regulated rate, which consists in impregnating carbid of calcium with a substance substantially insoluble in water at a pressure below that of the atmosphere, substantially as set forth.

3. Carbid of calcium impregnated with a substance substantially insoluble in water—such as stearin, as a new composition of matter, substantially as set forth.

4. The process of generating acetylene gas slowly and continuously as required for consumption in a lamp, which consists in first impregnating and filling the interstices of porous carbid of calcium at a pressure below that of the atmosphere with a substance substantially insoluble in water, and then subjecting the prepared mass to the action of water, substantially as set forth.

5. As a new article of manufacture for the production of acetylene gas, metallic carbid and an inert substance uniformly distributed throughout the carbid, whereby on exposure to water the reaction will be retarded.

6. The process of generating acetylene gas at a regulated rate, which consists in first impregnating metallic carbid with an inert substance, and then subjecting the prepared carbid to the action of water, whereby the production of acetylene gas is effected gradually.

In testimony whereof I have affixed my signature in presence of two witnesses.

WERNER BOLTON.

Witnesses:
RUDOLPH FRICKE,
OTTO DOEDERLEIN.

US 5915.351)

D7

United States Patent [19]

Nardi et al.

[11] Patent Number: 4,705,797

[45] Date of Patent: Nov. 10, 1987

[54] N-(3,3-DIPHENYLPROPYL) AMINOETHYL ESTERS OF 1,4-DIHYDRO-2,6-DIMETHYL-PYRIDINE-3,5-DICARBOXYLIC ACID, COMPOSITIONS AND USE

[75] Inventors: Dante Nardi; Amedeo Leonardi, both of Milan; Gabriele Graziani, Arese; Giorgio Bianchi, Milan, all of Italy

[73] Assignee: Recordati S.A., Chemical and Pharmaceutical Company, Chiasso, Switzerland

[21] Appl. No.: 701,672

[22] Filed: Feb. 14, 1985

[30] Foreign Application Priority Data

Feb. 14, 1984 [GB] United Kingdom 8403866

[51] Int. Cl.[4] A61K 31/455; C07D 211/90; C07D 413/04

[52] U.S. Cl. 514/356; 514/338; 546/271; 546/321

[58] Field of Search 546/321, 271; 514/338, 514/356

[56] References Cited

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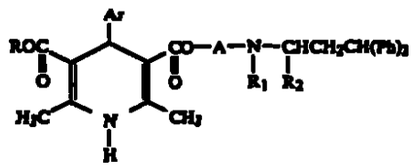
Bossert, et al. "4-Aryldihydropyridines" Angew. Chem. Int. Ed. Engl. 20 (1981) pp. 762–769.

Schramm, et al, "Novel Dihydropyridines with Positive Inotropic Action" Nature, vol. 303 (Jun. 9, 1983) pp. 535–537.

Primary Examiner—Henry R. Jiles
Assistant Examiner—Dale A. Bjorkman
Attorney, Agent, or Firm—Burns, Doane, Swecker & Mathis

[57] ABSTRACT

Novel antihypertensive and coronary dilating asymmetric diesters of 1,4-dihydro-2,6-dimethyl-pyridine-3,5-dicarboxylic acid (or the stereoisomers or pharmaceutically acceptable acid addition salts thereof) have the general formula (I):



(I)

wherein Ph is phenyl, Ar is 2-nitrophenyl, 3-nitrophenyl, 2,3-dichlorophenyl or benzofurazan-4-yl, A is a straight or branched chain alkylene radical having from 2 to 6 carbon atoms, R is a straight or branched chain alkyl radical having from 1 to 6 carbon atoms, optionally mono-substituted by an alkoxy substituent having from 1 to 6 carbon atoms, R_1 is hydrogen, hydroxy or an alkyl radical having from 1 to 4 carbon atoms, and R_2 is hydrogen or methyl. The subject diesters are facilely prepared from the aldehydes ArCHO and esters of acetoacetic and 3-aminocrotonic acids.

8 Claims, No Drawings

—N-(3,3-DIPHENYLPROPYL)-AMINOETHYL— ESTERS OF 1,4-DIHYDRO-2,6-DIMETHYL-PYRIDINE-3,5-DICARBOXYLIC ACID, COMPOSITIONS AND USE

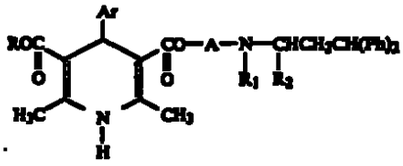
BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to novel asymmetric diesters of 1,4-dihydro-2,6-dimethyl-pyridine-3,5-dicarboxylic acid, to stereochemically isomeric forms and pharmaceutically acceptable acid addition salts thereof, to processes for the preparation of same and to pharmaceutical compositions comprised thereof.

SUMMARY OF THE INVENTION

A major object of the present invention is the provision of novel asymmetric diesters of 1,4-dihydro-2,6-dimethyl-pyridine-3,5-dicarboxylic acid, said diesters having the following general formula (I):



(I)

wherein Ph is phenyl, Ar is 2-nitrophenyl, 3-nitrophenyl, 2,3-dichlorophenyl or benzofurazan-4-yl, A is a straight or branched chain alkylene radical having from 2 to 6 carbon atoms, R is a straight or branched chain alkyl radical having from 1 to 6 carbon atoms, optionally mono-substituted by an alkoxy substituent having from 1 to 6 carbon atoms, R_1 is hydrogen, hydroxy or an alkyl radical having from 1 to 4 carbon atoms, and R_2 is hydrogen or methyl; as well as the pharmaceutically acceptable acid addition salts of such diesters.

Another object of the present invention is the provision of a process for the preparation of the esters of the general formula (I), said process comprising condensing a compound of the general formula (II):



(II)

wherein Ar is as defined above with a compound of the general formula (III):



(III)

wherein A_1 is either (a) a radical of the general formula (IV):



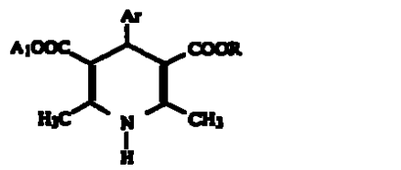
(IV)

wherein A, R_1, R_2 and Ph are as defined above, or (b) a radical of the general formula —AX wherein A is as defined above and X is a halogen atom, and then reacting the condensate thus formed with a compound of the general formula (V):



(V)

wherein R is as defined above, to give a compound of the general formula (VI):



(VI)

wherein A_1, R and Ar are as defined above, and, if A_1 is the radical —AX as defined above, then converting —AX to a radical of the general formula (IV), also as defined above.

DETAILED DESCRIPTION OF THE INVENTION

More particularly according to the present invention, it will be appreciated that a variety of syntheses are encompassed within the metes and bounds of the process hereinbefore outlined. The reaction scheme to follow illustrates a number of same.

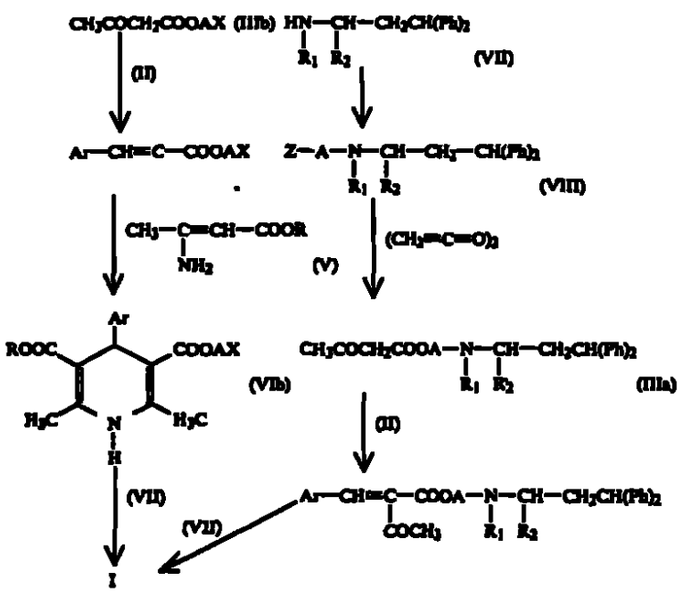
For example, the diesters (I) may be prepared by condensing a haloalkyl acetoacetate (IIIb) [III: A_1=—AX] with an aldehyde (II), reacting the condensate with an alkyl or alkoxyalkyl 3-aminocrotonate (V), and converting the radical —AX of the resultant 1,4-dihydropyridine derivative (VIb) [VI: A_1=—AX] to a radical (IV) by reaction with 3,3-diphenylpropylamine or a derivative thereof (VII). Alternatively, the radical (IV) may be introduced into the compound (III) prior to ring formation. This particular route begins with a compound (VIII):



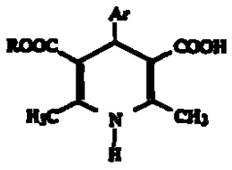
(VIII)

wherein Z is halogen or hydroxy and A, R_1, R_2 and Ph are as above defined. This is available from the amine (VII) by conventional alkylation to introduce haloalkyl or hydroxyalkyl group Z—A and reaction of the alkylated amine with diketene. Compound (IIIa) [III: A_1=IV] is condensed with an aldehyde (II) and the product is reacted with a 3-aminocrotonate (V).

Thus, said reaction scheme may be represented as:



The above reaction scheme includes a synthesis of the pyridine ring. If a 1,4-dihydropyridine derivative (VIb) is already present, it is only necessary to condense it with an amine (VII). Similarly, if a free acid of the formula (IX):



wherein R and Ar are as defined above is available, it is only necessary to condense it with a compound (VIII), or with a compound of the general formula YAX wherein Y is halogen and A and X are as above defined to give a 1,4-dihydropyridine derivative (VIb) for condensation with an amine (VII). These condensations are themselves within the ambit of the invention. When X is chlorine, they are preferably carried out in toluene or xylene under reflux, whereas when X is bromine they may be carried out in dimethylformamide at lower temperatures.

The diesters (I) obtained may be purified according to methods known per se, and crystallized as salts from suitable solvents in purified form. The pharmaceutically acceptable salts according to the invention may be prepared from the free bases in conventional manner. Preferred pharmaceutically acceptable acid addition salts are those of hydrochloric, sulfuric, maleic, succinic, citric, methanesulfonic and toluenesulfonic acids.

The diesters (I) and their salts according to the invention possess valuable antihypertensive activity and are also effective against coronary heart diseases. Accordingly, the invention also provides pharmaceutical compositions comprising diesters of the general formula (I) as above defined, or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable diluent or carrier.

The LD_{50} of the compounds according to the invention was determined in the mouse, both i.p. and per os, according to the method described by C. S. Weil [*Biometrics*, 8, 249 (1952)].

The antihypertensive activity of the diesters according to the invention was evaluated in male hypertensive rats (SHR, Wister-Kyoto strain, 15–25 weeks old). The determination of blood pressure was performed by an indirect method [M. Gerald et al., *Arzneim. Forsch.*, 18, 1825 (1968)]. The animals were prewarmed in a heating chamber at a temperature of from 35° C. to 37° C. for a period of 15 minutes before pressure determination. The compounds tested by oral route were dissolved or suspended in a 0.5% methylcellulose solution. Controls were given only the vehicle. Systolic blood pressure and heart rate were measured 1, 3, 5 and 7 hours after drug administration by means of a tail-cuff and a pulse transducer.

Coronary dilating activity was evaluated in anesthetized normotensive rats (weighing about 500 g), as the ability to antagonize methacholine induced coronary spasm. Rats were instrumented for methacholine infusion into the coronary ostium, while spastic activity was detected as ST segment elevation in D_2 ECG recording [K. Sakai et al., *J. Pharm. Meth.*, 5, 325 (1981)].

The compounds tested by i.v. infusion were dissolved in water: dimethylformamide (9:1 by volume). Activity was detected as normalization of ECG tracing after compounds administration during methacholine infusion. The former test indicates that the diesters possess valuable antihypertensive activities; according to the methacholine test the compounds according to the invention are also considered effective against coronary heart diseases.

Compound	LD_{50} mg/kg i.p.	LD_{50} mg/kg os	ED_{25} SHR os mg/kg	ED_{50} iv μg/kg
2279	121	279	7.3	297
2288	72	197	1.5	46
2263	171	254	3.4	197
2375	83	657	2.6	82

-continued

Compound	LD_{50} mg/kg i.p.	os	ED_{25} SHR os mg/kg	ED_{30} iv µg/kg
2361	99	493	7.2	—
2150	50	116	3.0	—
2329	63	401	3.4	—
2352	222	2195	6.9	—
2383	—	500	3.4	—

ED_{25} = antihypertensive activity
ED_{30} = coronary dilating activity
— = not tested

In order to further illustrate the present invention and the advantages thereof the following specific examples are given, it being understood that same are intended only as illustrative and in nowise limitative.

EXAMPLE 1

Preparation of 1-methyl-2-chloroethyl α-acetyl-3-nitrocinnamate

A solution containing 12.2 g of 3-nitrobenzaldehyde and 14.3 g of 1-methyl-2-chloroethyl acetoacetate in 80 ml of toluene, maintained at 0°–5° C., was saturated with hydrogen chloride gas. After two days at 20°–25° C., the residual hydrogen chloride was removed by bubbling nitrogen through the solution. The toluene was then evaporated off in vacuo. The residual oil was dissolved in dichloromethane and the solution thus obtained was washed with water until it was neutral. The organic phase which was separated and dried, was then evaporated to dryness under vacuum at 20° C. The residue was crystallized from 200 ml of isopropanol to give 20.70 g of the title compound, melting at 95°–96° C.

Following the procedure described above, but employing the appropriate aldehydes and acetoacetates, the following compounds were also prepared:

[i] 3-chloropropyl-α-acetyl-3-nitrocinnamate, m.p. 55°–60° C.;

[ii] 2-bromoethyl-α-acetyl-3-nitrocinnamate, m.p. 85°–95° C.;

[iii] 2-chloroethyl-α-acetyl-2,3-dichlorocinnamate, oil;

[iv] 1-methyl-2-chloroethyl α-acetyl-2,3-dichlorocinnamate, oil; and

[v] 2-chloroethyl-α-acetyl-β-(benzofurazan-4-yl)-acrylate, oil.

These compounds were mixtures of E/Z isomers and were employed as such in the further reactions, without separating the components.

EXAMPLE 2

Preparation of methyl 1-methyl-2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate

A solution comprising 18.72 g of 1-methyl-2-chloroethyl α-acetyl-3-nitrocinnamate, prepared as described in Example 1, and 7.12 g of methyl 3-aminocrotonate in 48 ml of isopropanol was refluxed under stirring for 210 minutes. After cooling, the reaction mixture was evaporated to dryness under vacuum and the oil thus obtained was chromatographed on silica gel (200 g) using chloroform as eluent. Evaporating the fractions containing a sole product (TLC, chloroform: ethylacetate 95:5) gave a thick oil, which was dissolved in diethyl ether. The solvent was then evaporated off at 20°–25° C. to give 15.75 g of the title compound, m.p. 95°–102° C., which could be employed without further purification.

Following the procedure described above, but using the appropriate 3-aminocrotonates and the α-acetyl cinnamates obtained as reported in Example 1, the following intermediates were obtained. They were all purified by column chromatography or by crystallization from suitable solvents:

[i] ethyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 158°–161° C.;

[ii] methyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 118°–120° C.;

[iii] isobutyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 146°–152° C.;

[iv] methyl 3-chloropropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 145°–146° C.;

[v] methyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-pyridine-3,5-dicarboxylate, m.p. 167°–69° C.;

[vi] methyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(benzofurazan-4-yl)-pyridine-3,5-dicarboxylate, m.p. 117°–118° C.;

[vii] isobutyl 1-methyl-2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-pyridine- 3,5-dicarboxylate, low-melting solid;

[viii] 2-propoxyethyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 104°–106° C.; and

[ix] methyl 2-bromoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 141–143° C.

EXAMPLE 3

Preparation of methyl 4-bromobutyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate

A suspension comprising 9.96 g of 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-5-methoxycarbonyl-pyridine-3-carboxylic acid, 5.4 ml of 1,4-dibromobutane and 2.07 g of potassium carbonate in 60 ml of dimethylformamide was heated at 50° C. under stirring for 2.5 hours. The mixture was then cooled to room temperature, poured into 400 ml of water and extracted with diethyl ether. The ethereal extracts were then dried on anhydrous sodium sulfate and evaporated to dryness to give an oil which was purified by flash chromatography on silica gel columns, employing first chloroform and then chloroform: ethyl acetate 97:3 by volume as eluent.

The unitary TLC fractions (chloroform: ethyl acetate 9:1 by volume) were evaporated to give 11.75 g (84%) of the title compound, m.p. 113°–118° C.

EXAMPLE 4

Preparation of methyl N-(3,3-diphenyl-1-methylpropyl)-N-methyl-3-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2202)

A solution of 3.94 g of methyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate and 4.78 g of 1,N-dimethyl-3,3-diphenylpropylamine in 12 ml of toluene was refluxed under stirring for 25 hours. Upon completion of the reaction the mixture was diluted with diethyl ether and the 1,N-dimethyl-3,3-diphenylpropylamine hydrochloride formed was collected by filtration. The filtrate was then evaporated, and the crude product thus obtained was purified by repeated chromatography on silica gel, using first chloroform, then chloroform: ethyl acetate (85:15 by volume) and lastly ethyl acetate as eluent. The

purified product was dissolved in methanol and ethanolic hydrogen chloride was added. The solution was filtered and evaporated to dryness. Repeated washings with warm diethyl ether: acetone (98:2 by volume) afforded 2.98 g of the title compound, m.p. 113°–120° C.

Following the procedure described above, but using N-methyl-3,3-diphenylpropylamine instead of 1,N-dimethyl-3,3-diphenylpropylamine, methyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (2206), m.p. 108°–113° C. was prepared.

The melting points of both compounds noted above refer to the respective hydrochlorides.

EXAMPLE 5

Preparation of methyl N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2279)

A solution comprising 5.92 g of methyl 2-chloroethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)- pyridine-3,5-dicarboxylate and 9.50 g of 3,3-diphenylpropylamine in 20 ml of xylene was refluxed under stirring for 105 minutes. The mixture was then cooled. By dilution with diethyl ether a semisolid compound was obtained, and it was separated from the liquid by decantation. The residue was treated with diethyl ether at 0°–4° C. and the supernatant was decanted. This procedure was repeated until a solid was obtained, and this was collected by filtration. Mother liquors and washings from the decantation were collected and evaporated to dryness under vacuum. The residue was chromatographed on a silica gel column (270 g) using a mixture of chloroform and acetone as eluent. Pure fractions were combined and the solvent was evaporated off. The residue was dissolved in diethyl ether and hydrogen chloride in diethyl ether was added. The solid (3.92 g) was crystallized from ethyl acetate or isopropyl acetate and recrystallized from ethyl acetate to give 3.46 g of the title compound, m.p. 128°–32° C.

Following the procedure described above, the following additional compounds were prepared:

[i] isobutyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 124°–129° C. (compound 2352);

[ii] methyl N-(3,3-diphenyl-1-methylpropyl)-N-methyl-3-aminopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 113°–118° C. (compound 2244);

[iii] methyl N-(3,3-diphenylpropyl)-N-methyl-3-aminopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 100°–107° C. (compound 2263);

[iv] ethyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 164°–167° C. (compound 2274);

[v] methyl 1, N-dimethyl-N-(3,3-diphenylpropyl)- 2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 114°–123° C. (compound 2288);

[vi] methyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-pyridine-3,5-dicarboxylate, m.p. 161° C. (compound 2306);

[vii] isobutyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 123°–124° C. (Compound 2329);

[viii] methyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(benzofurazan-4-yl)-pyridine-3,5-dicarboxylate, m.p. 161°–166° C. (compound 2344);

[ix] isopropyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, m.p. 160°–164° C. (compound 2350); and

[x] methyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate H_2O, m.p. 118°14 125° C. (compound 2332).

All melting points of the compounds noted above refer to the respective hydrochlorides.

EXAMPLE 6

Preparation of methyl N-(3,3-diphenylpropyl)-N-methyl-4-aminobutyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2361)

A solution comprising 9.34 g of methyl 4-bromobutyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (prepared as described in Example 3) and 9.0 g of N-methyl-3,3-diphenylpropylamine in 15 ml of dimethylformamide was maintained for 16 hours at room temperature. The solution was then diluted with 250 ml of diethyl ether, and the starting amine hydrobromide thus formed was collected by filtration.

The filtered solution was washed twice with water and the organic solvent was removed under vacuum. The oily residue was then purified by flash chromatography on silica gel columns using chloroform: acetone (from 95:5 to 7:3 by volume) mixtures as eluent. By evaporating the unitary TLC fractions (chloroform: diethyl ether 1:1 by volume) under vacuum, an oil was obtained. The oil was dissolved in methanol and the solution was acidified with hydrogen chloride in ethanol and evaporated under vacuum to give an oily residue. This was dissolved in acetone, and the solution was again evaporated in vacuo.

The solid residue thus obtained was dissolved in 300 ml of methyl acetate and the solution filtered and slowly diluted with 900 ml of diethyl ether, maintaining in the mixture a methyl acetate: diethyl ether ratio of 1:3 by volume. The mixture was then stirred at 0° C. to give a precipitate which was collected by filtration and purified.

7.0 g of the hydrochloride hemihydrate of the title compound, m.p. 91°–103° C., were obtained.

EXAMPLE 7

Preparation of methyl N-(3,3-diphenylpropyl)-N-hydroxy-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2410)

3 ml of triethylamine were added to a solution of 2.40 g of N-hydroxy-3,3-diphenylpropylamine in 17 ml of dimethylformamide. 4.39 g of methyl 2-bromoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate were added to the mixture. The mixture was then maintained in a nitrogen atmosphere and heated for 160 minutes at 105° C. After cooling, the mixture was poured into water and extracted with a diethyl ether: ethyl acetate mixture. The organic layer was washed many times with water and then dried. The solvent was evaporated off in vacuo. The oily residue obtained was purified first by chromatography on a

silica gel column with ethyl acetate gradient in toluene and then on a silica gel column containing ammonia at 5.5% by weight with about 6 N methanolic ammonia gradient in chloroform. The solid thus obtained was dissolved in a 2:1 by volume acetone: diethyl ether mixture and hydrogen chloride in diethyl ether was added. The hydrochloride hemihydrate of the title compound was filtered off, washed with isopropanol and dried at 100° C. and 0.5 mmHg for 1 hour. Yield 1.51 g, m.p. 100°–105° C.

EXAMPLE 8

Preparation of methyl 1,N-dimethyl-N-(3,3-diphenylpropyl)-3-aminopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2366)

A solution of 3.72 ml of 3-oxo-but-1-ene was added dropwise, over a period of 20 minutes and such that the temperature did not exceed 2°–3° C., to a cooled solution of 7.87 g of N-methyl-3,3-diphenylpropylamine in 7 ml of diethyl ether. The solution thus obtained was evaporated in vacuo at room temperature to give 10.3 g of N-methyl-N-(3,3-diphenylpropyl)-4-aminobutan-2-one (100%) as free base, shown to be practically pure by TLC (chloroform: methanol 9:1 by volume) which could be used as such for further reactions. This compound could be transformed into the corresponding hydrochloride (m.p. 133°–135° C.) by treating the free base with hydrogen chloride in diethyl ether, and crystallizing the product thus obtained first from acetone: ethyl acetate: isopropanol then from acetone.

To a solution of 9.16 g of the compound obtained as described above in 30 ml of methanol and maintained at 0° C., within 5 minutes 0.90 g of sodium borohydride were slowly added. When the addition was completed, the methanol was evaporated off in vacuo and the residue treated with water (about 100 ml) and diethyl ether (about 100 ml). The water was then washed with diethyl ether and the ethereal phases were combined, dried on anhydrous sulfate and evaporated to dryness in vacuo. The product (9.25 g) thus obtained, N-methyl-N-(3,3-diphenylpropyl)-4-aminobutan-2-ol, was a reddish oil unitary at TLC (chloroform: methanol 9:1 by volume).

A suspension comprising 7.44 g of the product from the last step, 12.46 g of 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-5-methoxycarbonyl-pyridine-3-carboxylic acid, 8.51 g of N,N'-dicyclohexylcarbodiimide and 0.54 g of 4-dimethylaminopyridine in 50 ml of dimethylformamide was stirred at room temperature for 8 days. The suspension was then diluted with 450 ml of diethyl ether and the N,N'-dicyclohexylurea thus precipitated was collected by filtration. The filtered solution was washed with water (3×200 ml). The organic phase was dried and then evaporated off in vacuo. The oil thus obtained was purified by flash chromatography on silica gel columns using chloroform: ethyl acetate mixtures (1:1 by volume) as eluent. The fractions which were unitary at TLC (ethyl acetate) were evaporated to give a semi-solid residue. This residue was dissolved in diethyl ether and the solution thus obtained was filtered and acidified with hydrogen chloride in diethyl ether to give a solid which was collected by filtration. The solution of this solid in 200 ml of ethyl acetate was diluted with 600 ml of ether, maintaining acetate: diethyl ether ratio of 1:3 by volume. The mixture was stirred at 0° C. until a precipitate was obtained. The operation was repeated three times, to give 8.50 g of the hydrochloride of the title compound, m.p. 93°–110° C.

EXAMPLE 9

Preparation of 2,N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol

A mixture of 16.87 g of N-methyl-3,3-diphenylpropylamine and 3.1 ml of 1-chloro-2-methyl-2-propanol in 20 ml of xylene was refluxed under stirring for 8 hours. After cooling at 15°–20° C., the suspension was diluted with diethyl ether and the starting amine hydrochloride thus obtained was collected by filtration. The solvent was removed from the filtrate in vacuo to give an oil which was then purified by flash chromatography on silica gel columns, employing chloroform with increasing amounts of methanol as eluent. The unitary TLC fractions (chloroform: methanol 94:6 by volume) were evaporated to give 7.8 g of the title compound as a brown oil.

EXAMPLE 10

Preparation of N-methyl-N-(3,3-diphenylpropyl)-1-amino-2-propanol

6.38 g of 1,2-epoxypropane was added to a solution of 22.5 g of N-methyl-3,3-diphenylpropylamine in 60 ml of methanol. The solution was maintained at 15°–20° C. for 3 days, and then an additional 1.16 g of 1,2-epoxypropane was added. This solution was maintained for 24 hours at room temperature. The methanol was evaporated off and the product purified by chromatography on a silica gel column (methanol gradient in chloroform), to give 24 g of the title compound as colorless oil.

EXAMPLE 11

Preparation of 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl acetoacetate

A solution of 13.10 g of the compound as prepared in Example 9 in 10 ml of toluene was heated to 85° C. 3.6 ml of diketene were added dropwise over a period of 10 minutes, maintaining the temperature below 100° C. The solution was then heated for 2 hours at 80° C. After cooling at 15°–20° C. the mixture was evaporated in vacuo and the oily residue was purified by flash chromatography on silica gel columns using chloroform containing increasing amounts of methanol as eluent. The unitary TLC fractions (chloroform: methanol 95:5 by volume) were evaporated to dryness to give 12.3 g of the title compound as a brown oil.

EXAMPLE 12

Preparation of 1,N-dimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl acetoacetate

Following the procedure described in Example 11, but using the compound prepared in Example 10 instead of that prepared in Example 9, the title compound was obtained as an oil.

EXAMPLE 13

Preparation of 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl α-acetyl-3-nitrocinnamate

Hydrogen chloride was bubbled into a solution of 3.78 g of 3-nitrobenzaldehyde and 9.54 g of the compound prepared in Example 11 in 25 ml of chloroform, cooled at 0° C., until the solution was saturated. The solution was maintained for 3 days at 15° C. It was then diluted with chloroform and washed with dilute aqueous sodium hydroxide solution until neutral. The organic phase was dried and the solvent was evaporated

off. The solid thus obtained was dissolved in ethyl acetate and the solution was cooled at 0° C. A slight excess of hydrogen chloride in diethyl ether was added. The solid thus formed was repeatedly treated with diethyl ether to give 12.50 g of the title compound, m.p. 65°–80° C. The product was an E/Z isomeric mixture, and was used as such in further reactions.

EXAMPLE 14

Preparation of 1,N-dimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl α-acetyl-3-nitrocinnamate

Following the procedure described in Example 13, but using the compound prepared in Example 12 instead of that prepared in Example 11, the title compound was obtained as an oil. The product was an E/Z isomeric mixture, and was used as such in further reactions.

EXAMPLE 15

Preparation of 1,N-dimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl α-acetyl-2,3-dichlorocinnamate

Following the procedure described in Example 14, but using 2,3-dichlorobenzaldehyde instead of 3-nitrobenzaldehyde, the title compound was obtained as an oil. The product was an E/Z isomeric mixture, and was used as such in further reactions.

EXAMPLE 16

Preparation of methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2375)

A solution of 2.37 g of methyl 3-aminocrotonate and 10.29 g of the compound prepared in Example 13 in 15 ml of isopropanol was refluxed for 3 hours. The mixture was then cooled and evaporated to dryness in vacuo. The oily residue thus obtained was purified by flash chromatography on silica gel columns, using chloroform with increasing amounts of acetone as eluent. The unitary TLC fractions (chloroform: acetone, 9:1 by volume) were evaporated and the solid thus obtained was dissolved in methanol. A slight excess of ethanolic hydrogen chloride was added and the solution was evaporated to dryness. The residue was dissolved in acetone and again evaporated in vacuo. The residue was crystallized from 2 liters of water containing 2 ml of 1 N hydrochloric acid and 5 ml of water saturated with sodium chloride, to give 4.8 g of the hydrochloride hemihydrate of the title compound, m.p. 119°–123° C.

EXAMPLE 17

Preparation of isobutyl 1,N-dimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-pyridine-3,5-dicarboxylate (compound 2383)

Following the procedure described in Example 16, but using isobutyl 3-aminocrotonate instead of methyl 3-aminocrotonate and the compound of Example 15 instead of that of Example 13, the hydrochloride of the title compound, m.p. 162°–164° C., was obtained.

EXAMPLE 18

Preparation of 2-propoxyethyl 1,N-dimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-pyridine-3,5-dicarboxylate (compound 2400)

Following the procedure described in Example 17, but using 2-propoxyethyl 3-aminocrotonate instead of isobutyl 3-aminocrotonate, the hydrochloride hydrate of the title compound, m.p. 108°–118° C., was obtained.

EXAMPLE 19

Preparation of 2-propoxyethyl 1,N-dimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2408)

Following the procedure described in Example 18, but using the compound of Example 14 instead of that of Example 15, the hydrochloride hemihydrate of the title compound, m.p. 85°–92° C., was obtained.

EXAMPLE 20

Preparation of 2-propoxy-1,1-dimethylethyl 1,N-dimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2409)

Following the procedure described in Example 19, but using 2-propoxy-1,1-dimethylethyl 3-aminocrotonate instead of 2-propoxyethyl 3-aminocrotonate, the hydrochloride hemihydrate of the title compound, m.p. 86°–95° C., was obtained.

EXAMPLE 21

Preparation of 2-propoxyethyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2411)

Following the procedure described in Example 16, but using 2-propoxyethyl 3-aminocrotonate instead of methyl 3-aminocrotonate, the hydrochloride hemihydrate of the title compound, m.p. 87°–92° C., was obtained.

EXAMPLE 22

Preparation of 2-propoxy-1,1-dimethylethyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (compound 2412)

Following the procedure described in Example 16, but using 2-propoxy-1,1-dimethylethyl 3-aminocrotonate instead of methyl 3-aminocrotonate, the hydrochloride hemihydrate of the title compound, m.p. 88°–92° C., was obtained.

While the invention has been described in terms of various preferred embodiments, the skilled artisan will appreciate that various modifications, substitutions, omissions, and changes may be made without departing from the spirit thereof. Accordingly, it is intended that the scope of the present invention be limited solely by the scope of the following claims.

We claim:

1. Methyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(benzofurazan-4-yl)-pyridine-3,5-dicarboxylate, or a stereoisomer or pharmaceutically acceptable acid addition salt thereof.

2. Methyl-N-(3,3-diphenylpropyl)-N-hydroxy-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, or a stereoisomer or pharmaceutically acceptable acid addition salt thereof.

3. Methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate, or a stereoisomer or pharmaceutically acceptable acid addition salt thereof.

4. 2-Propoxy-1,1-dimethylethyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate,

BNSDOCID: <US 4705797A1 |_>

or a stereoisomer or pharmaceutically acceptable acid addition salt thereof.

5. A pharmaceutical composition of matter for use as an antihypertensive or as a coronary dilator comprising an antihypertensive or coronary dilating amount of a member selected from the group consisting of

methyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(benzofurazan-4-yl)-pyridine-3,5-dicarboxylate;

methyl N-(3,3-diphenylpropyl)-N-hydroxy-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate;

methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3 -nitrophenyl)-pyridine-3,5-dicarboxylate; and

2-propoxy-1,1-dimethylethyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate;

or a stereoisomer or pharmaceutically acceptable acid addition salt thereof, and a pharmaceutically acceptable diluent or carrier therefor.

6. A pharmaceutical composition of matter for use as an antihypertensive or as a coronary dilator comprising an antihypertensive or coronary dilating amount of methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate or a stereoisomer or pharmaceutically acceptable acid addition salt thereof, and a pharmaceutically acceptable diluent or carrier therefor.

7. A method of eliciting an antihypertensive or coronary dilating response in a mammalian organism, comprising administering to a mammalian organism in need of such treatment a member selected from the group consisting of

methyl N-(3,3-diphenylpropyl)-N-methyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(benzofurazan-4-yl)-pyridine-3,5-dicarboxylate;

methyl N-(3,3-diphenylpropyl)-N-hydroxy-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate;

methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3 -nitrophenyl)-pyridine-3,5-dicarboxylate; and

2-propoxy-1,1-dimethylethyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate;

or a stereoisomer or a pharmaceutically acceptable acid addition salt thereof.

8. A method of eliciting an antihypertensive or coronary dilating response in a mammalian organism, comprising administering to a mammalian organism in need of such treatment

methyl 1,1,N-trimethyl-N-(3,3-diphenylpropyl)-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate; or a stereoisomer or pharmaceutically acceptable acid addition salt thereof.

* * * * *

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Gervasi, Gemma
NOTARBARTOLO & GERVASI
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ITALIE

NOTARBARTOLO & GERVASI
MILANO
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26 AGO. 2003

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	26.08.2003
Applicant's or agent's file reference 2808PTWO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP02/08699	05.08.2002	06.08.2001

Applicant
RECORDATI IRELAND LIMITED

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Parriche, S Tel. +49 89 2399-7890

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2608PTWO	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP02/08699	International filing date *(day/month/year)* 05.08.2002	Priority date *(day/month/year)* 06.08.2001
International Patent Classification (IPC) or both national classification and IPC C07D211/90		
Applicant RECORDATI IRELAND LIMITED		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.03.2003	26.08.2003
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Schuemacher, A Telephone No. +49 89 2399-7816

INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/EP02/08699

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-55 as originally filed

Claims, Numbers

1-74 received on 29.07.2003 with letter of 28.07.2003

Drawings, Sheets

1/19-19/19 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/EP02/08699

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22, 25, 26, 28, 31-60
	No:	Claims	23, 24, 27, 29, 30, 61-74
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-74
Industrial applicability (IA)	Yes:	Claims	1-74
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: LEONARDI, AMEDEO ET AL., EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY (1998), 33(5), 399-420, XP004127371
D2: WO 97 03669 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.;RECORDATI) 6 February 1997
D3: WO 96 35668 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.;RECORDATI) 14 November 1996
D4: MCCLELLAN K J ET AL.,DRUGS, ADIS INTERNATIONAL LTD, AT, vol. 5, no. 60, Nov. 2000, pages 1123-1140, XP008001723 ISSN: 0012-6667
D5: US-A-5 767 136 (TESTA RODOLFO ET AL) 16 June 1998; cited in the application
D6: US 596 139 A (RECORDATI SA) 9 December 1997; cited in the application
D7: US-A-4 705 797 (NARDI DANTE ET AL) 10 Nov.1987; cited in the application

1. Novelty, Article 33(2) PCT:

The present application concerns the different polymorphic forms of lercanidipine hydrochloride, the processes for their preparation and their use as antihypertensive agents.
The processes of new claims 3 and 12 can be considered to be novel over the above cited prior art documents since the isolating step c) which comprises respectively the steps i-viii) and i'-viii') has been defined more accurately and renders the processes new over the prior art.
Consequently, the processes of present claim 3 and 12 and the dependent claims 4-22 have not been anticipated by D1-D7 and can be considered to be new.
The crude lercanidipine hydrochloride solid forms (A) and (B) of present claims 1 and 2 are characterised by the specific ethyl acetate contents. According to the Applicant's letter, these compounds can only be obtained according to the new processes 3 and 12 and should not be seen as impure forms but as intermediates products permitting to obtain the pure crystalline form (I) and (II) of lercanidipine hydrochloride. Thus, the subject-matter of claims 1 and 2 is considered to satisfy the criteria of Article 33(2) PCT.
The method for producing lercanidipine hydrochloride crystalline form (II) of claim 42, 48 and 52 have not been anticipated by the prior art documents: present claim 42 differ from

the known processes because of the use of acetonitrile as solvent for the recrystallisation, claim 48 because of the step e'''), in which the solution should be cooled to a temperature between 20 and 40°C and claim 52 because of the protic polar or aprotic dipolar solvent containing up to 50% by weight of water. .
Thus, the subject-matter of claims 25, 26, 42-60 are new.

As mentioned in the Applicant's letter dated the 28.07.2003, Example 1 of D3, Example 3 of D5 correspond to Example 3 of D2 and describe the preparation of lercanidipine hydrochloride (with M.p. 186-188°C) starting from the acyl chloride and the aminoalcohol compounds in dichloromethane and DMF, followed by the working-up in ethyl acetate and the **recrystallisation from either absolute or 99% ethanol.**
The crystalline hydrochloride form (I) of claim 23, prepared according to the method of claim 27 seems therefore to be anticipated by D2, D3 and D5 : even if the physical data characterising the crystalline form (I) in claim 23, 24 or 73 have not been disclosed in the prior art document, it is assumed that the process as disclosed in claim 27 corresponds in fact to a recrystallisation with a C_1-C_5 alcohol solvent containing a max. of 5% water. Since it is not sufficiently clarified if the crystalline hydrochloride form (I) of present claims 23, 24 and obtained according to present claim 27 is different to the compound of the prior art D2, D3 and D5 it is considered that crystalline hydrochloride form (I) of claims 23, 24 and 27 is the same as the compound of D2, D3 or D5 just defined in other wording : a further definition of the same compound by further parameters is insufficient to establish novelty.
Thus, compound-claims 23 and 24, process-claims for their preparation and their use as antihypertensive agents in claims 61-74 are not considered to meet the requirements for novelty of article 33(2) PCT.

2. Inventive step, article 33(3) PCT:

The problem to be solved by the present invention may be regarded as to develop different processes in order to obtain different polymorphic forms of lercanidipine hydrochloride, which are useful as antihypertensive agents.
Prior art document D2 is already considered to provide a solution to the above mentioned technical problem because it also discloses the preparation of lercanidipine hydrochloride crystalline form. The difference of the subject-matter of the claims 23, 24 and 27 vis-à-vis the state of the art is not at present apparent and appropriate information to clarify where an inventive step lies is missing in the present application (e.g. comparative data with the compound obtained according to D2).
Furthermore, it is considered as common technical knowledge to obtain different

polymorphic forms of a compound by changing the crystallisation conditions like the nature of the solvents or the temperature.

The problem underlying the present application is therefore to be seen the provision of crystalline forms having unexpected advantageous effect with regard to the prior art.

Indeed, the crude forms (A) and (B) claim to have a higher solubility a faster drying rate and a simplified crystallisation process in comparison to the known crude form (C). However, there is no evidence of such advantages in the application.

Additionally, crystalline forms (I) and (II) are claimed to be obtained with a higher purity and uniformity which also allows a better dosage forms. However, there is neither an indication in the present application that the form (I) is indeed more suited for immediate release formulations and dosage forms nor that form (II) shows a higher bioavailability in comaprison to the crystalline compound disclosed in D2 .

In the absence of evidence of such unexpected effect, the present application cannot be considered to involve an inventive step and does not meet the requirements of Article 33(3) PCT.

CLAIMS

1. Crude lercanidipine hydrochloride solid Form (A), having a melting point of about 150-152°C (DSC peak) and comprising about 3-4% (w/w) ethyl acetate.

2. Crude lercanidipine hydrochloride solid Form (B), having a melting point of about 131-135°C (DSC peak) and comprising about 0.3-0.7% (w/w) ethyl acetate.

3. A method of producing the crude lercanidipine hydrochloride Form of claim 1, comprising the steps of:

a) reacting 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid with a chloride selected from the group consisting of thionyl chloride and oxalyl chloride in an aprotic dipolar solvent and an aprotic polar solvent to produce the corresponding carbonyl chloride;

b) reacting, *in-situ*, the chloride of step a) with 2, N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propyl alcohol to form crude lercanidipine hydrochloride; and

c) isolating the crude lercanidipine hydrochloride of step b and recovering crude lercanidipine hydrochloride Form (A),

wherein step (c) consists essentially of the following steps:

i. washing the crude lercanidipine hydrochloride of step b) with water;
ii. removing the water from step i) to produce a mixture;
iii. concentrating the mixture of step ii) and adding solvent to produce a suspension having about the same volume as the initial volume of the mixture of step ii) and a water content, according to Karl Fischer, of between 0.10 and 0.15%;
iv. cooling the suspension obtained in step iii) to obtain a solid;
v. filtering the solid from step iv);
vi. re-suspending the solid of step v) in ethyl acetate;
vii. cooling the suspension of step vi) ; and
viii. filtering and drying the precipitate of step vii) to produce the crude lercanidipine hydrochloride Form (A).

4. The method of claim 3 wherein the reacting step b) is conducted at a temperature between -5 and +5°C.

5. The method of claim 3 wherein the chloride in step a) is thionyl chloride.

AMENDED SHEET

6. The method of claim 3 wherein in step c) ii) comprises removing the water from step c) i) by azeotropic distillation under vacuum within the range 200-300 mm Hg, at a temperature not higher than 60°C, to produce a mixture.

7. The method of claim 3 wherein the resuspending step c) vi) comprises stirring at 60-65°C for about 1 hour.

8. The method of claim 3 wherein the drying in step c) viii) is in an oven at 70°C.

9. The method of claim 3, wherein the washing step c) i) is with water; the mixture in step c) iii) is concentrated to 1/3 of its initial volume and solvent is added to produce a suspension having about the same volume as the initial volume of said mixture; and the water content of said suspension according to Karl Fischer , is between 0.1 and 0.15%.

10. The method of claim 3, wherein cooling in step c) iv) is carried out up to a temperature within the range of 0-5°C.

11. The method of claim 3 wherein cooling in step vii) is carried out up to a temperature within range of 5-10°C.

12. A method of producing the crude lercanidipine hydrochloride Form (B) of claim 2, comprising the steps of:

a) reacting 2,6-dimethyl-5-methoxycarbonyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylic acid with a chloride selected from the group consisting of thionyl chloride and oxalyl chloride in an aprotic dipolar solvent and an aprotic polar solvent to produce the corresponding carbonyl chloride;

b) reacting, *in-situ*, the chloride of step a) with 2, N-dimethyl-N-(3,3-diphenylpropyl)-1-amino-2-propyl alcohol to yield crude lercanidipine hydrochloride; and

c) isolating the crude lercanidipine hydrochloride of step b) and recovering crude lercanidipine hydrochloride Form (B), wherein step (c) consists essentially of the following steps:

i') washing the crude lercanidipine hydrochloride of step b) with water,

ii') removing the water from step i') to produce a mixture having a water content of about 2%, measured according to Karl Fischer;

iii') concentrating the mixture of step ii') and adding solvent to produce a solution having about the same volume as the initial volume of the mixture

of step ii') and a water content, according to Karl Fischer, of between 0.9 and 1.1%;

iv') cooling the solution of step iii') to obtain a solid;

v') filtering the solid of step iv');

vi') re-suspending the solid of step v') in a solvent;

vii') cooling the suspension of step vi'); and

viii') filtering and drying the solid obtained to produce the crude lercanidipine hydrochloride Form (B).

13. The method of claim 12 wherein the reacting step b) is conducted at a temperature between -5 and +5°C.

14. The method of claim 12 wherein the chloride is thionyl chloride.

15. The method of claim 12 wherein step c) ii') comprises removing the water from step i') by azeotropic reflux to produce said mixture.

16. The method of claim 12 wherein step c) iii') comprises concentrating said mixture to 3/4 of its initial volume.

17. The method of claim 12 wherein said solvent in steps c) iii') and vi') is ethyl acetate.

18. The method of claim 12 wherein the step c) iv') comprises cooling the solution to a temperature within the range of 0-5°C.

19. The method of claim 12 wherein said step c) vi') further comprises stirring the suspension at 60-65°C for about one hour.

20. The method of claim 18 wherein said step c) vii') further comprises cooling the solid to a temperature between 5 and 10°C.

21. The method of claim 12 wherein said drying in step viii') is in an oven at approximately 70°C.

22. The method of any one of claims 3-21, wherein said aprotic dipolar solvent is dimethylformamide and said aprotic polar solvent is ethyl acetate.

23. Isolated lercanidipine hydrochloride crystalline Form (I), which has the X-ray diffraction pattern, at wavelength Kα, as shown in Figure 11.

24. The lercanidipine crystalline Form of claim 23, wherein distances, (I/Io) ratios, and 2 θ angles of significant peaks in Figure 11 are:

D (X)	Relative Intensity (I/Io)	2 θ angle
16.3	83	5.4
6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

25. Isolated lercanidipine hydrochloride crystalline Form (II), which has an X-ray diffraction pattern, at wavelength Kα, as shown in Figure 12.

26. The lercanidipine crystalline Form of claim 25, wherein distances, (I/Io) ratios, and 2 θ angles of significant peaks in Figure 12 are:

D (X)	Relative Intensity (I/Io)	2 θ angle
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8
3.81	41	23.4
3.77	100	23.6
3.58	44	24.8
3.54	29	25.2

27. A method of producing lercanidipine hydrochloride crystalline Form (I), as claimed in anyone of claims 23 and 24 , which comprises:

d) adding a C1-C5 alcohol solvent containing a maximum of 5% water (v/v) to a crude lercanidipine hydrochloride Form and heating under reflux and with stirring to produce a clear solution;

e) cooling the solution of step d) and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is ≤ 2%; and

f) recovering the solid obtained from step e), and drying said solid to produce the lercanidipine hydrochloride crystalline Form (I).

AMENDED SHEET

28. The method of claim 27, wherein step f) comprises filtering the solid obtained from step e), washing the solid with isopropanol and re-filtering the solid before drying.

29. The method of claim 27 wherein the alcohol of step d) is selected from the group consisting of isopropanol, ethanol and anhydrous ethanol.

30. The method of claim 27, wherein the crude Form is lercanidipine hydrochloride crude Form (A), lercanidipine hydrochloride crude Form (B) or lercanidipine crude Form (C)

31. The method of claim 27 wherein said step d) further comprises filtering the heated solution.

32. The method of claim 29 wherein when the C1-C5 alcohol is isopropanol said step e) comprises cooling the solution to a temperature between 30 and 40°C.

33. The method of claim 32 wherein said step e) further comprises stirring for a period of time of 12-48 hours.

34. The method of claim 27 wherein said drying in step f) takes place in an oven.

35. The method according to claim 29, wherein when the C1-C5 alcohol is ethanol it comprises the following steps

d') providing a mixture of ethanol and lercanidipine hydrochloride, refluxing under stirring and cooling to 20°C and adding crystalline seeds of Form (I);

e') further cooling the seeded mixture of step d') and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is ≤ 2%; and

f') recovering the solid of step e') to form lercanidipine hydrochloride Form (I).

36. The method of claim 35, wherein the ratio of lercanidipine hydrochloride to volume of solvent in step d') on a weight volume ratio is within the range of about 1:4 to 1:6.

37. The method of claim 35 wherein said ratio is 1:4.

38. The method of claim 35 wherein said step d') further comprises filtering the heated solution.

39. The method of claim 35 wherein cooling in said step e') is carried out up to a temperature between 10 and 15°C.

40. The method of claim 35 wherein the drying in said step f') takes place in an oven at 70°C.

41. The method of claim 35 wherein authentic seeds of lercanidipine Form (I) are added at the end of cooling in steps e') and d').

42. A method of producing lercanidipine hydrochloride crystalline Form (II), which has an x-ray diffraction pattern, at wavelength Kα, as shown in Figure 12, as claimed in anyone of claims 25 and 26 the method comprising the steps of:

d") adding acetonitrile to lercanidipine hydrochloride and heating the mixture thus obtained to form a solution;

e") cooling of the solution of step d") and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is ≤ 2%; and

f") recovering the solid of step e") and drying said solid to produce the lercanidipine hydrochloride Form (II).

43. The method of claim 42 wherein said step d") comprises heating said mixture under reflux with stirring.

44. The method of claim 42 wherein said step e") comprises cooling the solution to room temperature.

45. The method of claim 42 wherein said step e") comprises stirring the solution at room temperature for 24 hours.

46. The method of claim 42 wherein drying step f") takes place in an oven.

47. The method of claim 42, wherein the crude Form is lercanidipine hydrochloride crude Form (A), lercanidipine hydrochloride crude Form (B) or lercanidipine crude Form (C).

48. A method of producing lercanidipine hydrochloride crystalline Form (II), which has an X-ray diffraction pattern, at wavelength Kα, as shown in Figure 12, according to anyone of claims 25 and 26, which comprises:

d"') adding ethanol or isopropanol with a water content below 10% by weight to lercanidipine hydrochloride and refluxing to produce a solution;

e"') cooling the solution to a temperature between 20 and 40°C and stirring until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is ≤ 2%; and

f"') recovering the solid produced in step e"') to produce lercanidipine hydrochloride Form (II).

49. The method of claim 48 wherein ethanol is added in said step d"').

50. The method of claims 48 wherein the water content of the solvent in step d''') is between 5 and 10%.

51. The method of claim 48 wherein step f''') comprises filtering said solid and drying in an oven.

52. A method of producing the lercanidipine hydrochloride crystalline Form (II), which has an x-ray diffraction pattern, at wavelength Kα, as shown in Figure 12, according to anyone of claims 25 and 26 which comprises:

d'''') dissolving crude lercanidipine hydrochloride or lercanidipine hydrochloride crystalline Form (I) in a protic polar or an aprotic dipolar solvent containing up to 50% by weight of water to produce a solution;

e'''') stirring the solution of step d'''') until the concentration of lercanidipine hydrochloride dissolved in the crystallization solvent is ≤ 2%; and

f'''') recovering the solid of step e'''') to produce lercanidipine Form (II).

53. The method of claim 52, further comprising irradiating with ultrasound and/or adding crystalline seeds of Form (II) to step e'''').

54. The method of claim 52, further comprising adding up to 60% water to the solution of step d'''').

55. The method of claim 52, wherein the protic polar solvent is an alcohol solvent.

56. The method of claim 52, wherein the alcohol solvent is selected from the group consisting of methanol, ethanol, n-propanol, isopropanol.

57. The method of claim 52, wherein the aprotic dipolar solvent is N-methyl-pyrrolidone.

58. The method of claim 52, wherein the temperature of said step d'''') is between 20 and 70°C.

59. The method of claim 52, wherein stirring in said step e'''') takes place at a temperature between 20 and 25°C.

60. The method of claim 52, wherein drying in said step f'''') takes place at 70°C.

61.An antihypertensive pharmaceutical composition comprising (1) crystalline lercanidipine hydrochloride and optionally other forms of lercanidipine, wherein the crystalline lercanidipine hydrochloride is selected from the group consisting of lercanidipine hydrochloride crystalline Form (I), lercanidipine hydrochloride crystalline Form (II) and combination thereof comprising a predetermined content

of each crystalline form, and (2) at least one component selected from the group consisting of a pharmaceutically acceptable carrier or diluent, a flavorant, a sweetener, a preservative, a dye, a binder, a suspending agent, a dispersing agent, a colorant, a disintegrant, an excipient, a lubricant, a plasticizer, and an edible oil.

62. A unit dosage form comprising the antihypertensive pharmaceutical composition of claim 61.

63. The unit dosage form of claim 62 wherein the dosage form is a lercanidipine immediate release dosage form.

64. The unit dosage form of claim 62 wherein the dosage form is a lercanidipine sustained release dosage form.

65. The unit dosage form of claim 64 wherein the dosage form comprises a lercanidipine immediate release phase and a lercanidipine sustained release phase.

66. The unit dosage form of claim 65, wherein the composition comprises 0.1 to 400 mg lercanidipine hydrochloride.

67. The unit dosage form of claim 66, wherein the composition comprises 1 to 200 mg lercanidipine hydrochloride.

68. The unit dosage form of claim 67, wherein the composition comprises 5 to 40 mg lercanidipine hydrochloride.

69. An antihypertensive composition comprising predetermined amounts of lercanidipine hydrochloride crystalline Form (I) and lercanidipine hydrochloride crystalline Form (II)

70. The antihypertensive composition of claim 69 wherein the lercanidipine hydrochloride crystalline Form (I) has a melting point of about 197-201°C and the lercanidipine hydrochloride crystalline Form (II) has a melting point of about 207-211°C, when said melting points are determined as DSC peaks.

71. The antihypertensive composition of claim 69 and 70, wherein the ratio of form (I) to form (II) is between 1:9 to 9:1.

72. The antihypertensive composition of claim 71 wherein the ratio of Form (I) : Form (II) is selected from the group consisting of 9.1, 7:3 1:1, 3:7 and 1:9.

73. The isolated lercanidipine crystal Form of any one of claims 23, 24, 25 or 26

comprising an average particle size of D (50%) 2-8 µm and D (90%) < 15 µm.

74. The antihypertensive pharmaceutical composition of claim 62 wherein said lercanidipine hydrochloride crystalline Forms (I) and (II) each have an average particle size of D (50%) 2-8 µm and D (90%) < 15 µm.

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PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
Gervasi, Gemma
NOTARBARTOLO & GERVASI
Corso di Porta Vittoria, 9
I-20122 Milano
ITALIE

PCT

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing (day/month/year)	11 SEP 2002
Applicant's or agent's file reference: 2608PTWO/scb	IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/EP 02/ 08699	05/08/2002	06/08/2001

Applicant: RECORDATI IRELAND LIMITED

Title of the invention:

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.
2. The applicant is further notified that the record copy of the international application was transmitted to the International Bureau on the above date of mailing.

3. ☒ Other:

We hereby draw your attention to the fact that US has not been designated in this international application.
Form PCT/RO/101, Box No. II and Box No. III have been changed accordingly by this Receiving Office.
If you want to designate US, this is still possible under Rule 4.9 (precautionary designation).
Please, see enclosed form PCT/RO/144.

ho già scritto
SC 16.09.02

NOTARBARTOLO & GERVASI
MILANO
RECEIVED
13 SET. 2002
SBC

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

Name and mailing address of the Receiving Office	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040 Fax: (+31-70) 340-3016	Y. Marinus-v.d. Nouweland

Form PCT/RO/105 (Juli 1992)

PATENT COOPERATION TREATY

PCT

NOTICE OF CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

(to be filed with the receiving Office)

(PCT Rules 4.9(c) and 15.5)

Applicant's or agent's file reference	International filing date *(day/month/year)*
International application No.	(Earliest) Priority date *(day/month/year)*
Applicant	

1. The applicant hereby confirms the following designations made under Rule 4.9(b):

Name of State *(specify if a regional patent and/or another kind of protection or treatment is/are desired)*	Name of Applicant(s) for that State

2. **Prescribed fees** *(Applicants from certain States are entitled to a reduction of 75% of the designation fee and the confirmation fee. Where the applicant is (or all applicants are) so entitled, the total to be entered in the TOTAL box is 25% of the sum of the amounts entered at D and C. See Notes to the Fee Calculation Sheet as annexed to the Request Form, PCT/RO/101, for details.)*

for receiving Office use only

_____ x _____ = _____ D

Number of designations confirmed | Amount of designation fee | Total designation fee

Confirmation fee (50% of the above total) = _____ C

Total fees payable (D + C) = TOTAL

Mode of payment *(payment must accompany this notice)*:

- ☐ authorization to charge deposit account (see below)
- ☐ bank draft
- ☐ coupons
- ☐ cheque
- ☐ cash
- ☐ other *(specify)*:
- ☐ postal money order
- ☐ revenue stamps

3. **Signature of applicant or agent**

Deposit account authorization

The RO/ _____ ☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☐ is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

Deposit Account Number	Date *(day/month/year)*	Signature

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. PCT/EP 02/08699

International Filing Date (05.08.2002) 05 AUG 2002

EUROPEAN PATENT OFFICE
PCT INTERNATIONAL APPLICATION
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 2608PTWO/sbc

Box No. I TITLE OF INVENTION

Novel crude and crystalline forms of lercanidipine hydrochloride

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

RECORDATI IRELAND LIMITED
17, The Crescent, LIMERICK - IRELAND

Telephone No.

Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IE

State *(that is, country)* of residence: IE

This person is applicant for the purposes of: ☒ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Rdep

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BONIFACIO Fausto
Via G. d'Arezzo, 9
04100 LATINA - ITALY

This person is:
☐ applicant only
☒ applicant and inventor
☒ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Rdep

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

GERVASI Gemma
c/o NOTARBARTOLO & GERVASI
Corso di Porta Vittoria, 9
20122 MILAN - ITALY

Telephone No. +39025417991

Facsimile No. +390254179920

Teleprinter No.

Agent's registration No. with the Office

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

PCT/EP02/08699

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Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CAMPANA Francesco
Via Mediana
00040 ROCCA PRIORA (Prov. of ROME) ITALY

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☒ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

RO/EP

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

DE IASI Gianluca
Via dei Bersaglieri, 27
04011 APRILIA (Province of LATINA) - ITALY

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☒ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

RO/EP

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LEONARDI Amedeo
Via Poliziano, 16 -
20154 MILAN - ITALY

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☒ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IT

State *(that is, country)* of residence: IT

RO/EP

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☐ the United States of America only
- ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

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Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

☒ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZM Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

☒ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent:** AT Austria, BE Belgium, BG Bulgaria, CH & LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, SK Slovakia, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AG Antigua and Barbuda
☒ AL Albania
☒ AM Armenia
☒ AT Austria
☒ AU Australia
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina
☒ BB Barbados
☒ BG Bulgaria
☒ BR Brazil
☒ BY Belarus
☒ BZ Belize
☐ CA Canada
☒ CH & LI Switzerland and Liechtenstein
☒ CN China
☒ CO Colombia
☒ CR Costa Rica
☒ CU Cuba
☒ CZ Czech Republic
☒ DE Germany
☒ DK Denmark
☒ DM Dominica
☒ DZ Algeria
☒ EC Ecuador
☒ EE Estonia
☒ ES Spain
☒ FI Finland
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia
☒ GH Ghana
☒ GM Gambia
☒ HR Croatia
☒ HU Hungary
☒ ID Indonesia
☒ IL Israel
☒ IN India
☒ IS Iceland
☒ JP Japan
☒ KE Kenya
☒ KG Kyrgyzstan
☒ KP Democratic People's Republic of Korea
☒ KR Republic of Korea
☒ KZ Kazakhstan
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco
☒ MD Republic of Moldova
☒ MG Madagascar
☒ MK The former Yugoslav Republic of Macedonia
☒ MN Mongolia
☒ MW Malawi
☒ MX Mexico
☒ MZ Mozambique
☒ NO Norway
☒ NZ New Zealand
☒ OM Oman
☒ PH Philippines
☒ PL Poland
☒ PT Portugal
☒ RO Romania
☒ RU Russian Federation
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia
☒ SK Slovakia
☒ SL Sierra Leone
☒ TJ Tajikistan
☒ TM Turkmenistan
☒ TN Tunisia
☒ TR Turkey
☒ TT Trinidad and Tobago
☒ TZ United Republic of Tanzania
☒ UA Ukraine
☒ UG Uganda
☐ US United States of America
☒ UZ Uzbekistan
☒ VN Viet Nam
☒ YU Yugoslavia
☒ ZA South Africa
☒ ZM Zambia
☒ ZW Zimbabwe

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ ..... ☐ ..... ☐ .....
☐ ..... ☐ ..... ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) (06.08.01) doug 2001	MI2001A001726	IT		
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* ....

.....

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / .....

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*:

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*:		Number of declarations
☐ Box No. VIII (i)	Declaration as to the identity of the inventor	:
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application contains:			This international application is accompanied by the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*:	Number of items
(a) the following number of sheets in paper form:				
request (including declaration sheets)	:	5	1. ☐ fee calculation sheet :	
description (excluding sequence listing part)	:	55	2. ☒ original separate power of attorney :	2
claims	:	13	3. ☐ original general power of attorney :	
abstract	:	1	4. ☐ copy of general power of attorney; reference number, if any: ..... :	
drawings	:	19	5. ☐ statement explaining lack of signature :	
Sub-total number of sheets	:	93	6. ☒ priority document(s) identified in Box No. VI as item(s): ..... :	1
sequence listing part of description *(actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (b) below)*	:		7. ☐ translation of international application into *(language)*: ..... :	
Total number of sheets	:	93	8. ☐ separate indications concerning deposited microorganism or other biological material :	
(b) sequence listing part of description filed in computer readable form			9. ☐ sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other))	
(i) ☐ only (under Section 801(a)(i))			(i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application) :	
(ii) ☐ in addition to being filed in paper form (under Section 801(a)(ii))			(ii) ☐ *(only where check-box (b)(i) or (b)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter* :	
Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained *(additional copies to be indicated under item 9(ii), in right column)*:			(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column :	
			10. ☒ other *(specify)*: ACCOMPANYING LETTER ..... :	1

Figure of the drawings which should accompany the abstract:	Language of filing of the international application: english

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Milan, Italy, August 2, 2002 GERVASI Gemma

For receiving Office use only

1. Date of actual receipt of the purported international application:	05.08.02	05 AUG 2002
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:		
4. Date of timely receipt of the required corrections under PCT Article 11(2):		
5. International Searching Authority (if two or more are competent): ISA /	6. ☒ Transmittal of search copy delayed until search fee is paid	

2. Drawings: ☒ received: ☐ not received:

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

GERVASI, Gemma
Notarbartolo & Gervasi
Corso di Porta Vittoria, 9
I-20122 Milan
ITALIE



Date of mailing *(day/month/year)*	
20 February 2003 (20.02.03)	
Applicant's or agent's file reference	IMPORTANT NOTICE
2608PTWO/sbc	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP02/008699	05 August 2002 (05.08.02)	06 August 2001 (06.08.01)

Applicant: RECORDATI IRELAND LIMITED

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

   KP, KR

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AE, AG, AL, AM, AP, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EA, EC, EE, EP, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OA, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-*bis*)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 20 February 2003 (20.02.03) under No. WO 03/014084.

4. **TIME LIMITS for filing a demand for international preliminary examination and for entry into the national phase**

   The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be **30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 19 months from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

   In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

   For filing a demand for international preliminary examination, see the *PCT Applicant's Guide*, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

   It is the applicant's sole responsibility to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Judith Zahra
Facsimile No.(41-22) 740.14.35	Telephone No.(41-22) 338.91.11

Form PCT/IB/308 (April 2002)

(12) SOLICITUD INTERNACIONAL PUBLICADA CONFORME AL TRATADO DE COOPERACION EN MATERIA DE PATENTES (PCT)

(19) Organización Mundial de la Propiedad Intelectual
Oficina Internacional

(43) Fecha de Publicación Internacional
20 de Febrero de 2003 (20.02.2003)

PCT

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(25) Idioma de Presentación: Inglés

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(30) Fecha de Prioridad:
MI2001A001726 6 de Agosto de 2001 (06.08.2001) IT

(71) Solicitante: RECORDATI IRELAND LIMITED [IE/IE]; 17, The Crescent, Limerick (IE).

(72) Inventores: BONIFACIO, Fausto; Via G. D'Annun, 9, I-04100 Latina (IT). CAMPANA, Francesco; Via Madiano, I-00040 Rocca Priora (IT). DE IASI, Gianluca, Via dei Berseglieri, 27, I-04011 Aprilia (IT). LEONARDI, Amedeo; Via Polesano, 16, I-20154 Milano (IT).

(74) Agente: GERVASI, Gemma; Notarbartolo y Gervasi, Corso di Porta Vittoria, 9, I-20122 Milán (IT).

(81) Estados Designados: *(nacional)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.

(84) Estados Designados: *(regional)*: Patente ARIPO (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW). Patente eurasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM). Patente europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, *IT, LU,* MC, NL, PT, SE, SK, TR). Patente OAPI (BF, BJ, CF, CG, , CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

Publicada
— con reporte de búsqueda internacional

Para códigos de dos letras y otras abreviaciones, refiérase a las "Notas de Guía sobre Códigos y Abreviaciones" que aparecen al principio de cada ejemplar regular de la gaceta del PCT

(54) Título: NUEVAS FORMAS POLIMÓRFICAS, CRISTALINAS DE CLORHIDRATO DE LERCANIDIPINA Y PROCEDIMIENTO PARA PREPARARLAS

(57) Resumen: La invención describe las nuevas formas crudas (A) y (B) de lercanidipino, las nuevas formas cristalinas (I) y (II) de clorhidrato de lercanidipino obtenidas a partir de las formas crudas, composiciones antihipertensivas, farmacéuticas que contienen como agente activo al menos una de las formas cristalinas (I) y (II) de clorhidrato de lercanidipino y métodos de uso de las mismas.

FEB-02-2004 13:59 DE :UNTHOFT,GOMEZ UEGA 5208850T R :571 2859919 P.2/5

PCT

PETITORIO

El abajo firmante solicita que la presente solicitud Internacional sea procesada de acuerdo al Tratado de Cooperación en Materia de Patentes

Para uso de la Oficina receptora solamente
PCT/EP 02/08699
No. de Solicitud Internacional
(05.08.2002) 05 DE AGOSTO DE 2002
Fecha de presentación internacional
OFICINA EUROPEA DE PATENTES SOLICITUD INTERNACIONAL DEL PCT
Nombre de la oficina receptora y "Solicitud Internacional de PCT"

Referencia de archivo del solicitante o apoderado (*si se desea*) (*12 caracteres máximo*): 2608PTWO/sbc

Cuadro No. I TITULO DE LA INVENCIÓN

Nuevas formas crudas y cristalinas de clorhidrato de lercanidipina

Cuadro No. II SOLICITANTE ☐ Esta persona es también inventor

Nombre y dirección: (*Apellido seguido por nombre propio; para una entidad legal, designación oficial completa. La dirección debe incluir código postal y nombre del país*).
El país de la dirección indicada en este Cuadro es el Estado del solicitante (es decir país) de residencia si no se indica el Estado de residencia posteriormente)

RECORDATI IRELAND LIMITED
17, The Crescent, LIMERICK - IRLANDA

No. de teléfono:

No. de facsímil:

No. de teleimpresor:

No. de registro del solicitante con la Oficina

Estado (es decir, país) de nacionalidad: IE

Estado (es decir, país) de residencia: IE

Este persona es solicitante para los propósitos de: ☒ todos los Estados designados ☐ todos los Estados designados excepto los Estados Unidos de América ☐ únicamente los Estados Unidos de América ☐ los Estados indicados en el cuadro suplementario

Cuadro No. III SOLICITANTE(S) ADICIONAL(ES) Y/O INVENTOR(ES) (ADICIONALES)

Nombre y dirección: (*Apellido seguido por nombre propio; para una entidad legal, designación oficial completa. La dirección debe incluir código postal y nombre del país). El país de la dirección indicada en este Cuadro es el Estado del solicitante (es decir país) de residencia si no se indica el Estado de residencia posteriormente)*

BONIFACIO Fausto
Via G. d'Arezzo, 9
04100 LATINA - ITALIA

Esta persona es:

☐ solicitante solamente

☐ solicitante e inventor

☒ inventor solamente (si se marca este cuadro, no se debe llenar lo que sigue)

No. de registro del solicitante con la Oficina

Estado (es decir, país) de nacionalidad:

Estado (es decir, país) de residencia:

Esta persona es solicitante para los propósitos de: ☐ todos los Estados designados ☐ todos los Estados designados excepto los Estados Unidos de América ☐ únicamente los Estados Unidos de América ☐ los Estados indicados en el cuadro suplementario

☐ Solicitantes adicionales y/o inventores (adicionales) se indican en una hoja de continuación.

Cuadro No. IV APODERADO O REPRESENTANTE COMUN; O DIRECCION PARA CORRESPONDENCIA

La persona identificada posteriormente es/ha sido citada para actuar en nombre del(los) solicitante(s) ante las Autoridades Internacionales competentes como: ☒ agente ☐ representante común

Nombre y dirección: (*Apellido seguido por nombre propio; para una entidad legal, designación oficial completa. La dirección debe incluir código postal y nombre del país*)

GERVASI Gemma
c/o NOTARBARTOLO & GERVASI
Corso di Porta Vittoria, 9
20122 MILÁN - ITALIA

No. de teléfono: +39025417991

No. de facsímil: +390254179920

No. de teleimpresor

No. de registro del solicitante con la Oficina

☐ Marque este cuadro cuando ningún apoderado o representante común es/haya sido citado y el espacio anterior se usa en su lugar para indicar una dirección especial a la cual se debe enviar la correspondencia.

Forma PCT/RO/101 (primera hoja) (Marzo de 2001; reimpresión en Julio de 2002) *Véanse notas a la forma del petitorio*

Hoja No. 2

Continuación del Cuadro No. III SOLICITANTE(S) ADICIONALES Y/O INVENTOR(ES) (ADICIONALES)

Si ninguno de los siguientes subcuadros se utilizan, esta hoja no se debe incluir en el petitorio

Nombre y dirección: *(Apellido seguido por nombre propio; para una entidad legal, designación oficial completa. La dirección debe incluir código postal y nombre del país). El país de la dirección indicada en este Cuadro es el Estado del solicitante (es decir país) de residencia si no se indica el Estado de residencia posteriormente)*

CAMPANA Francesco
Via Mediana
00040 ROCCA PRIORA (Provincia of ROMA) ITALIA

Esta persona es:

☐ solicitante solamente

☐ solicitante e inventor

☒ inventor solamente (si se marca este cuadro, no llenar lo que sigue)

No. de registro del solicitante con la Oficina

Estado (es decir, *país*) de nacionalidad:

Estado (es decir, *país*) de residencia:

Esta persona es solicitante para los propósitos de: ☐ todos los Estados designados ☐ todos los Estados designados excepto los Estados Unidos de América ☐ únicamente los Estados Unidos de América ☐ los Estados indicados en el cuadro suplementario

Nombre y dirección: *(Apellido seguido por nombre propio; para una entidad legal, designación oficial completa. La dirección debe incluir código postal y nombre del país). El país de la dirección indicada en este Cuadro es el Estado del solicitante (es decir país) de residencia si no se indica el Estado de residencia posteriormente)*

DE IASI Gianluca
Via dei Bersaglieri, 27
04011 APRILIA (Provincia de LATINA) - ITALIA

Esta persona es:

☐ solicitante solamente

☐ solicitante e inventor

☒ inventor solamente (si se marca este cuadro, no llenar lo que sigue)

No. de registro del solicitante con la Oficina

Estado (es decir, *país*) de nacionalidad:

Estado (es decir, *país*) de residencia:

Esta persona es solicitante para los propósitos de: ☐ todos los Estados designados ☐ todos los Estados designados excepto los Estados Unidos de América ☐ únicamente los Estados Unidos de América ☐ los Estados indicados en el cuadro suplementario

Nombre y dirección: *(Apellido seguido por nombre propio; para una entidad legal, designación oficial completa. La dirección debe incluir código postal y nombre del país). El país de la dirección indicada en este Cuadro es el Estado del solicitante (es decir país) de residencia si no se indica el Estado de residencia posteriormente)*

LEONARDI Amedeo
Via Poliziano, 16 -
20154 MILÁN - ITALIA

Esta persona es:

☐ solicitante solamente

☐ solicitante e inventor

☒ inventor solamente (si se marca este cuadro, no llenar lo que sigue)

No. de registro del solicitante con la Oficina

Estado (es decir, *país*) de nacionalidad:

Estado (es decir, *país*) de residencia:

Esta persona es solicitante para los propósitos de: ☐ todos los Estados designados ☐ todos los Estados designados excepto los Estados Unidos de América ☐ únicamente los Estados Unidos de América ☐ los Estados indicados en el cuadro suplementario

Nombre y dirección: *(Apellido seguido por nombre propio; para una entidad legal, designación oficial completa. La dirección debe incluir código postal y nombre del país). El país de la dirección indicada en este Cuadro es el Estado del solicitante (es decir país) de residencia si no se indica el Estado de residencia posteriormente)*

Esta persona es:

☐ solicitante solamente

☐ solicitante e inventor

☐ inventor solamente (si se marca este cuadro, no llenar lo que sigue)

No. de registro del solicitante con la Oficina

Estado (es decir, *país*) de nacionalidad:

Estado (es decir, *país*) de residencia:

Esta persona es solicitante para los propósitos de: ☐ todos los Estados designados ☐ todos los Estados designados excepto los Estados Unidos de América ☐ únicamente los Estados Unidos de América ☐ los Estados indicados en el cuadro suplementario

☐ **Solicitantes adicionales y/o inventores (adicionales) se indican en una hoja de continuación.**

Hoja No. 3

Cuadro No. V **DESIGNACION DE ESTADOS** Márquense las casillas adecuadas; debe marcarse por lo menos una

A continuación se hacen las siguientes designaciones conforme a la Regla 4.9(a):

Patente Regional

☒ AP Patente ARIPO: GH Ghana, GM Gambia, KE Kenya, LS Lesoto, MW Malawi, MZ Mozambique, SD Sudán, SL Sierra Leona, SZ Swazilandia, TZ República Unida de Tanzanía, UG Uganda, ZM Zambia, ZW Zimbabwe y cualquier otro estado contratante del Protocolo de Harare y del PCT *(si se desea otra forma de protección o de tramitación especifíquese en la línea de puntos)*.....

☒ EA Patente Euroasiática: AM Armenia, AZ Azerbaiyan, BY Belorrusia, KG Kyrgyzstan, KZ Kazajstán, MD República de Moldova, RU Federación Rusa, TJ Tayikistán, TM Turkmenistán, y cualquier otro Estado contratante del Convenio de Patentes Euroasiático y del PCT

☒ EP Patente Europea: AT Austria, BE Bélgica, CH y LI Suiza y Liechtenstein, CY Chipre, DE Alemania, DK Dinamarca, ES España, FI Finlandia, FR Francia, GB Reino Unido, GR Grecia, IE Irlanda, IT Italia, LU Luxemburgo, MC Mónaco, NL Países Bajos, PT Portugal, SE Suecia, TR Turquía y cualquier otro Estado contratante del Convenio de Patentes Europeo y del PCT

☒ OA Patente OAPI: BF Burkina Faso, BJ Benin, CF República Centroafricana, CG Congo, CI Côte d'Ivoire, CM Camerún, GA Gabón, GN Guinea, GQ Guinea Ecuatorial, GW Guinea-Bissau, ML Malí, MR Mauritania, NE Níger, SN Senegal, TD Chad, TG Togo y cualquier otro Estado que sea Estado miembro de la OAPI y que sea un Estado contratante del PCT *(si se desea otra forma de protección o de tramitación, especifíquese en la línea de puntos)*.....

Patente Nacional *(si se desea otra forma de protección o de tramitación especifíquese en la línea de puntos):*

☒	AE	Emiratos Árabes Unidos	☒	GE	Georgia	☒	MZ	Mozambique
☒	AG	Antigua y Barbuda	☒	GH	Ghana	☒	NO	Noruega
☒	AL	Albania	☒	GM	Gambia	☒	NZ	Nueva Zelanda
☒	AM	Armenia	☒	HR	Croacia	☒	OM	Omán
☒	AT	Austria	☒	HU	Hungría	☒	PH	Filipinas
☒	AU	Australia	☒	ID	Indonesia	☒	PL	Polonia
☒	AZ	Azerbaiyán	☒	IL	Israel	☒	PT	Portugal
☒	BA	Bosnia y Herzegovina	☒	IN	India	☒	RO	Rumania
☒	BB	Barbados	☒	IS	Islandia	☒	RU	Federación Rusa
☒	BG	Bulgaria	☒	JP	Japón	☒	SD	Sudán
☒	BR	Brasil	☒	KE	Kenia	☒	SE	Suecia
☒	BY	Bielorrusia	☒	KG	Kirguizistán	☒	SG	Singapur
☒	BZ	Belice	☒	KP	República Popular	☒	SI	Eslovenia
☐	CA	Canadá			Democrática de Corea	☒	SK	Eslovaquia
☒	CH e	IL Suiza y Liechtenstein	☒	KR	República de Corea	☒	SL	Sierra Leona
☒	CN	China	☒	KZ	Kazajstán	☒	TJ	Tayikistán
☒	CO	Colombia	☒	LC	Santa Lucía	☒	TM	Turkmenistán
☒	CR	Costa Rica	☒	LK	Sri Lanka	☒	TN	Tunicia
☒	CU	Cuba	☒	LR	Liberia	☒	TR	Turquía
☒	CZ	República Checa	☒	LS	Lesoto	☒	TT	Trinidad y Tobago
☒	DE	Alemania	☒	LT	Lituania	☒	TZ	República Unida de Tanzania
☒	DK	Dinamarca	☒	LU	Luxemburgo	☒	UA	Ucrania
☒	DM	Dominica	☒	LV	Letonia	☒	UG	Uganda
☒	DZ	Argelia	☒	MA	Marruecos	☐	US	Estados Unidos de América
☒	EC	Ecuador	☒	MD	República de Moldavia	☒	UZ	Uzbekistán
☒	EE	Estonia	☒	MG	Madagascar	☒	VN	Vietnam
☒	ES	España	☒	MK	Macedonia	☒	YU	Yugoslavia
☒	FI	Finlandia	☒	MN	Mongolia	☒	ZA	Sudáfrica
☒	GB	Reino Unido	☒	MW	Malawi	☒	ZM	Zambia
☒	GD	Granada	☒	MX	México	☒	ZW	Zimbabwe

Casillas reservadas para los Estados los cuales han llegado a ser parte del PCT después de la publicación de esta hoja.

☐	☐	☐
☐	.....	.....
	☐	☐
	.....	.....

Declaración de Designación Preventiva: Además de las designaciones arriba efectuadas, el solicitante efectuará también en virtud de la Regla 4.9 (b), todas las designaciones que estén permitidas con arreglo al PCT, salvo la designación(es) indicadas en el Cuadro Suplementario como se excluye del alcance de esta declaración. El solicitante declara que estas designaciones adicionales están sujetas a confirmación y que cualquier designación que no se confirme antes de que expiren los 15 meses a partir de la fecha prioritaria se considerará retirada por el solicitante al expirar dicho plazo. *(La confirmación [incluyendo [cuando] debería llegar a la Oficina receptora dentro del plazo de 15 meses)*

Cuadro No. VI REIVINDICACIÓN DE PRIORIDAD.

La prioridad de la o las siguientes solicitudes anteriores es reivindicada:

Fecha de presentación de la solicitud anterior (*día/mes/año*)	Número de solicitud anterior	De donde la solicitud anterior es:		
		Solicitud nacional: país	Solicitud regional:* Oficina regional	Solicitud internacional: Oficina receptora
cuadro (1) (06.08.01) 06 de Agosto de 2001	MI2001A001726	IT		
cuadro (2)				
cuadro (3)				
cuadro (4)				
cuadro (5)				

☐ Las reivindicaciones de prioridad adicionales se indican en el Cuadro Suplementario.

La Oficina receptora es notificada mediante la presente para que prepare y transmita a la Oficina Internacional una copia certificada de la(s) solicitud(es) anterior(es) identificada(s) anteriormente (únicamente si la solicitud anterior fue presentada con la Oficina la cual para los propósitos de la presente solicitud internacional es la Oficina receptora) identificada como cuadro(s):

☐ todos los cuadros ☐ cuadro (1) ☐ cuadro (2) ☐ cuadro (3) ☐ cuadro (4) ☐ cuadro (5) ☐ otros, ver el cuadro suplementario

***** *Donde la solicitud anterior es una solicitud ARIPO, es obligatorio indicar en el Cuadro Suplementario por lo menos un país parte de la Convención de París para la Protección de la Propiedad Industrial para la cual la solicitud anterior fue presentada (Regla 4.10(b)(ii))*.....

Cuadro No. VII AUTORIDAD DE BÚSQUEDA INTERNACIONAL

Elección de la Autoridad de Búsqueda Internacional (ISA) (Si dos o más Autoridades de Búsqueda Internacional son competentes para llevar a cabo la búsqueda internacional, indique la Autoridad escogida; se puede utilizar el código de dos letras):

ISA/ .....

La petición para el uso de los resultados de la búsqueda anterior; con referencia a la búsqueda (*si la búsqueda anterior se ha llevado a cabo por o solicitaría de la Autoridad de Búsqueda Internacional*):

Fecha (*día/mes/año*) **Número** **País (*u Oficina regional*)**

Cuadro No. VIII DECLARACIONES

Las declaraciones siguientes figuran en los cuadros No. VIII (i) a (v) (*marcar la o las casillas apropiadas e indicar la columna de la derecha el nombre del tipo de cada declaración*): **Número de declaraciones**

☐ cuadro no. VIII (i)	Declaración con relación a la identidad del inventor	:
☐ cuadro no. VIII (ii)	Declaración con relación al derecho de depósito, a la fecha de depósito internacional, para solicitar y obtener una solicitud	:
☐ cuadro no. VIII (iii)	Declaración con relación al derecho de depósito, a la fecha de depósito internacional, para reivindicar la prioridad de una solicitud anterior	:
☐ cuadro no. VIII (iv)	Declaración con respecto a la calidad inventiva (solamente con fines de la designación de los Estados Unidos de Norteamérica)	:
☐ cuadro no. VIII (v)	Declaración con relación a las descripciones que tienen algunos accesos que no se oponen o tienen excepciones al defecto de novedad	:

Cuadro No. IX HOJA DE DECLARACIÓN: IDIOMA DE DEPÓSITO

La presente solicitud internacional contiene:

(a) el número de hojas siguientes conforme a la forma de pago:

Petitorio (y comprende la o las hojas para declaración)	:	5
Descripción (a excepción de la parte reservada para el listado de secuencias)	:	55
Reivindicaciones	:	13
Resumen	:	1
Dibujos	:	19
Total del número de hojas		93
Parte de la descripción reserva un listado de secuencias (*nombre real de las hojas si son parte de este depósito, conforme a la forma de pago, que está o no igualmente disponible conforme a la forma leíble en computadora; ver (b) siguiente*):	:	
Número total de hojas		93

(b) parte de la descripción se reserva al listado de secuencias depositado conforme a la forma leíble en computadora

(i) ☐ solamente (en virtud de la instrucción 801(a)(i))

(ii) ☐ e igualmente conforme a la forma de pago (en virtud de la instrucción 801(a)(i))

Tipo y nombre de soporte (disquete, CD-ROM, CD-R y otros) los cuales representan la parte reservada al listado de secuencias (*copias suplementarias e indicadas en el punto 9(i)*), en la columna de la derecha):

El o los elementos siguientes están acompañados por la presente solicitud internacional (verificar el o los casos apropiados e indicarlos en la columna de la derecha el número de elementos verificados):

		No. de elementos
1. ☐ costo de la hoja de cálculo	:	
2. ☒ poder del apoderado firmado separado	:	2
3. ☐ original del poder general	:	
4. ☐ copia del poder general del apoderado, número de referencia, si la hay etc.	:	
5. ☐ declaración que explique carencia de firma	:	
6. ☒ documento(s) de prioridad identificado en el cuadro No. VI como en el cuadro(s):	:	1
7. ☐ traducción de la solicitud internacional en (idioma):	:	
8. ☐ indicaciones separadas concernientes a los microorganismos depositados u otro material biológico.	:	
9. ☐ listado de secuencia conforme a la forma leíble en computadora (indicar también el tipo y el nombre de soporte (disquete))	:	
(i) ☐ copia proporcionada al final de la búsqueda internacional en virtud de la regla 13*ter* únicamente (y no como parte de la solicitud internacional)	:	
(ii) ☐ (únicamente cuando el caso (b)(i) o (b)(ii) de la columna de la izquierda es marcado) hojas suplementarias, incluyendo si fuera necesario, la hoja del documento al final de la búsqueda internacional en virtud de la regla 13*ter*		
(iii) ☐ una declaración pertinente con respecto a la identidad de la copia o copias suplementarias y la parte reservada para el listado de secuencias mencionado en la columna de la izquierda.	:	
10. ☒ otros (*especificar*) CARTA ADJUNTA	:	1

La figura de los dibujos la cual debe acompañar al resumen:

Idioma del archivo de la solicitud internacional: Inglés

Cuadro No. IX FIRMA DEL SOLICITANTE O AGENTE
Enseguida de cada firma, indique el nombre de la persona que firma y la capacidad en la cual la persona firma (si tal capacidad no es obvia de la lectura del petitorio).

Milán, Italia, 2 de Agosto de 2002 GERVASI Gemma

Para uso de la Oficina receptora solamente

1. Fecha de recepción actual de la solicitud internacional alegada: (05.08.02) 05 DE AGOSTO DE 2002	**2. Dibujos** ☒ **recibidos:** ☐ **no recibidos:**
3. Fecha corregida del vencimiento de recepción actual a los documentos o dibujos recibidos posteriormente pero a tiempo que complementan la solicitud internacional alegada:	
4. Fecha de recepción a tiempo de las correcciones requeridas conforme al artículo 11(2) de PCT:	
5. Autoridad de Búsqueda Internacional especificada por la solicitante: ISA/	**6.** ☒ **Transmisión de copia de búsqueda retardada hasta que la cuota de búsqueda sea pagada**

Para uso de la Oficina Internacional solamente

Fecha de recepción de la copia de registro por la Oficina Internacional:

REPORTE DE BÚSQUEDA INTERNACIONAL

No. de solicitud internacional
PCT/EP 02/08699

A. CLASIFICACION DEL TEMA
IPC 7 C07D211/90 A61K31/44 A61P9/12

De acuerdo a la Clasificación de Patentes Internacional (IPC) o a tanto la clasificación nacional como la IPC

B. CAMPOS BUSCADOS

Documentación mínima buscada (sistema de clasificación seguido por los símbolos de clasificación):
IPC 7 C07D A61K

Documentación buscada diferente a la documentación mínima, al grado que tales documentos estan incluidos en los campos buscados:

Base de datos electrónica consultada durante la búsqueda internacional (nombre de la base de datos y, en donde sea practico, terminos de busqueda utilizados):
CHEM ABS Data, EPO-Internal

C. DOCUMENTOS CONSIDERADOS QUE SON RELEVANTES

Categoría*	Cita del documento, con indicación, en donde sea apropiado, de los pasajes relevantes	Pertinente para la reivindicación No.
X	LEONARDI, AMEDEO Y COLABORADORES: "Asymmetric N-(3,3-diphenylpropyl)aminoalkyl esters of 4-aryl-2,6-dimethyl 1,4-dihydropyridine-3,5-dicarboxylic acids with antihypertensive activity" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY (1998), 33(5), 399-420, XP004127371 véase compuesto 9u en la Tabla I conclusiones página 405 método B2 página 415 y preparación de 9u, 9ul y 9v, página 416 --- -/--	1-78

[X] Los documentos adicionales se listan en la continuación del cuadro C [X] Los miembros de la familia de patentes se listan en el anexo

*categorías especiales de los documentos citados.

"A" documento que define el estado general de la técnica, el cual no se considera que es de particular relevancia.

"E" primer documento publicado en o después de la fecha de presentación internacional.

"L" documento el cual puede arrojar dudas sobre la(s) reivindicación(es) de prioridad o el cual se cita para establecer la fecha de publicación de otra cita u otra razón especial (como se especifica)

"O" documento que se refiere a una descripción oral, uso, exhibición u otro medio

"P" documento publicado antes de la fecha de representación internacional pero después de la fecha de prioridad reivindicada.

"T" documento posterior publicado después de la fecha de presentación internacional o la fecha de prioridad y no en conflicto con la solicitud, pero citado para entender el principio o teoría fundamental de la invención.

"X" documento de relevancia particular; la invención reclamada no puede ser considerada nueva o no se puede considerar que involucra una paso inventivo cuando el documento se toma solo.

"Y" documento de particular relevancia; no se puede considerar que la invención reclamada involucra un paso inventivo cuando el documento es combinado con uno o más de tales documentos, tal combinación que es obvia para una persona experimentada en la técnica

"&" documento miembro de la misma familia de patentes.

Fecha de la terminación actual de la búsqueda internacional	Fecha de envío del reporte de búsqueda internacional
4 de Noviembre de 2002	18/11/2002

Nombre y dirección de correo de la ISA	Funcionario autorizado
Oficina Europea de Patentes, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schuemacher, A

Forma PCT/ISA/210 (segunda hoja) (Julio de 1992)

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REPORTE DE BÚSQUEDA INTERNACIONAL

No. de solicitud internacional
PCT/EP 02/08699

C (Continuación) DOCUMENTOS QUE SE CONSIDERAN PERTINENTES

Categoría*	Citación del documento, con indicación, donde es apropiado, de los pasajes pertinentes	Pertinente para la reivindicación No.
X	WO 97 03669 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.; RECORDATI) 6 de Febrero de 1997 (06-02-1997) esquema de reacción 2, página 4 página 5; ejemplos 1-3	1-78
X	WO 96 35668 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.; RECORDATI) 14 de Noviembre de 1996 (14-11-1996) el documento completo	1-78
X	MCCLELLAN K J Y COLABORADORES: "LERCANIDIPINE A REVIEW OF ITS USE IN HYPERTENSION" DRUGS, ADIS INTERNATIONAL LTD, AT, Vol. 5, no. 60, Noviembre de 2000 (01-2000), página 1123-1140, XP008001723 ISSN: 0012-667 véase abstracto del resumen página 1124	65-76
X	US 5 767 136 A (TESTA RODOLFO Y COLABORADORES) 16 de Junio de 1998 (16-06-1998) citada en la solicitud columna 7, línea 31 - línea 65; ejemplos 1-3, 11B columna 8, línea 38 - línea 48	1-78
X	US 596 139 A (RECORDATI SA) 9 de Diciembre de 1997 (09-12-1997) citada en la solicitud columna 3, línea 31 - línea 65; ejemplo 1	1-78
X	US 4 705 797 A (NARDI DANTE Y COLABORADORES) 10 de Noviembre de 1987 (10-11-1987) citada en la solicitud reivindicaciones 1-8; ejemplo 16	1, 2

Forma PCT/ISA/210 (Continuación de la segunda hoja) (Julio de 1992)

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REPORTE DE BÚSQUEDA INTERNACIONAL

Información de miembros de familia de la patente

No. de Solicitud Internacional
PCT/EP 02/08699

Documento de patente citado en el reporte de busqueda	Fecha de publicación	Miembro(s) de Familia de patente(s)		Fecha de publicación
WO 9703669 A	06-02-1997	IT	MI951613 A1	14-01-1997
		AT	183644 T	15-09-1999
		AU	690471 B2	23-04-1998
		AU	6516496 A	18-02-1997
		CA	2219501 A1	06-02-1997
		CN	1190345 A	12-08-1998
		CZ	9800116 A3	17-06-1998
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		DK	839036 T3	24-01-2000
		WO	9703669 A1	06-02-1997
		EP	0839036 A1	06-05-1998
		ES	2138359 T3	01-01-2000
		GR	3031096 T3	31-12-1999
		HU	9802736 A2	29-03-1999
		IL	122302 A	13-08-2000
		JP	11509214 T	17-08-1999
		NO	980171 A	14-01-1998
		SK	4198 A3	06-05-1998
		US	5912351 A	15-06-1999
		US	5767136 A	16-06-1998
		ZA	9605924 A	30-01-1997
WO 9635668 A	14-11-1996	IT	MI950957 A1	12-11-1996
		AT	221050 T	15-08-2002
		AU	694046 B2	09-07-1998
		AU	5898596 A	29-11-1996
		BG	62400 B1	29-10-1999
		BG	102034 A	30-04-1998
		BR	9608374 A	24-08-1999
		CA	2217849 A1	14-11-1996
		CN	1184468 A	10-06-1998
		CZ	9703567 A3	15-04-1998
		DE	69622552 D1	29-08-2002
		EE	3351 B1	15-02-2001
		EG	21755 A	27-02-2002
		WO	9635668 A1	14-11-1996
		EP	0824517 A1	25-02-1998
		HU	9801913 A2	28-12-1998
		IL	118143 A	14-06-2001
		JP	11504932 T	11-05-1999
		NO	975176 A	11-11-1997
		NZ	309059 A	28-10-1998
		PL	323236 A1	16-03-1998
		SK	151497 A3	06-05-1998
		TW	404940 B	11-09-2000
		US	5912351 A	15-06-1999
		US	5767136 A	16-06-1998
		ZA	9603716 A	20-11-1996
US 5767136 A	16-06-1998	IT	MI950957 A1	12-11-1996
		IT	MI951513 A1	14-01-1997
		AT	221050 T	15-08-2002
		AU	694046 B2	09-07-1998
		AU	5898596 A	29-11-1996
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Forma PCT/ISA/210 (anexo de familia de patentes) (Julio de 1992)

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REPORTE DE BÚSQUEDA INTERNACIONAL

Información de miembros de familia de la patente

No. de Solicitud Internacional
PCT/EP 02/08699

Documento de patente citado en el reporte de búsqueda		Fecha de publicación	Miembro(s) de Familia de patentes			Fecha de publicación
US 5767136	A		CA	2217849	A1	14-11-1996
			CN	1184468	A	10-06-1998
			CZ	9703567	A3	15-04-1998
			DE	69622552	D1	29-08-2002
			EE	3351	B1	15-02-2001
			EG	21755	A	27-02-2002
			WO	9635668	A1	14-11-1996
			EP	0824517	A1	25-02-1998
			HU	9801913	A2	28-12-1998
			IL	118143	A	14-06-2001
			JP	11504932	T	11-05-1999
			NO	975176	A	11-11-1997
			NZ	309059	A	28-10-1998
			PL	323236	A1	16-03-1998
			SK	151497	A3	06-05-1998
			TW	404940	B	11-09-2000
			US	5912351	A	15-06-1999
			ZA	9603716	A	20-11-1996
			AT	183644	T	15-09-1999
			AU	690471	B2	23-04-1998
			AU	6516496	A	18-02-1997
			CA	2219601	A1	06-02-1997
			CN	1190345	A	12-08-1998
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			DE	69603968	D1	30-09-1999
			DE	69603968	T2	06-04-2000
			DK	839036	T3	24-01-2000
			WO	9703669	A1	06-02-1997
			EP	0839036	A1	06-05-1998
			ES	2138359	T3	01-01-2000
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US 4705797	A	10-11-1987	AR	240804	A1	28-02-1991
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			AU	3868985	A	12-09-1985
			CA	1277666	A1	11-12-1990
			DE	3577703	D1	21-06-1990
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			EG	17772	A	30-08-1991
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			HU	37399	A2	28-12-1985
			IE	57716	B1	10-03-1993
			IL	74238	A	31-07-1988
			JP	1811001	C	27-12-1993
			JP	5017908	B	10-03-1993

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REPORTE DE BÚSQUEDA INTERNACIONAL

Información de miembros de familia de la patente

No. de Solicitud Internacional
PCT/EP 02/08699

Documento de patente citado en el reporte de búsqueda	Fecha de publicación	Miembro(s) de Familia de patentes	Fecha de publicación
US 4706797 A		JP 60199874 A	09-10-1985
		KR 9105231 B1	24-07-1991
		LU 90069 A9	23-07-1997
		MX 166874 A	08-10-1988
		NO 850521 A ,B,	15-08-1985
		NZ 210866 A	28-07-1988
		PH 20725 A	30-03-1987
		PT 79963 A ,B	01-03-1985
		SG 48590 G	17-08-1990
		US 4772621 A	20-09-1988
		US 4968832 A	06-11-1990
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Forma PCT/ISA/210 (anexo de familia de patentes) (Julio de 1992)

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TRATADO DE COOPERACION EN MATERIA DE PATENTES

PCT

REPORTE DE EXAMEN PRELIMINAR INTERNACIONAL

(Artículo 36 y Regla 70 del PCT)

Referencia de registro del solicitante o del apoderado	PARA UNA ACCION ADICIONAL	Véase la Notificación de Transmisión del Reporte de Examen Preliminar Internacional (Forma PCT/IPEA/416)
260RPTWO		
No. de solicitud internacional:	Fecha de presentación internacional (día/mes/año):	Fecha de prioridad (día/mes/año):
PCT/EP02/08699	05.08.2002	06.08.2001

Clasificación de Patente Internacional (IPC) o clasificación nacional e IPC
C07D211/90

Solicitante:
RECORDATI IRELAND LIMITED

1. Este reporte de examen preliminar internacional ha sido preparado por esta Autoridad de Examen Preliminar Internacional y se transmite al solicitante de acuerdo con el Artículo 36

2. Este REPORTE consiste de un total de 6 hojas, incluyendo esta portada.

[X] Este reporte también está acompañado de ANEXOS, es decir, hojas de la descripción, las reivindicaciones y/o los dibujos, los cuales han sido enmendados y son la base de este reporte y/o las hojas que contienen las rectificaciones hechas antes de esta Autoridad. (Véase la Regla 70.16 y la Sección 607 de las Instrucciones Administrativas conforme al PCT).

Estos anexos consisten de un total de 9 hojas.

3. Este reporte contiene las indicaciones que se relacionan con los siguientes puntos:

I [X] Base del reporte

II [ ] Prioridad

III [ ] No se establece opinión con respecto a la novedad, paso inventivo o aplicabilidad industrial, citas y explicaciones que soportan tal declaración

IV [ ] Carencia de unidad de la invención

V [X] Declaración razonada conforme al Artículo 35(2) con respecto a la novedad, paso inventivo o aplicabilidad industrial; citas y explicaciones que soportan tal declaración

VI [ ] Ciertos documentos citados

VII [ ] Ciertos defectos en la solicitud internacional

VIII [ ] Ciertas observaciones en la solicitud internacional.

Fecha de presentación de la solicitud	Fecha de terminación de este reporte
04.03.2003	26.08.2003
Nombre y dirección de correspondencia de la autoridad de examen preliminar internacional Oficina Europea de Patentes D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Funcionario autorizado: Schuemacher, A No. de Teléfono +49 89 2399-7816

Forma PCT/IPEA/409 (portada) (Enero de 1994)

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REPORTE DE EXAMEN PRELIMINAR INTERNACIONAL

No. de Solicitud Internacional PCT/EP02/08699

I. Bases del reporte

1. Con respecto a los elementos de la solicitud internacional (las hojas de reemplazo que se han suministrado a la Oficina receptora en respuesta a una invitación conforme al Artículo 14 se refieren en este reporte como "*presentadas originalmente*" y no se anexan a este reporte puesto que no contienen enmiendas (Reglas 70.16 y 70.17)):

Descripción, Páginas

1-55 como se presentaron originalmente

Reivindicaciones, Números

1-74 como se presentaron originalmente

Dibujos, Hojas

1/19-19/19 como se presentaron originalmente

2. Con respecto al idioma, todos los elementos marcados anteriormente fueron disponibles o proporcionados a esta Autoridad en el idioma en el cual la solicitud internacional se presentó, a menos que se indique de otra manera bajo este artículo.

Estos elementos fueron disponibles o proporcionados a esta Autoridad en el siguiente idioma: ,el cual es:

☐ el idioma de una traducción proporcionada para los propósitos de la búsqueda internacional (conforme a la Regla 23.1(b)).

☐ el idioma de publicación de la solicitud internacional (conforme a la Regla 48.3(b)).

☐ el idioma de la traducción proporcionada para los propósitos del examen preliminar internacional (conforme a las Reglas 55.2 y/o 55.3).

3. Con respecto a cualquier secuencia de nucleótidos y/o aminoácidos descrita en la solicitud internacional, el examen preliminar internacional se llevó a cabo en base del listado de secuencias:

☐ contenido en la solicitud internacional en forma escrita.

☐ presentado junto con la solicitud internacional en forma leíble por computadora.

☐ proporcionado subsecuentemente a la Autoridad en forma escrita.

☐ proporcionado subsecuentemente a la Autoridad en forma leible por computadora.

☐ Ha sido proporcionada la declaración de que el listado de secuencias escrito, subsecuentemente proporcionado no va más allá de la descripción en la solicitud internacional como se presentó.

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☐ Ha sido proporcionada la declaración de que la información registrada en forma leíble por computadora es idéntica al listado de secuencias escrito.

4. Las enmiendas han dado por resultado la cancelación de:

☐ la descripción, páginas
☐ las reivindicaciones, Nos.
☐ los dibujos, hojas

5. ☐ Este reporte ha sido redactado como si (alguna de) las enmiendas no hubieran sido hechas, puesto que éstas han sido consideradas que van más allá de la descripción como se presentó, (Regla 70.2(c)).

(Cualquier hoja de reemplazo que contenga tales declaraciones debe ser referida conforme al artículo 1 y anexada a este reporte)

6. Observaciones adicionales, si es necesario:

V. Declaración razonada conforme al Artículo 35(2) con respecto a la novedad, paso inventivo o aplicabilidad industrial; citaciones y explicaciones que soportan esta declaración.

1. Declaración

Novedad (N)	Si: Reivindicaciones 1-22, 25, 26, 28, 31-60
	No: Reivindicaciones 23, 24, 27, 29, 30, 61-74
Paso Inventivo (PI)	Si: Reivindicaciones
	No: Reivindicaciones 1-74
Aplicabilidad Industrial (AI)	Si: Reivindicaciones 1-74
	No: Reivindicaciones

2. Citaciones y explicaciones

véase hoja separada

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Con Referencia al Artículo V

Declaración razonada conforme a la Regla 66.2(a)(ii) con respecto a la novedad, paso inventivo o aplicabilidad industrial; citaciones y explicaciones que soportan esta declaración

Se hace referencia a los siguientes documentos:

D1: LEONARDI, AMEDEO Y COLABORADORES, EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY (1998), 33(5), 399-420, XP004127371

D2: WO 97 03669 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.; RECORDATI) 6 de Febrero de 1997

D3: WO 96 35668 A (RECORDATI S.A., CHEMICAL AND PHARMACEUTICAL COMPANY, SWITZ.; RECORDATI) 14 de Noviembre de 1996

D4: MCCLELLAN K J Y COLABORADORES, DRUGS, ADIS INTERNATIONAL LTD, AT, volumen 5, no. 60, Noviembre de 2000, páginas 1123-1140, XP008001723 ISSN: 0012-6667

D5: US-A-5 767 136 (TESTA RODOLFO Y COLABORADORES) 16 de Junio de 1998; citada en la solicitud

D6: US 596 139 A (RECORDATI SA) 9 de Diciembre de 1997; citada en la solicitud

D7: US-A-4 705 797 (NARLI DANTE Y COLABORADORES) 10 de Noviembre de 1987; citada en la solicitud

1. Novedad, Artículo 33(2) del PCT

La presente solicitud se refiere a diferentes formas

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polimórficas de clorhidrato de lercanidipina, los procedimientos para su preparación y su uso como agentes antihipertensivos. Los procedimientos de las nuevas reivindicaciones 3 y 12 se pueden considerar como novedosos sobre los documentos de la técnica anterior citados anteriormente puesto que el paso de aislamiento c) que comprende respectivamente los pasos i-viii) y i'-viii') ha sido definido más precisamente y vuelve nuevos a los procedimientos sobre la técnica anterior.

Consecuentemente, los procedimientos de las presentes reivindicaciones 3 y 12 y las reivindicaciones dependientes 4-22 no han sido anticipados por D1-D7 y se puede considerar que son nuevos.

Las formas sólidas, crudas (A) y (B) de clorhidrato de lercanidipina de las presentes reivindicaciones 1 y 2 son caracterizadas por los contenidos especificos de acetato de etilo. De acuerdo con la carta del solicitante, estos compuestos solo se pueden obtener de acuerdo con los nuevos procedimientos 3 y 12 y no se deben observar como formas impuras sino como productos intermediarios que permiten obtener las formas cristalinas, puras (I) y (II) de clorhidrato de lercanidipina. De esta manera, se considera que el objeto de las reivindicaciones 1 y 2 satisface los criterios del Artículo 33(2) del PCT.

El método para producir la forma cristalina (II) de clorhidrato de lercanidipina de conformidad con las reivindicaciones 42, 48 y 52 no ha sido anticipado por los documentos de la técnica anterior: la presente reivindicación 42 difiere de los procedimientos conocidos

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debido al uso de acetonitrilo como solvente para la recristalización, la reivindicación 48 debido al paso e"'), en el cual la solución debe ser enfriada a una temperatura entre 20 y 40°C y la reivindicación 52 debido al solvente polar, prótico o dipolar, aprótico que contiene hasta 50% en peso de agua.
De esta manera, el objeto de las reivindicaciones 25, 26 y 42-60 es nuevo.

Como se menciona en la carta de solicitante fechada el 28.07.2003, Ejemplo 1 de D3, Ejemplo 3 de D5 corresponden al Ejemplo 3 de D2 y describen la preparación de clorhidrato de lercanidipina (con P.f. de 186-188°C) iniciando a partir del cloruro de acilo y los compuestos aminoalcohólicos en diclorometano y DMF, seguido por la elaboración en acetato de etilo y la **recristalización a partir de etanol ya sea puro o al 99%.**
Por lo tanto, la forma cristalina (I) del clorhidrato de la reivindicación 23, preparada de acuerdo con el método de la reivindicación 27, parece ser anticipada por D2, D3 y D5: aún si los datos físicos que caracterizan la forma cristalina (I) en la reivindicación 23, 24 o 73 no han sido descritos en el documento de la técnica anterior, se asume que el procedimiento descrito en la reivindicación 27 corresponde de hecho a una recristalización con un solvente de alcohol de 1 a 5 átomos de carbono que contiene un máximo de 5% de agua. Puesto que no se aclara suficientemente si la forma cristalina (I) del clorhidrato de las presentes reivindicaciones 23, 24 y obtenida de

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acuerdo con la presente reivindicación 27 es diferente al compuesto de D2, D3 y D5 de la técnica anterior, se considera que la forma cristalina (I) del clorhidrato de las reivindicaciones 23, 24 y 27 es la misma como el compuesto de D2, D3 o D5 que se acaba de definir en el otro párrafo: una definición adicional del mismo compuesto por parámetros adicionales es insuficiente para establecer la novedad. De esta manera, las reivindicaciones de compuesto 23 y 24, las reivindicaciones de proceso para su preparación y su uso como agentes antihipertensivos en las reivindicaciones 61-74 no se considera que cumplan con los requerimientos de novedad del Artículo 33(2) del PCT.

2. Paso inventivo, artículo 33(3) del PCT:

El problema a resolverse por la presente invención puede ser considerado como desarrollar diferentes procedimientos a fin de obtener diferentes formas polimórficas de clorhidrato de lercanidipina, que sean útiles como agentes antihipertensivos.

El documento D2 de la técnica anterior ya se considera que proporciona una solución para el problema técnico mencionado anteriormente debido a que también describe la preparación de una forma cristalina de clorhidrato de lercanidipina. La diferencia del objeto de las reivindicaciones 23, 24 y 27 en comparación con el estado de la técnica no es aparente en la actualidad y en la presente solicitud falta la información apropiada para aclarar donde se encuentra un paso inventivo (por ejemplo,

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los datos comparativos con el compuesto obtenido de acuerdo con D2).

Además, se considera como un conocimiento técnico común obtener diferentes formas polimórficas de un compuesto al cambiar las condiciones de cristalización como la naturaleza de los solventes o la temperatura.

Por lo tanto, se debe observar que el problema subyacente a la presente solicitud es la provisión de formas cristalinas que tienen un efecto ventajoso, inesperado con respecto a la técnica anterior. En realidad, las formas crudas (A) y (B) reclaman tener una solubilidad más alta, una velocidad de secado más rápida y un procedimiento de cristalización simplificado en comparación con la forma cruda, conocida (C). Sin embargo, no existe evidencia de tales ventajas en la solicitud.

Adicionalmente, se reclama que las formas cristalinas (I) y (II) se obtienen con una pureza y uniformidad superior que también permite mejores formas de dosificación. Sin embargo, ni existe una indicación en la presente solicitud que la forma (I) sea en realidad más adecuada para las formulaciones y formas de dosificación de liberación inmediata ni que la forma (II) muestre una biodisponibilidad superior en comparación con el compuesto cristalino descrito en D2.

Debido a la falta de evidencia de tal efecto inesperado, no se puede considerar que la presente solicitud involucre un paso inventivo y no cumple con los requerimientos del Artículo 33(3) del PCT.

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REIVINDICACIONES

1. Una forma sólida, cruda (A) de clorhidrato de lercanidipina, caracterizada porque tiene un punto de fusión de aproximadamente 150-152°C (pico de DSC) y que comprende aproximadamente 3-4% (p/p) de acetato de etilo.

2. La forma sólida, cruda (B) de clorhidrato de lercanidipina, caracterizada porque tiene un punto de fusión de aproximadamente 131-135°C (pico de DSC) y que comprende aproximadamente 0.3-0.7% (p/p) de acetato de etilo.

3. Un método para producir la forma cruda de clorhidrato de lercanidipina de conformidad con la reivindicación 1, caracterizado porque comprende los pasos de:

a) hacer reaccionar el ácido 2,6-dimetil-5-metoxicarbonil-4-(3-nitrofenil)-1,4-dihidropiridin-3-carboxílico con un cloruro seleccionado del grupo que consiste de cloruro de tionilo y cloruro de oxalilo en un solvente dipolar, aprótico y un solvente polar, aprótico para producir el cloruro de carbonilo correspondiente;

(b) hacer reaccionar, in situ, el cloruro del paso a) con el alcohol de 2,N-dimetil-N-(3,3-difenilpropil)-1-amino-2-propilo para formar el clorhidrato de lercanidipina crudo; y

c) aislar el clorhidrato de lercanidipina crudo del paso b) y recuperar la forma cruda (A) de clorhidrato de

lercanidipina,

en donde el paso (c) consiste esencialmente de los siguientes pasos:

i. lavar el clorhidrato de lercanidipina crudo del paso (b) con agua;

ii. remover el agua del paso i), para producir una mezcla;

iii. concentrar la mezcla del paso ii) y adicionar un solvente para producir una suspensión que tiene aproximadamente el mismo volumen como el volumen inicial de la mezcla del paso ii) y un contenido de agua, de acuerdo con Karl Fischer, de entre 0.10 y 0.15%;

iv. enfriar la suspensión obtenida en el paso iii) para obtener un sólido;

v. filtrar el sólido del paso iv);

vi. resuspender el sólido del paso v) en acetato de etilo;

vii. enfriar la suspensión del paso vi); y

viii. filtrar y secar el producto precipitado del paso vii) para producir la forma cruda (A) de clorhidrato de lercanidipina.

4. El método de conformidad con la reivindicación 3, caracterizado porque el paso de reacción b) se conduce a una temperatura entre -5 y +5°C.

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5. El método de conformidad con la reivindicación 3, caracterizado porque el cloruro en el paso a) es cloruro de tionilo.

6. El método de conformidad con la reivindicación 3, caracterizado porque el paso c) ii) comprende la remoción del agua del paso c) i) por medio de la destilación azeotrópica bajo vacio dentro del rango de 200-300 mmHg, a una temperatura no mayor de 60°C, para producir una mezcla.

7. El método de conformidad con la reivindicación 3, caracterizado porque el paso de resuspensión c) vi) comprende la agitación a 60-65°C durante aproximadamente 1 hora.

8. El método de conformidad con la reivindicación 3, caracterizado porque el secado en el paso c) viii) es un horno a 70°C.

9. El método de conformidad con la reivindicación 3, caracterizado porque el paso de lavado c) i) es con agua; la mezcla en el paso c) iii) es concentrada a 1/3 de su volumen inicial y el solvente se adiciona para producir una suspensión que tiene aproximadamente el mismo volumen como el volumen inicial de la mezcla; y el contenido de agua de la suspensión, de acuerdo con Karl Fischer, es entre 0.1 y 0.15%.

10. El método de conformidad con la reivindicación 3, caracterizado porque el enfriamiento en el paso c) iv) se

lleva a cabo a una temperatura dentro del rango de 0-5°C.

11. El método de conformidad con la reivindicación 3, caracterizado porque el enfriamiento en el paso vii) se lleva a cabo a una temperatura dentro del rango de 5-10°C.

12. Un método para producir la forma cruda (B) de clorhidrato de lercanidipina de conformidad con la reivindicación 2, caracterizado porque comprende los pasos de:

a) hacer reaccionar el ácido 2,6-dimetil-5-metoxicarbonil-4-(3-nitrofenil)-1,4-dihidropiridin-3-carboxilico con un cloruro seleccionado del grupo que consiste de cloruro de tionilo y cloruro de oxalilo en un solvente dipolar, aprótico y un solvente polar, aprótico para producir el cloruro de carbonilo correspondiente;

(b) hacer reaccionar, in situ, el cloruro del paso a) con el alcohol de 2,N-dimetil-N-(3,3-difenilpropil)-1-amino-2-propilo para producir el clorhidrato de lercanidipina crudo; y

c) aislar el clorhidrato de lercanidipina crudo del paso b) y recuperar la forma cruda (B) de clorhidrato de lercanidipina, en donde el paso (c) consiste esencialmente de los siguientes pasos:

i') lavar el clorhidrato de lercanidipina crudo del paso (b) con agua;

ii') remover el agua del paso i'), para producir una

mezcla que tiene un contenido de agua de aproximadamente 2%, medido de acuerdo con Karl Fischer;

iii') concentrar la mezcla del paso ii') y adicionar un solvente para producir una solución que tiene aproximadamente el mismo volumen como el volumen inicial de la mezcla del paso ii') y un contenido de agua, de acuerdo con Karl Fischer, de entre 0.9 y 1.1%;

iv') enfriar la solución del paso iii') para obtener un sólido;

v') filtrar el sólido del paso iv');

vi') resuspender el sólido del paso v') en un solvente;

vii') enfriar la suspensión del paso vi'); y

viii') filtrar y secar el sólido obtenido para producir la forma cruda (B) de clorhidrato de lercanidipina.

13. El método de conformidad con la reivindicación 12, caracterizado porque el paso de reacción b) es conducido a una temperatura entre -5 y +5°C.

14. El método de conformidad con la reivindicación 12, caracterizado porque el cloruro es cloruro de tionilo.

15. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) ii') comprende la

remoción de agua del paso i') mediante el calentamiento a reflujo azeotrópico para producir la mezcla.

16. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) iii') comprende la concentración de la mezcla a 3/4 de su volumen inicial.

17. El método de conformidad con la reivindicación 12, caracterizado porque el solvente en los pasos c) iii') y vi') es acetato de etilo.

18. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) iv') comprende el enfriamiento de la solución a una temperatura dentro del rango de 0-5°C.

19. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) vi') además comprende agitar la suspensión a 60-65°C durante aproximadamente 1 hora.

20. El método de conformidad con la reivindicación 18, caracterizado porque el paso c) vii') además comprende el enfriamiento del sólido a una temperatura entre 5 y 10°C.

21. El método de conformidad con la reivindicación 12, caracterizado porque el secado en el paso viii') es en un horno a aproximadamente 70°C.

22. El método de conformidad con cualquiera de las reivindicaciones 3-21, caracterizado porque el solvente dipolar, aprótico es dimetilformamida y el solvente polar,

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aprótico es acetato de etilo.

23. La forma cristalina, aislada (I) de clorhidrato de lercanidipina, caracterizada porque tiene el patrón de difracción de rayos X, a una longitud de onda Kα, como se muestra en la figura 11.

24. La forma cristalina de lercanidipina de conformidad con la reivindicación 23, caracterizada porque las distancias, relaciones (I/Io), y ángulos 2θ de los picos significantes en la figura 11 son:

D(X)	Intensidad Relativa (I/Io)	ángulo 2θ
16.3	83	5.4
6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

25. La forma cristalina, aislada (II) de clorhidrato de lercanidipina, caracterizada porque tiene un patrón de difracción de rayos X, a la longitud de onda Kα, como se muestra en la figura 12.

26. La forma cristalina de lercanidipina de conformidad con la reivindicación 25, caracterizada porque las distancias, relaciones (I/Io), y ángulos 2θ de los picos significantes en la figura 12 son:

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D(X)	Intensidad Relativa (I/Io)	ángulo 2θ
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8
3.81	41	23.4
3.77	100	23.6
3.58	44	24.8
3.54	29	25.2

27. Un método para producir la forma cristalina (I) de clorhidrato de lercanidipina de conformidad con cualquiera de las reivindicaciones 23 y 24, caracterizado porque comprende:

d) adicionar un solvente de alcohol de 1 a 5 átomos de carbono que contiene un máximo de 5% de agua (v/v) a una forma cruda de clorhidrato de lercanidipina y calentar bajo reflujo y con agitación producir una solución clara;

e) enfriar la solución del paso d) y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f) recuperar el sólido obtenido del paso e), y secar el sólido para producir la forma cristalina (I) de clorhidrato de lercanidipina.

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28. El método de conformidad con la reivindicación 27, caracterizado porque el paso f) comprende filtrar el sólido obtenido del paso e), lavar el sólido con isopropanol y filtrar nuevamente el sólido antes del secado.

29. El método de conformidad con la reivindicación 27, caracterizado porque el alcohol del paso d) se selecciona del grupo que consiste de isopropanol, etanol y etanol anhidro.

30. El método de conformidad con la reivindicación 27, caracterizado porque la forma cruda es la forma cruda (A) de clorhidrato de lercanidipina, la forma cruda (B) de clorhidrato de lercanidipina o la forma cruda (C) de lercanidipina.

31. El método de conformidad con la reivindicación 27, caracterizado porque el paso d) además comprende filtrar la solución calentada.

32. El método de conformidad con la reivindicación 29, caracterizado porque cuando el alcohol de 1 a 5 átomos de carbono es isopropanol, el paso e) comprende enfriar la solución a una temperatura entre 30 y 40°C.

33. El método de conformidad con la reivindicación 32, caracterizado porque el paso e) además comprende agitar durante un periodo de tiempo de 12-48 horas.

34. El método de conformidad con la reivindicación 27, caracterizado porque el secado en el paso f) toma lugar

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en un horno.

35. El método de conformidad con la reivindicación 29, caracterizado porque cuando el alcohol de 1 a 5 átomos de carbono es etanol, éste comprende los siguientes pasos

d') proporcionar una mezcla de etanol y clorhidrato de lercanidipina, calentar a reflujo bajo agitación y enfriar a 20°C y adicionar las semillas cristalinas de la forma (I);

e') enfriar adicionalmente la mezcla sembrada del paso d') y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f') recuperar el sólido del paso e') para formar la forma (I) de clorhidrato de lercanidipina.

36. El método de conformidad con la reivindicación 35, caracterizado porque la relación de clorhidrato de lercanidipina con el volumen del solvente en el paso d') en una relación de volumen en peso está dentro del rango de aproximadamente 1:4 a 1:6.

37. El método de conformidad con la reivindicación 35, caracterizado porque la relación de 1:4.

38. El método de conformidad con la reivindicación 35, caracterizado porque d') además comprende filtrar la solución calentada.

39. El método de conformidad con la reivindicación

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35, caracterizado porque el enfriamiento en el paso e') se lleva a cabo a una temperatura entre 10 y 15°C.

40. El método de conformidad con la reivindicación 35, caracterizado porque el secado en el paso f') toma lugar en un horno a 70°C.

41. El método de conformidad con la reivindicación 35, caracterizado porque las semillas auténticas de la forma (I) de lercanidipina se adicionan al final del enfriamiento en los pasos e') y d').

42. Un método para producir la forma cristalina (II) de clorhidrato de lercanidipina, que tiene un patrón de difracción de rayos X, en la longitud de onda Kα, como se muestra en la figura 12, de conformidad con cualquiera de las reivindicaciones 25 y 26, el método está caracterizado porque comprende los pasos de:

d") adicionar acetonitrilo al clorhidrato de lercanidipina y calentar la mezcla obtenida de esta manera para formar una solución;

e") enfriar la solución del paso d") y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f") recuperar el sólido del paso e") y secar el sólido para producir la forma (II) de clorhidrato de lercanidipina.

43. El método de conformidad con la reivindicación

42, caracterizado porque el paso d") comprende calentar la mezcla bajo reflujo con agitación.

44. El método de conformidad con la reivindicación 42, caracterizado porque el paso e") comprende enfriar la solución a temperatura ambiente.

45. El método de conformidad con la reivindicación 42, caracterizado porque el paso e") comprende agitar la solución a temperatura ambiente durante 24 horas.

46. El método de conformidad con la reivindicación 42, caracterizado porque el paso de secado f") toma lugar en un horno.

47. El método de conformidad con la reivindicación 42, caracterizado porque la forma cruda es la forma cruda (A) de clorhidrato de lercanidipina, la forma cruda (B) de clorhidrato de lercanidipina o la forma cruda (C) de lercanidipina.

48. Un método para producir la forma cristalina (II) de clorhidrato de lercanidipina, que tiene un patrón de difracción de rayos X, en la longitud de onda Kα, como se muestra en la figura 12, de conformidad con cualquiera de las reivindicaciones 25 y 26, caracterizado porque comprende:

d"') adicionar etanol o isopropanol con un contenido de agua inferior a 10% en peso al clorhidrato de lercanidipina y calentar a reflujo para producir una solución;

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e‴') enfriar la solución a una temperatura entre 20 y 40°C y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f‴') recuperar el sólido producido del paso e‴) para producir la forma (II) de clorhidrato de lercanidipina.

49. El método de conformidad con la reivindicación 48, caracterizado porque el etanol se adiciona en el paso d‴').

50. El método de conformidad con la reivindicación 48, caracterizado porque el contenido de agua del solvente en el paso d‴') es entre 5 y 10%.

51. El método de conformidad con la reivindicación 48, caracterizado porque el paso f‴') comprende filtrar el sólido y secar en un horno.

52. Un método para producir la forma cristalina (II) de clorhidrato de lercanidipina, que tiene un patrón de difracción de rayos X, en la longitud de onda Kα, como se muestra en la figura 12, de conformidad con cualquiera de las reivindicaciones 25 y 26, caracterizado porque comprende:

d⁗) disolver el clorhidrato de lercanidipina crudo o la forma cristalina (I) de clorhidrato de lercanidipina en un solvente polar, prótico o dipolar aprótico que contiene hasta 50% en peso de agua para producir una solución;

e⁗) agitar la solución del paso d⁗) hasta que la concentración de clorhidrato de lercanidipina disuelto en el

solvente de cristalización es ≤ 2%; y

f"") recuperar el sólido del paso e"") para producir la forma (II) de lercanidipina.

53. El método de conformidad con la reivindicación 52, caracterizado porque además comprende irradiar con ultrasonido y/o adicionar semillas cristalinas de la forma (II) al paso e"").

54. El método de conformidad con la reivindicación 52, caracterizado porque además comprende adicionar hasta 60% de agua a la solución del paso d"").

55. El método de conformidad con la reivindicación 52, caracterizado porque el solvente polar, prótico es un solvente de alcohol.

56. El método de conformidad con la reivindicación 52, caracterizado porque el solvente de alcohol se selecciona del grupo que consiste de metanol, etanol, n-propanol e isopropanol.

57. El método de conformidad con la reivindicación 52, caracterizado porque el solvente dipolar, aprótico es N-metilpirrolidona.

58. El método de conformidad con la reivindicación 52, caracterizado porque la temperatura del paso d"") es entre 20 y 70°C.

59. El método de conformidad con la reivindicación 52, caracterizado porque la agitación en el paso e"") toma

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lugar a una temperatura entre 20 y 25°C.

60. El método de conformidad con la reivindicación 52, caracterizado porque el secado en el paso f"") toma lugar a 70°C.

61. Una composición farmacéutica antihipertensiva, caracterizada porque comprende (1) clorhidrato de lercanidipina cristalino y opcionalmente otras formas de lercanidipina, en donde el clorhidrato de lercanidipina cristalino se selecciona del grupo que consiste de la forma cristalina (I) de clorhidrato de lercanidipina, la forma cristalina (II) de clorhidrato de lercanidipina y combinaciones de las mismas que comprenden un contenido predeterminado de cada forma cristalina y (2) al menos un componente seleccionado del grupo que consiste de un portador o diluyente farmacéuticamente aceptable, un saborizante, edulcorante, conservador, tinte, sustancia aglutinante, agente de suspensión, agente de dispersión, colorante, desintegrante, excipiente, lubricante, plastificante y aceite comestible.

62. Una forma de dosificación unitaria, caracterizada porque comprende la composición farmacéutica, antihipertensiva de conformidad con la reivindicación 61.

63. La forma de dosificación unitaria de conformidad con la reivindicación 62, caracterizada porque la forma de dosificación es una forma de dosificación de

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liberación inmediata de lercanidipina.

64. La forma de dosificación unitaria de conformidad con la reivindicación 62, caracterizada porque la forma de dosificación es una forma de dosificación de liberación sostenida de lercanidipina.

65. La forma de dosificación unitaria de conformidad con la reivindicación 64, caracterizada porque la forma de dosificación comprende una fase de liberación inmediata de lercanidipina y una fase de liberación sostenida de lercanidipina.

66. La forma de dosificación unitaria de conformidad con la reivindicación 65, caracterizada porque la composición comprende 0.1 a 400 mg de clorhidrato de lercanidipina.

67. La forma de dosificación unitaria de conformidad con la reivindicación 66, caracterizada porque la composición comprende 1 a 200 mg de clorhidrato de lercanidipina.

68. La forma de dosificación unitaria de conformidad con la reivindicación 67, caracterizada porque la composición comprende 5 a 40 mg de clorhidrato de lercanidipina.

69. Una composición antihipertensiva, caracterizada porque comprende cantidades predeterminadas de la forma cristalina (I) de clorhidrato de lercanidipina y la

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forma cristalina (II) de clorhidrato de lercanidipina.

70. La composición antihipertensiva de conformidad con la reivindicación 69, caracterizada porque la forma cristalina (I) de clorhidrato de lercanidipina tiene un punto de fusión de aproximadamente 197-201°C y la forma cristalina (II) de clorhidrato de lercanidipina tiene un punto de fusión de aproximadamente 207-211°C, cuando los puntos de fusión son determinados como los picos de DSC.

71. La composición antihipertensiva de conformidad con las reivindicaciones 69 y 70, caracterizada porque la relación de la forma (I) con la forma (II) es entre 1:9 y 9:1.

72. La composición antihipertensiva de conformidad con la reivindicación 71, caracterizada porque la relación de la forma (I):forma (II) se selecciona del grupo que consiste de 9:1, 7:3, 1:1, 3:7 y 1:9.

73. La forma cristalina, aislada de lercanidipina de conformidad con cualquiera de las reivindicaciones 23, 24, 25 o 26, caracterizada porque comprende un tamaño de partícula promedio de D (50%) 2-8 µm y D (90%) <15 µm.

74. La composición farmacéutica, antihipertensiva de conformidad con la reivindicación 62, caracterizada porque las formas cristalinas (I) y (II) de clorhidrato de lercanidipina tienen cada una un tamaño de partícula promedio de D (50%) 2-8 µm y D (90%) < 15 µm.

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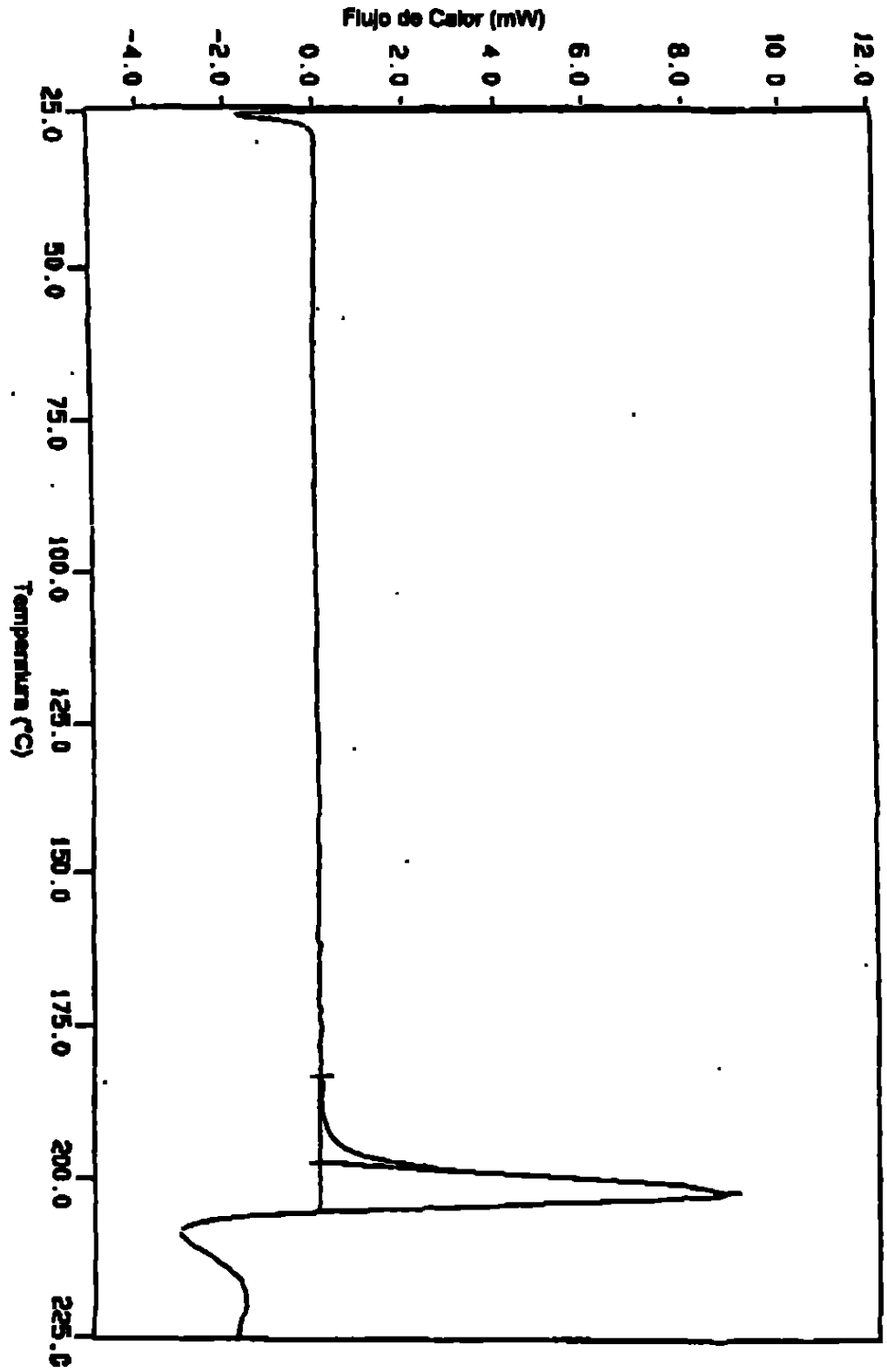

Flujo de Calor (mW)
-4.0
-2.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
Temperatura (°C)
25.0
50.0
75.0
100.0
125.0
150.0
175.0
200.0
225.0

FIG. 1

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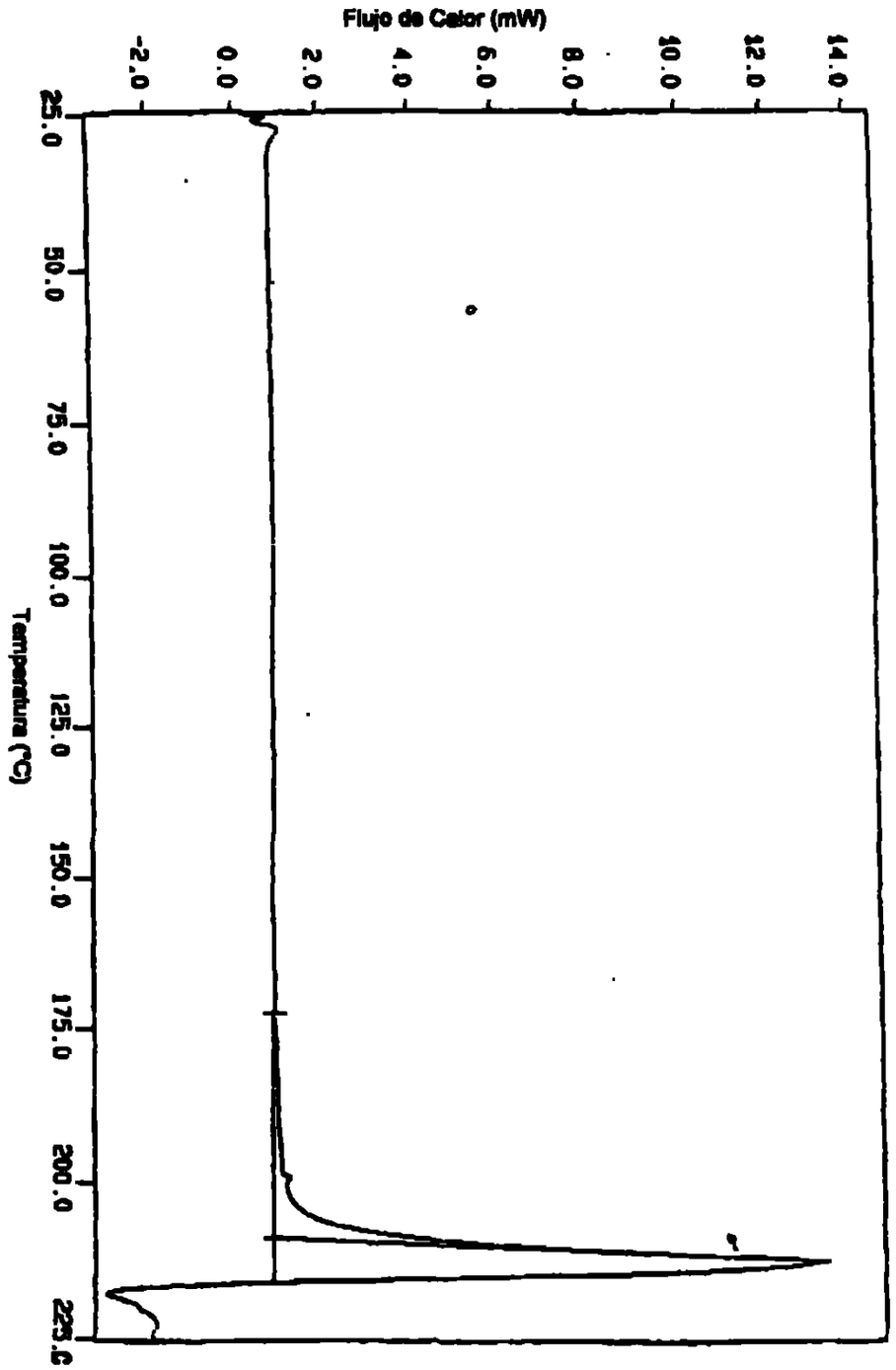

Flujo de Calor (mW)
-2.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
Temperatura (°C)
25.0
50.0
75.0
100.0
125.0
150.0
175.0
200.0
225.0

FIG. 2

183

181

Forma I
Forma II
105
100
95
90
85
80
75
70
% de Masse
50
100
150
200
Temperature (°C)

FIG. 3

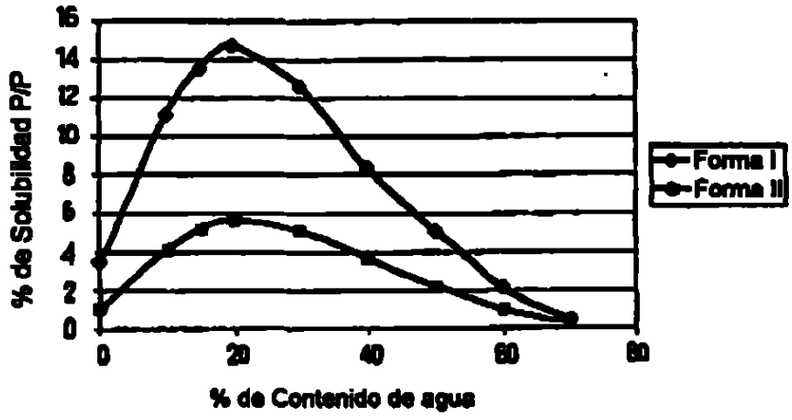

% de Solubilidad P/P
16
14
12
10
8
6
4
2
0
0
20
40
60
80
% de Contenido de agua
Forma I
Forma II

Fig. 4

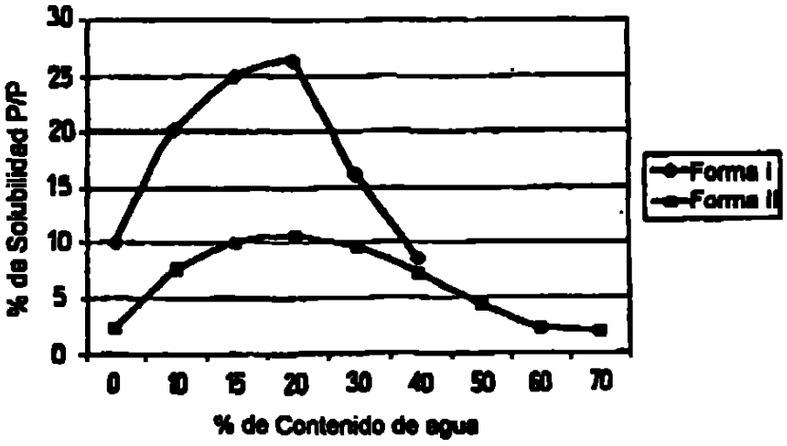

% de Solubilidad P/P
30
25
20
15
10
5
0
0
10
15
20
30
40
50
60
70
% de Contenido de agua
Forma I
Forma II

Fig. 5

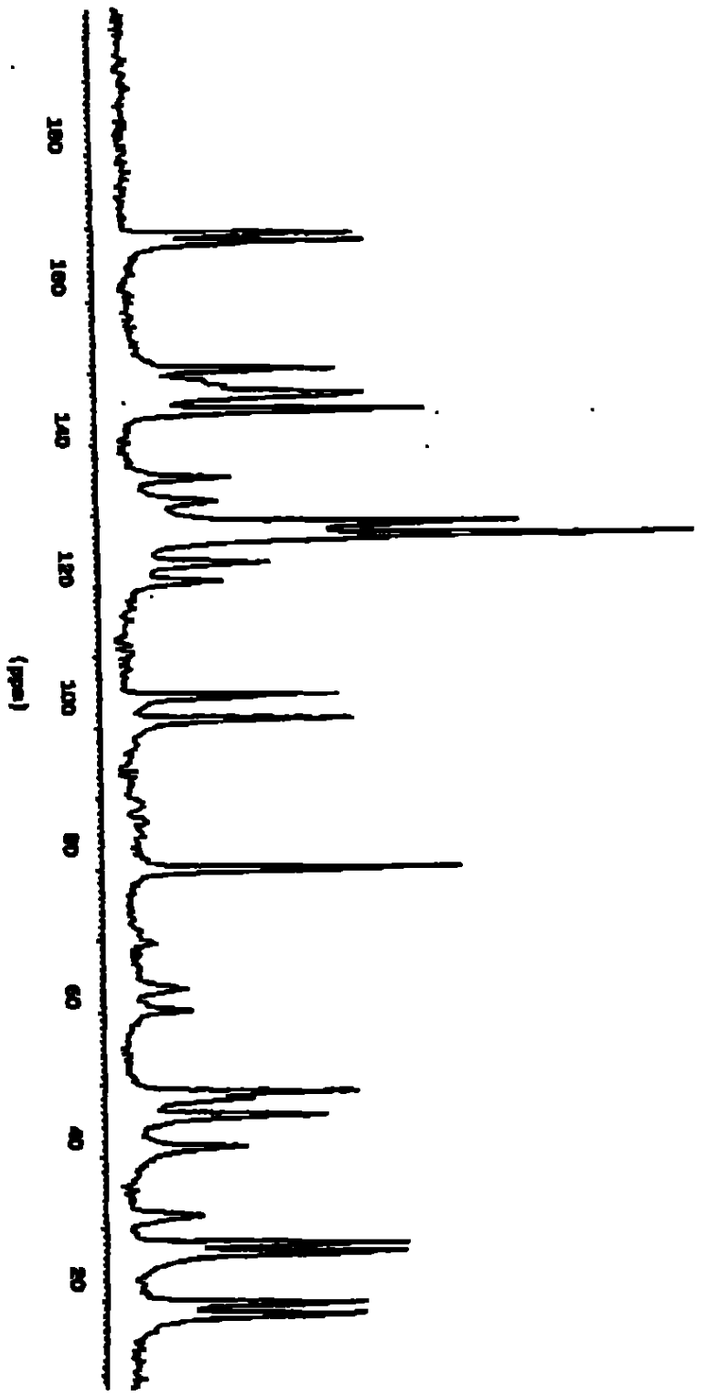


FIG. 6

186

186

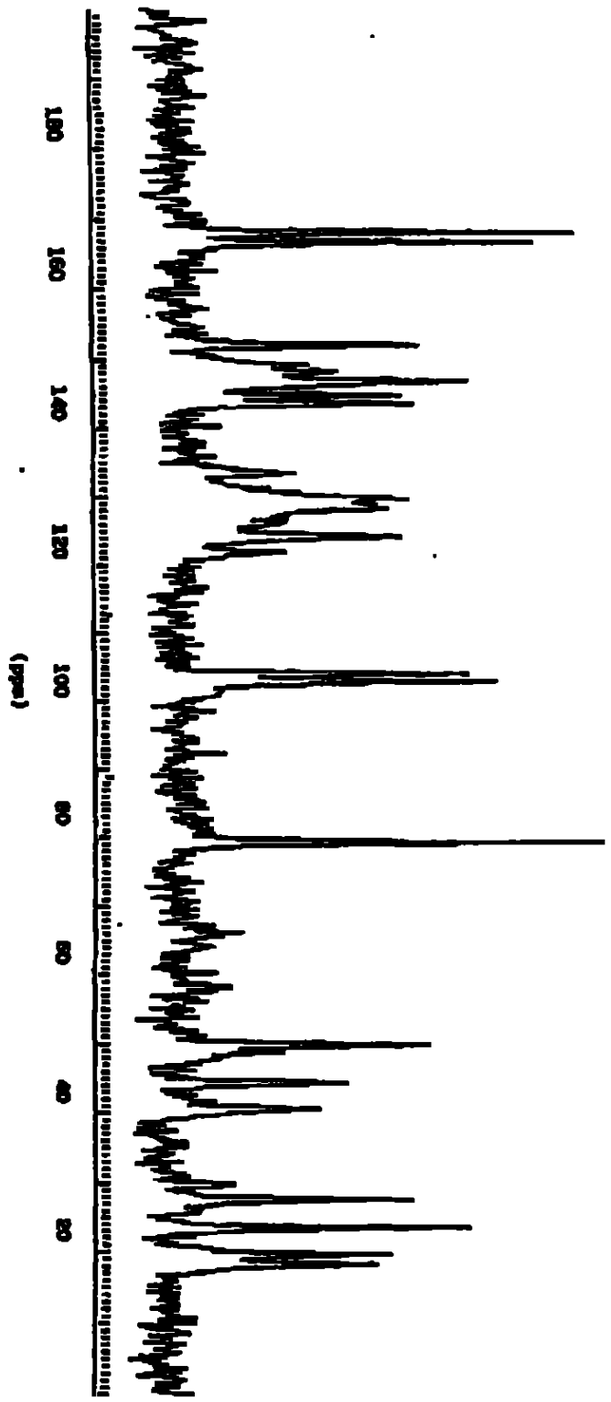


FIG.7

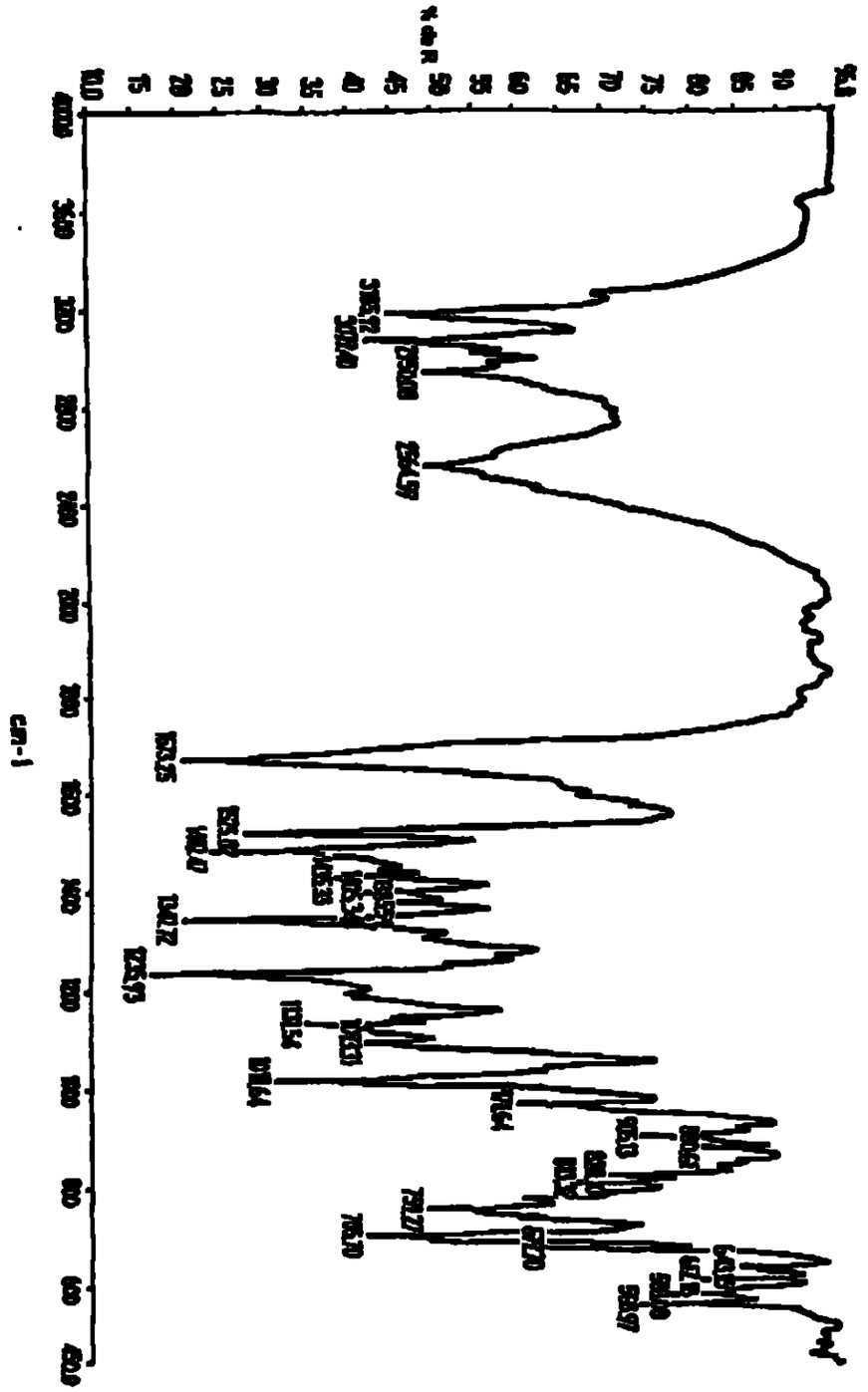


Fig. 8

884

881

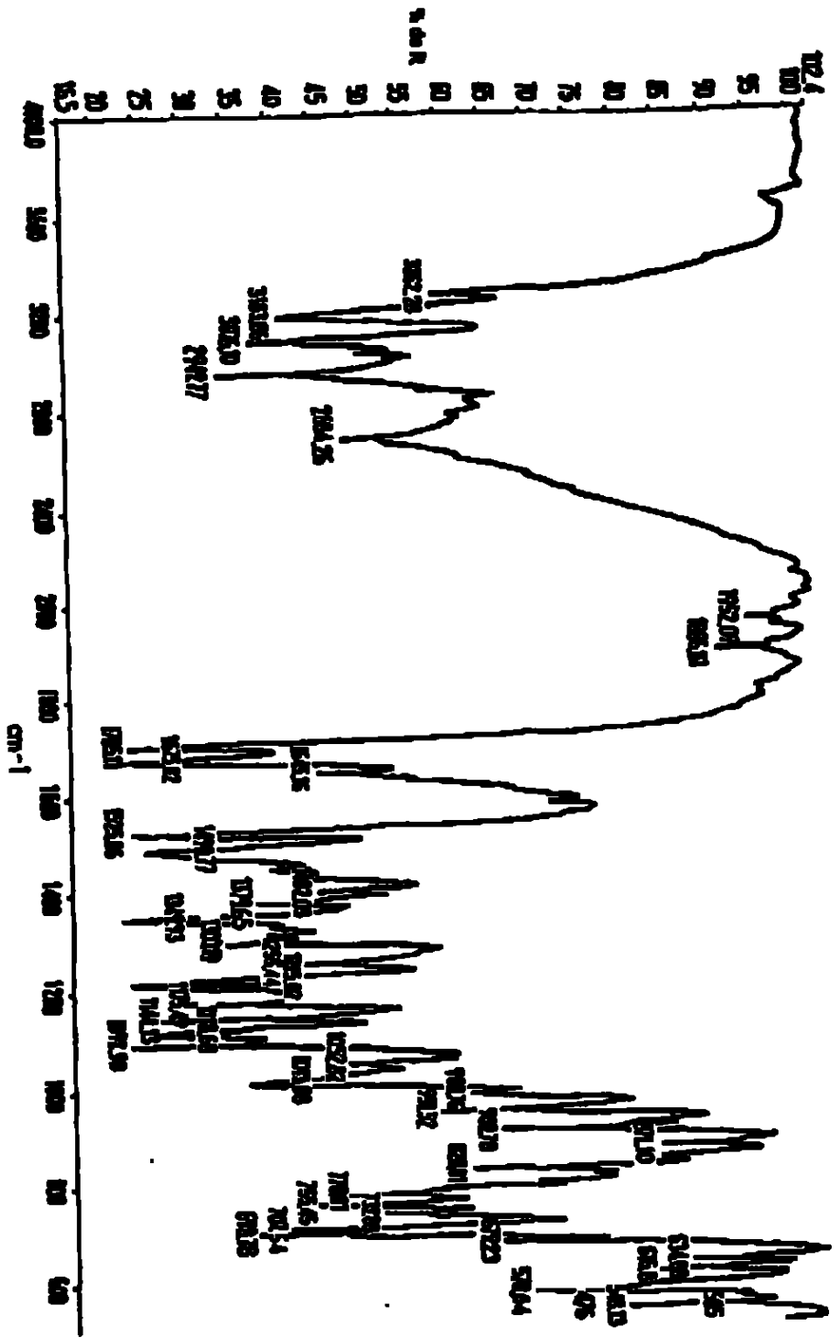


FIG. 9

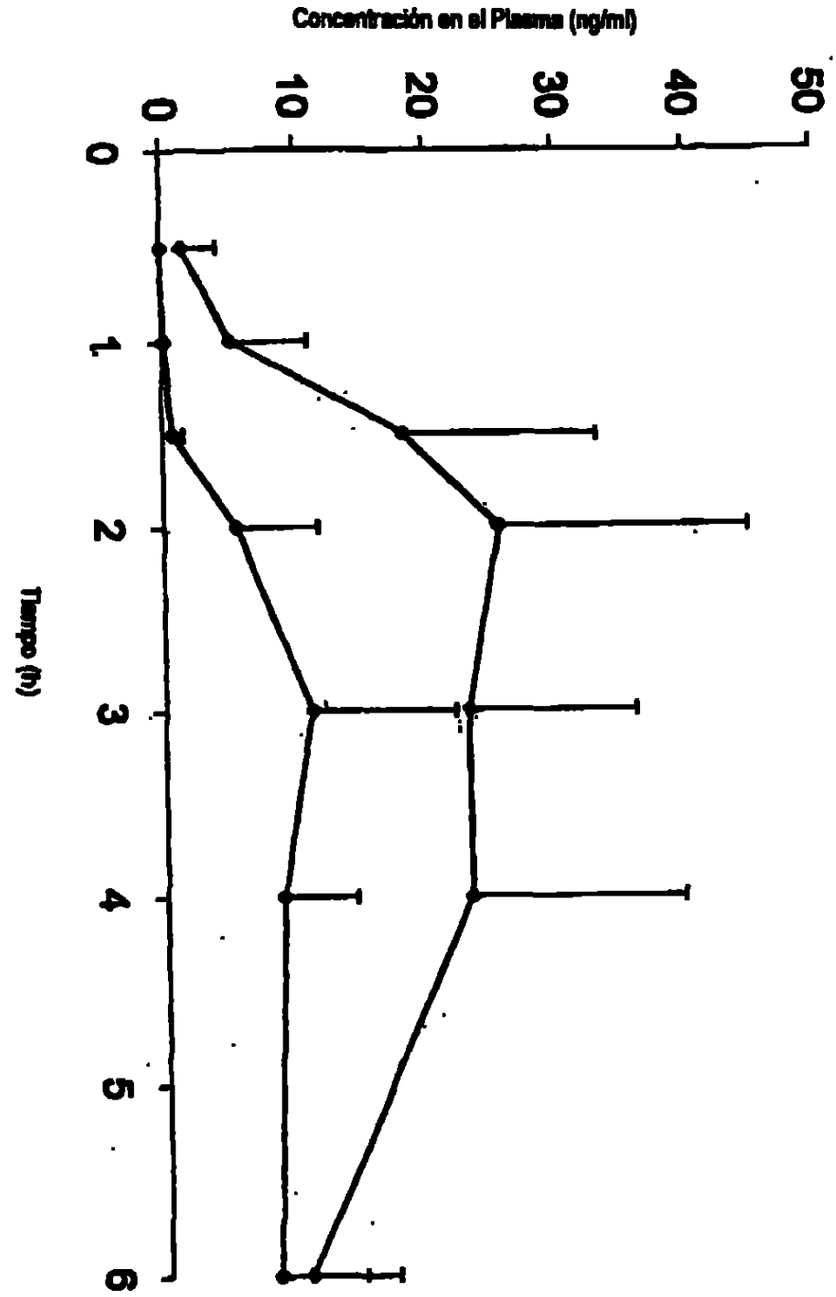


FIG. 10

190

190

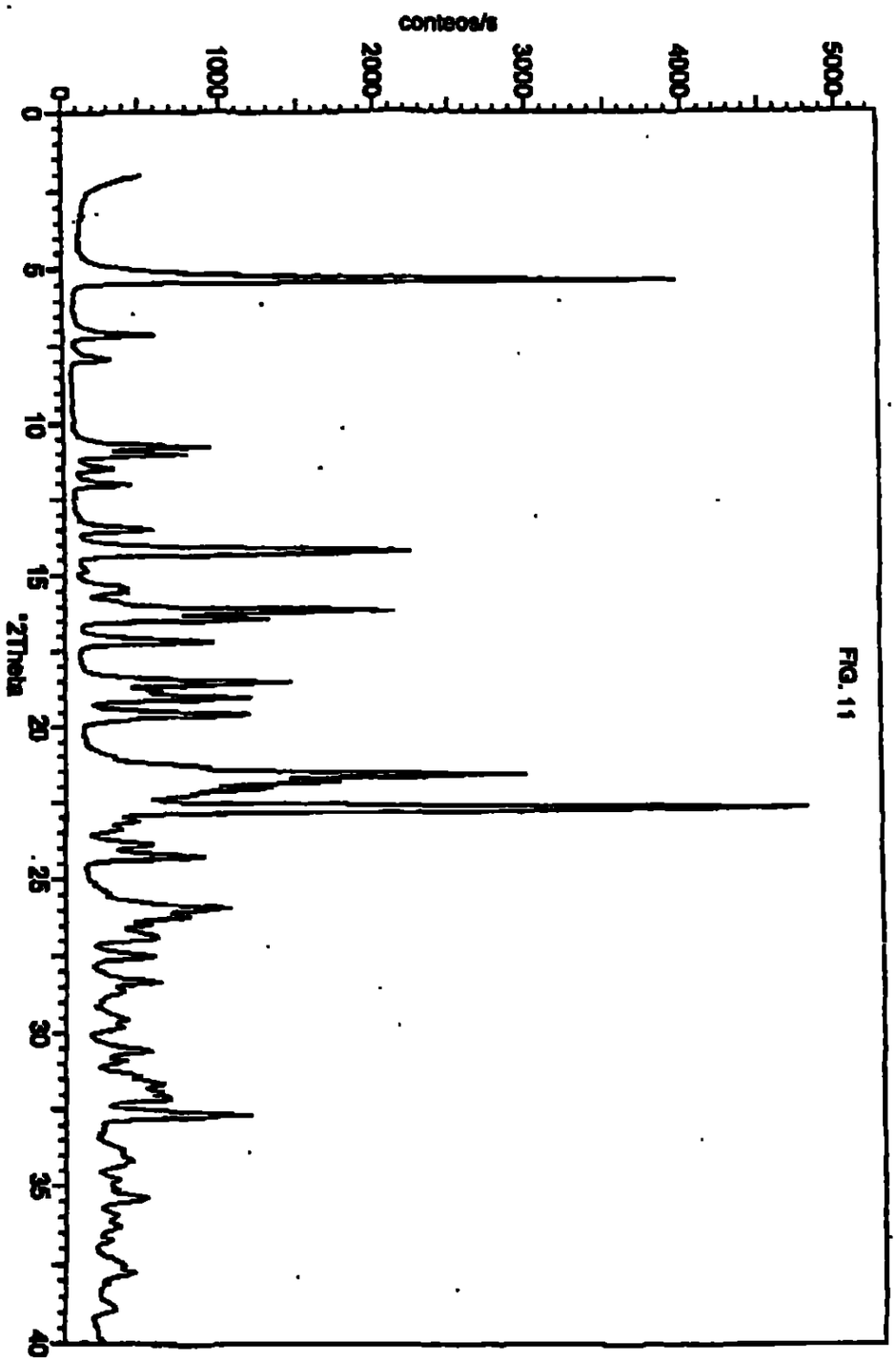

conteos/s
0
1000
2000
3000
4000
5000
0
5
10
15
20
25
30
35
40
°2Theta

FIG. 11

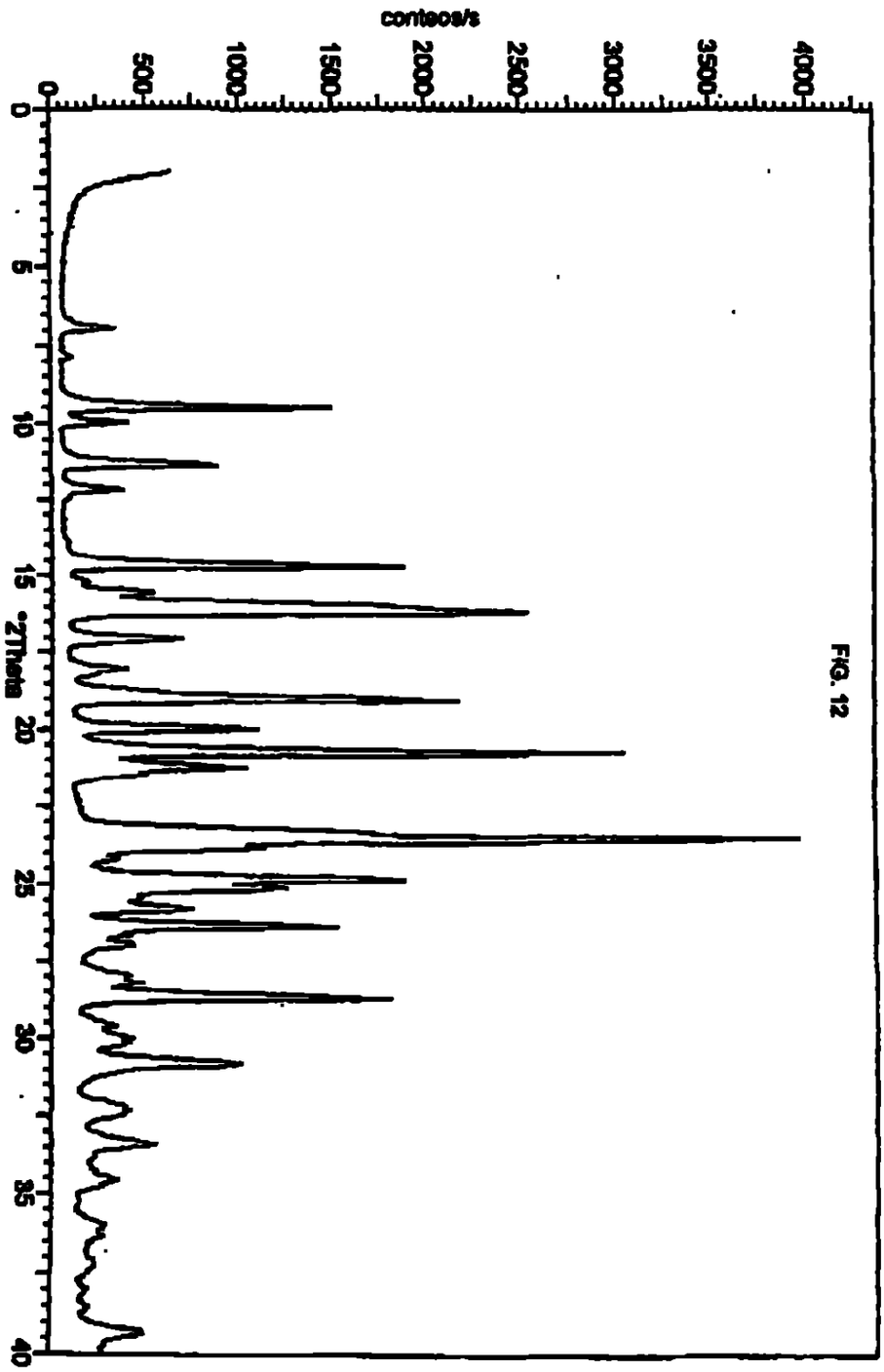


FIG. 12

192

192

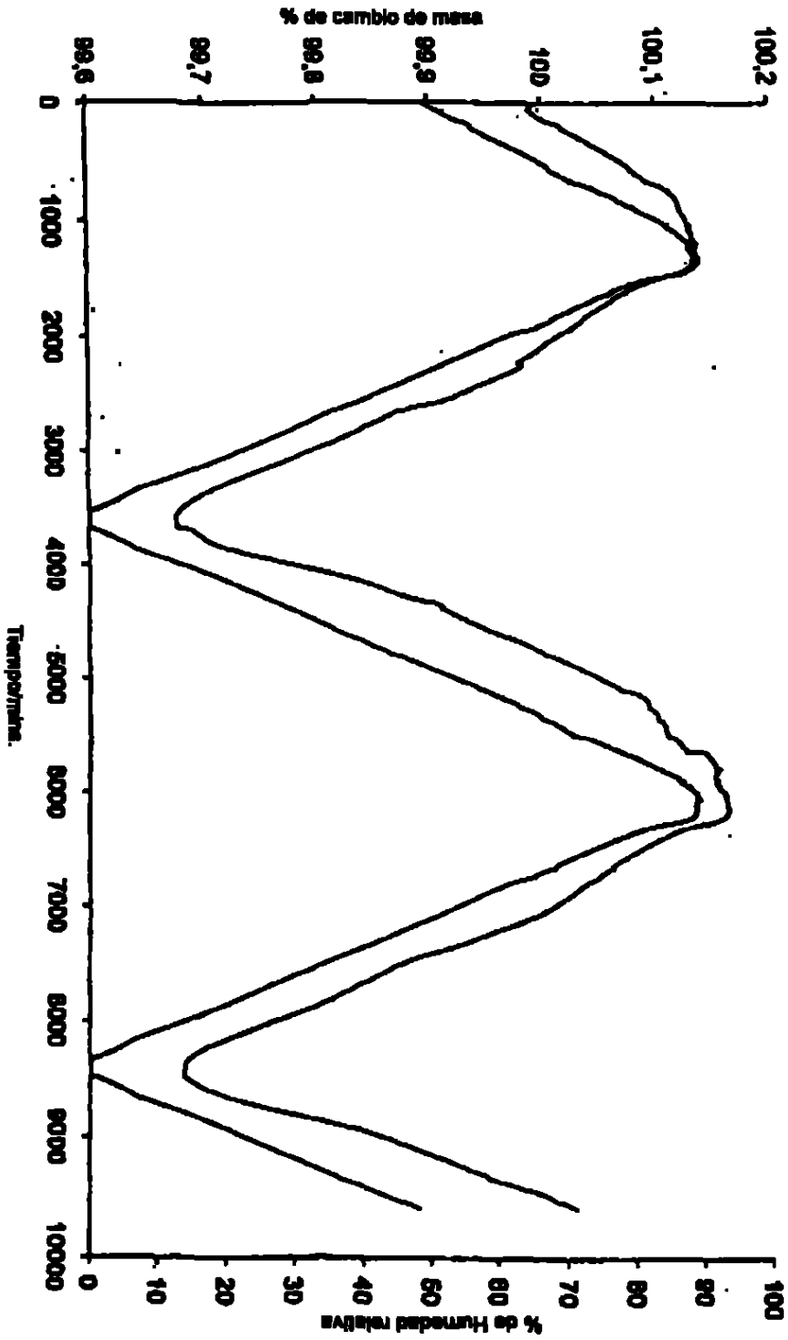


FIG. 13

193

193

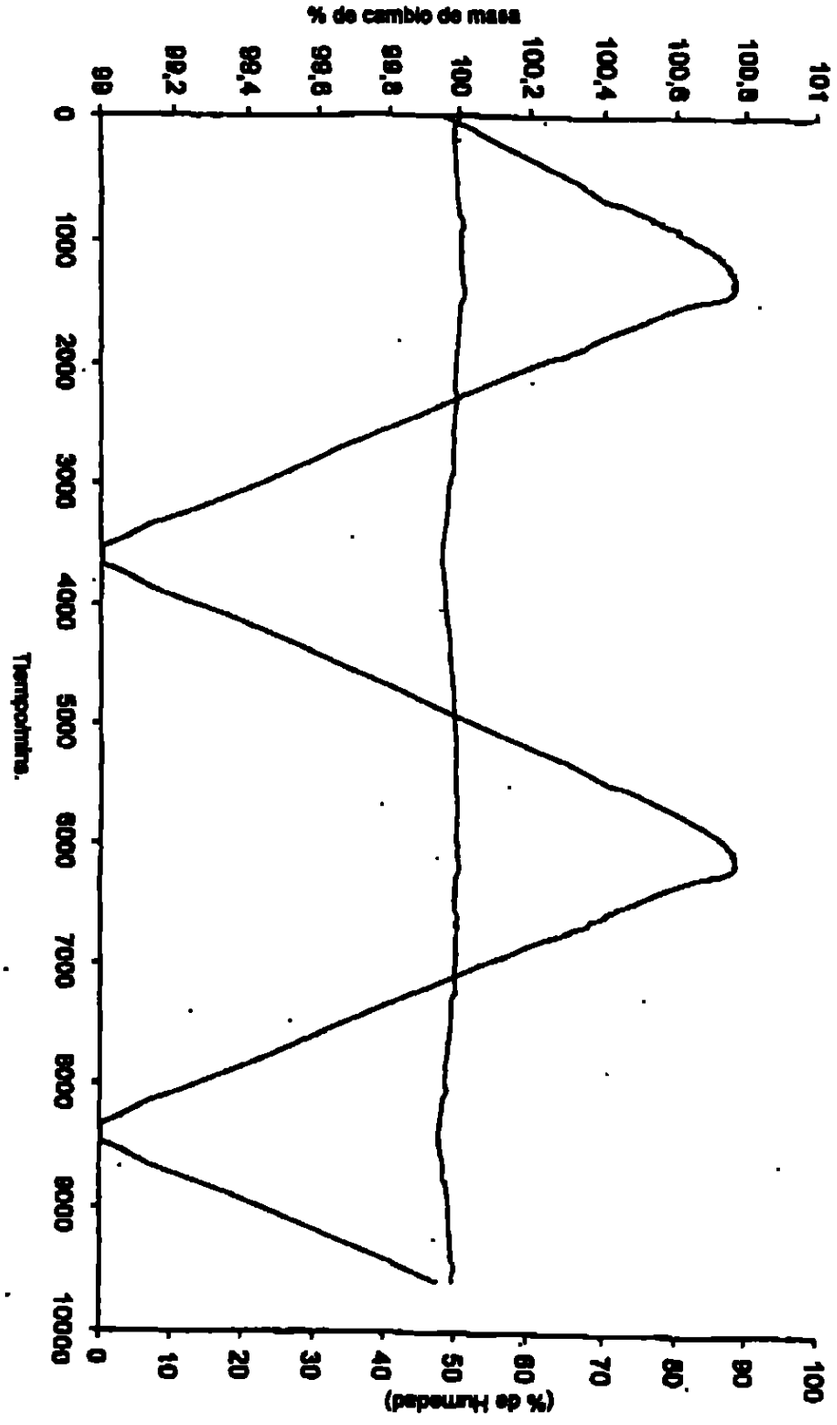

% de cambio de masa
98
98,2
98,4
98,6
98,8
100
100,2
100,4
100,6
100,8
101
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
Temperatura.
0
10
20
30
40
50
60
70
80
90
100
(% de Humedad)

FIG. 14

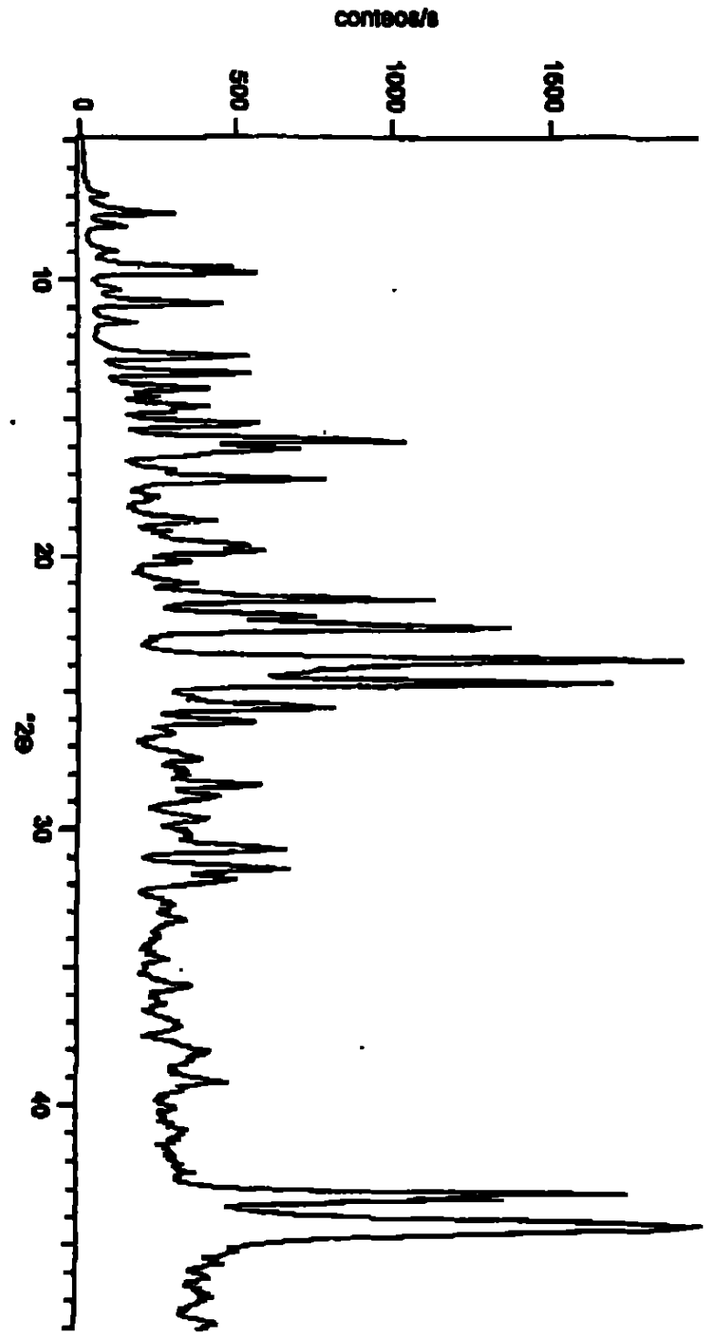


FIG. 15

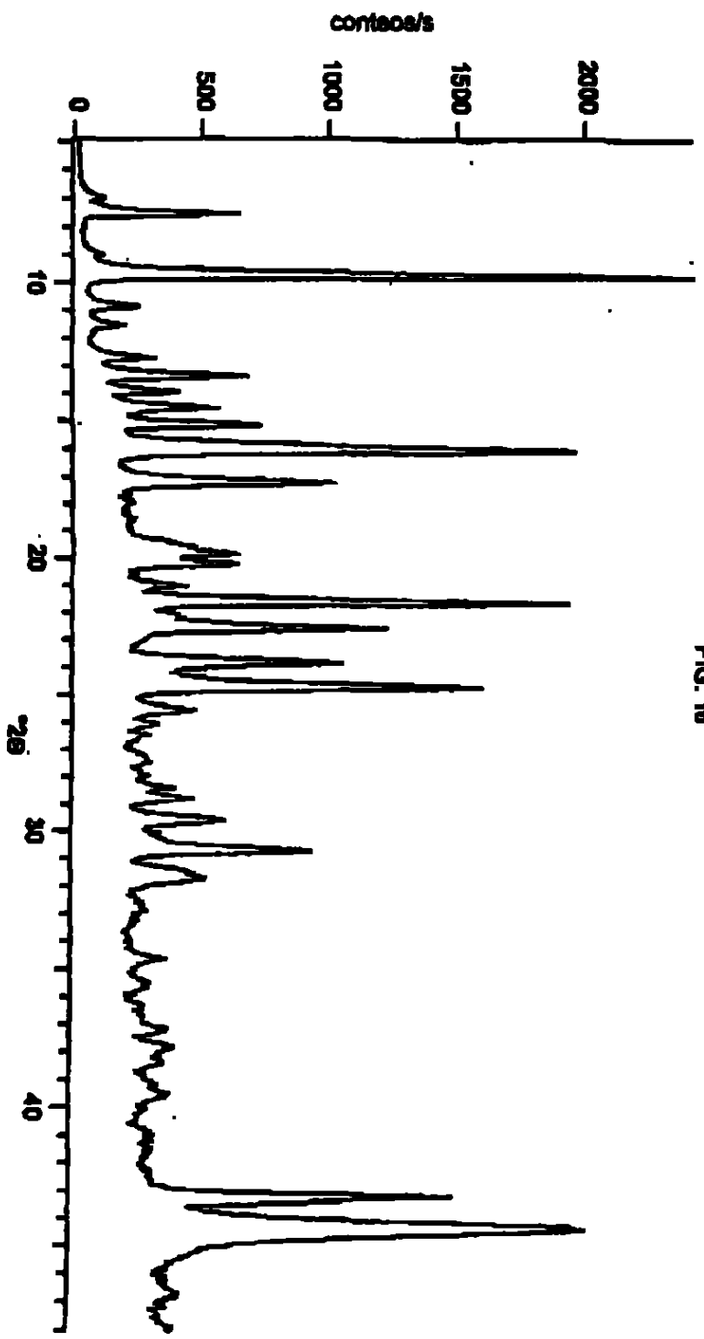


FIG. 18

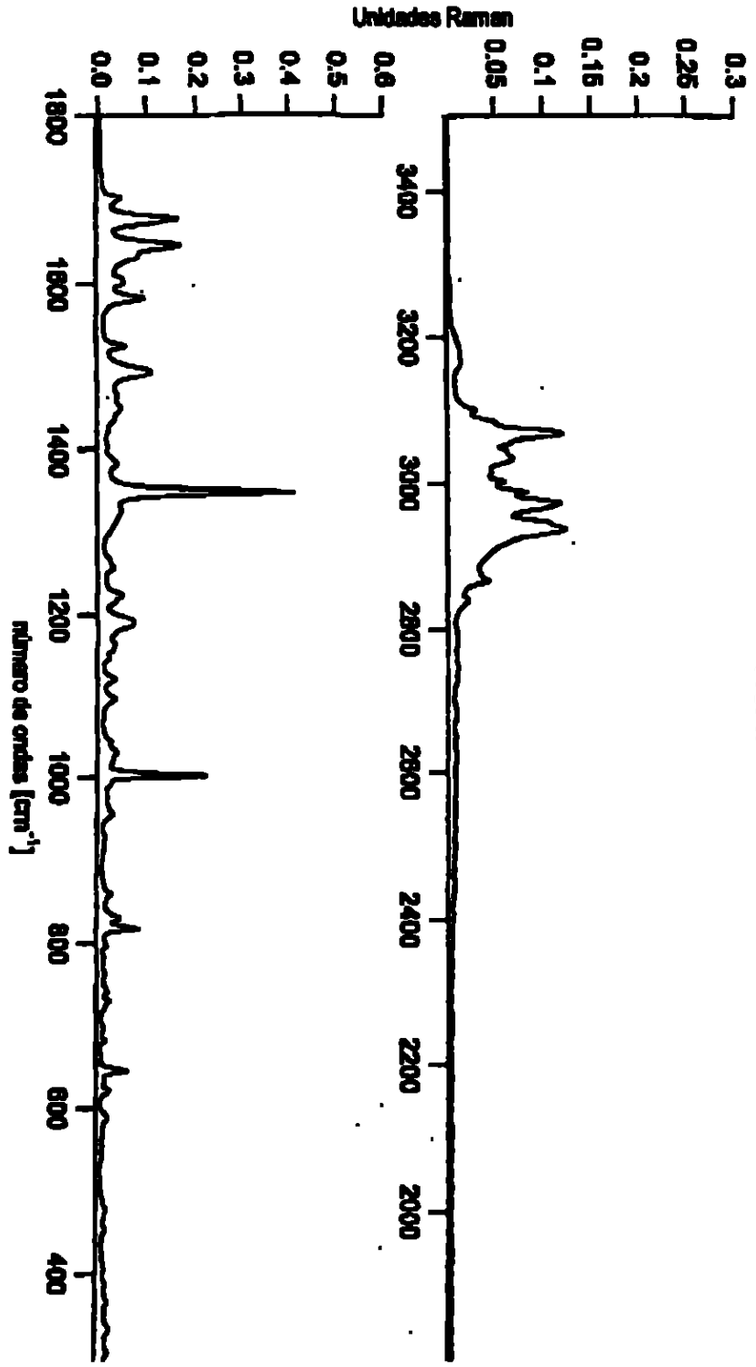

Unidades Raman
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.05
0.1
0.15
0.2
0.25
0.3
1800
1600
1400
1200
1000
800
600
400
3400
3200
3000
2800
2600
2400
2200
2000
número de ondas [cm-1]

FIG. 17

19

191

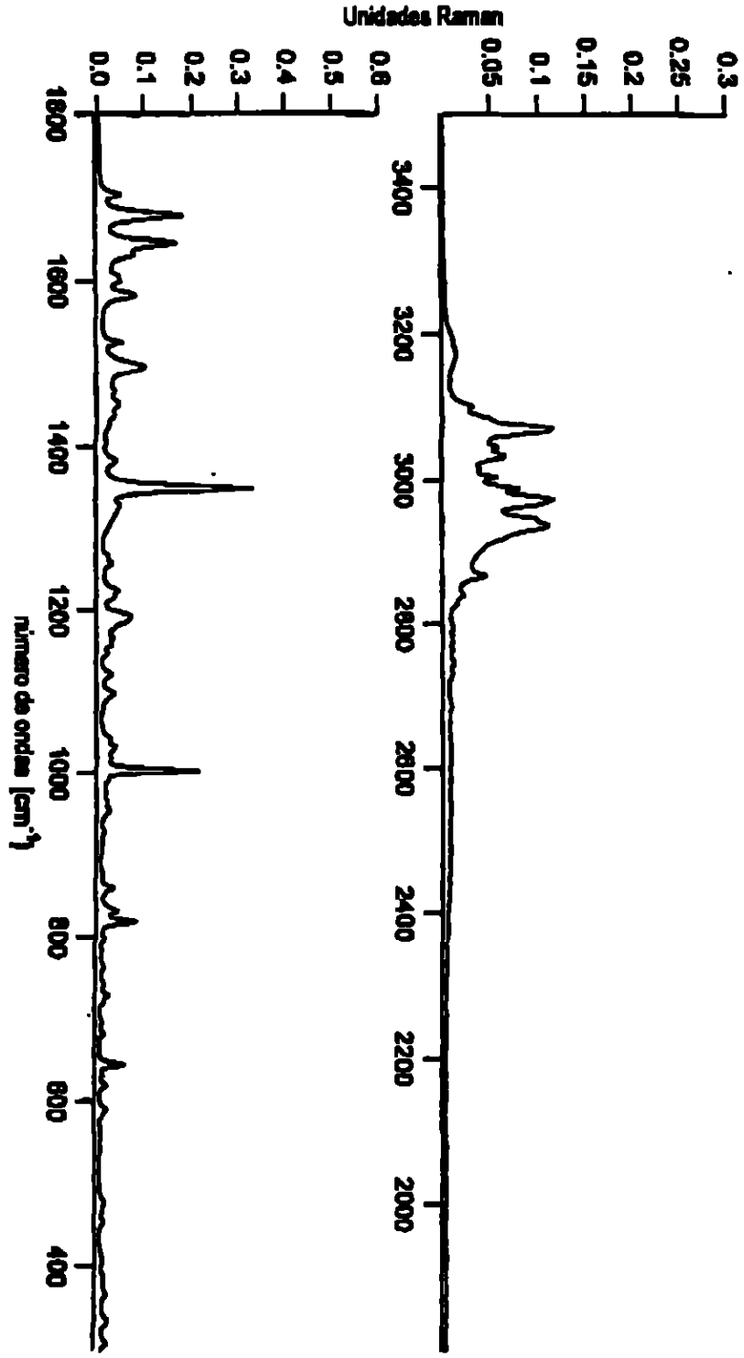

Unidades Raman
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.05
0.1
0.15
0.2
0.25
0.3
1800
1600
1400
1200
1000
800
600
400
3400
3200
3000
2800
2600
2400
2200
2000
número de ondas [cm-1]

FIG. 18

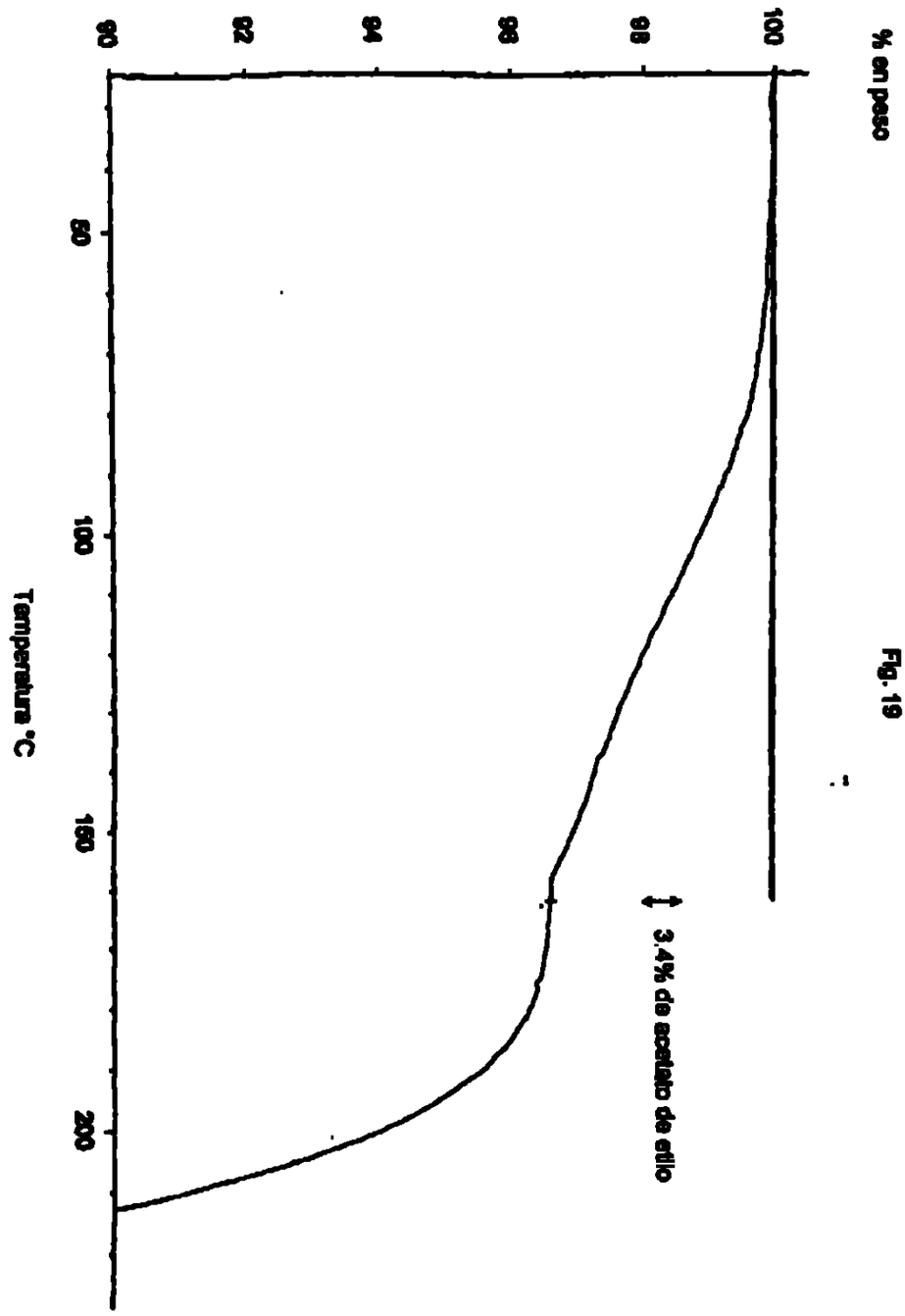


Fig. 19

199

199

200.

205

Fig. 20
% en peso
100.5
100.0
99.5
99.0
98.5
98.0
97.5
50
100
150
200
Temperatura °C
-0.3% de agotado de ciclo
(aproximadamente 0.1% de agua)

NUEVAS FORMAS POLIMORFICAS CRISTALINAS DE CLORHIDRATO DE LERCANIDIPINA Y PROCESO PARA SU PREPARACION

CAMPO DE LA INVENCIÓN

La invención se dirige a nuevas formas crudas y formas cristalinas de clorhidrato de lercanidipina y a procedimientos para la preparación de estas formas. También se contemplan las composiciones farmacéuticas que comprenden las nuevas formas cristalinas.

ANTECEDENTES DE LA INVENCIÓN

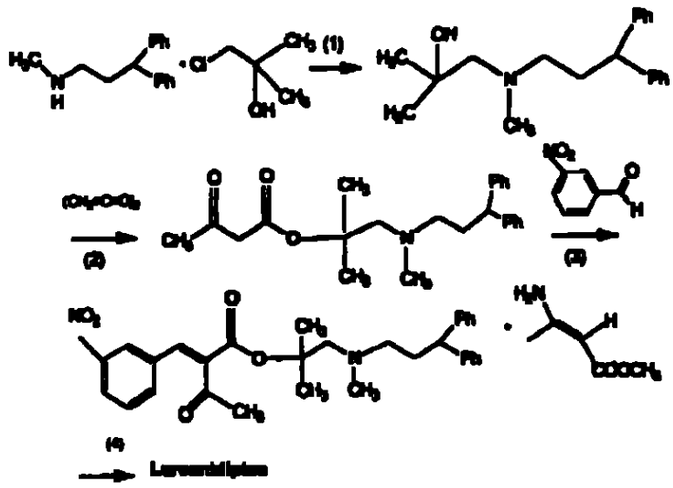
La lercanidipina (1,1,N-trimetil-N-(3,3-difenilpropil)-2-aminoetil-1,4-dihidro-2,6-dimetil-4-(3-nitrofenil)piridin-3,5-dicarboxilato de metilo) es un antagonista cálcico de dihidropiridina altamente lipofílico con una larga duración de acción y alta selectividad vascular. Su mecanismo de actividad antihipertensiva se atribuye a un efecto relajante, directo sobre el músculo liso vascular, lo cual disminuye la resistencia periférica total. La dosis de inicio recomendada de lercanidipina como monoterapia es 10 mg al dia por la ruta oral, con una titulación del fármaco como sea necesario para 20 mg al dia. La lercanidipina es adsorbida rápidamente después de la administración oral con niveles pico en el plasma que ocurren 2-3 horas después de la dosificación. La eliminación es

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esencialmente por medio de la ruta hepática.

En virtud de su lipofilicidad alta y coeficiente en las membranas alto, la lercanidipina combina un corto periodo de vida útil en el plasma con una larga duración de acción. De hecho, la distribución preferencial del fármaco en membranas de las células de músculo liso da por resultado la farmacocinética controlada por la membrana que está caracterizada por un efecto farmacológico prolongado. En comparación a otros antagonistas cálcicos, la lercanidipina está caracterizada por un comienzo gradual y duración prolongada de acción a pesar de la disminución de los niveles en el plasma. Los estudios *in vitro* muestran que la respuesta de la aorta aislada de rata a K^+ alto puede ser atenuada por la lercanidipina, aún después de que el fármaco ha sido removido del ambiente del tejido aórtico durante 6 horas.

La lercanidipina es comercialmente disponible de Recordati S.p.A. (Milán, Italia) y ha sido descrita junto con métodos para elaborarla y resolverla en enantiómeros individuales en las patentes norteamericanas Nos. 4,705,797; 5,767,136; 4,968,832; 5,912,351 y 5,696,139. Un procedimiento para preparar la lercanidipina descrito en la patente norteamericana No. 4,705,797 involucra el siguiente esquema de reacción:



(1): xileno a reflujo. (2): tolueno, 85°C; (3) HCl + $CHCl_3$; 0°C; (4) HO-$CH(CH_3)_2$ a reflujo

La lercanidipina cruda es un residuo oleoso que debe ser purificado mediante la cromatografía con evaporación instantánea utilizando cloroformo, que contiene cantidades crecientes de acetona, como el eluyente. El solvente luego es evaporado a sequedad y el residuo restante es disuelto en metanol adicionando un pequeño exceso de ácido clorhídrico en etanol. Después de la evaporación del solvente, la sal de clorhidrato semihidratada es preparada mediante el tratamiento con ácido clorhídrico diluido en presencia de cloruro de sodio.

Una desventaja principal del procedimiento para preparar la lercanidipina, como se describe en la patente norteamericana No. 4,705,797, es que la reacción de ciclización descrita genera varios subproductos, lo cual da

por resultado un rendimiento inferior para el producto deseado. Además, la purificación y aislamiento de la lercanidipina de la mezcla de reacción son muy complejos, puesto que requieren numerosos tratamientos con diferentes solventes. Finalmente, los pasos de purificación y aislamiento son difíciles de realizar a una escala industrial debido a la necesidad de purificar el producto mediante la cromatografía en columna.

La patente norteamericana No. 5,912,351 describe un proceso más simple para la preparación de clorhidrato de lercanidipina. Éste involucra la reacción del ácido 1,4-dihidro-2,6-dimetil-5-metoxicarbonil-4-(3-nitrofenil)-piridin-3-carboxílico con cloruro de tionilo en diclorometano y dimetilformamida a una temperatura entre -4 y +1°C y la esterificación subsecuente del cloruro de ácido obtenido con el alcohol de 2,N-dimetil-N-(3,3-difenilpropil)-1-amino-2-propilo a una temperatura entre -10 y 0°C. El procedimiento produce clorhidrato de lercanidipina en una forma cristalina, no higroscópica, anhidra y evita la formación de subproductos indeseados y la purificación subsecuente en columnas para la cromatografía.

Sin embargo, el aislamiento de cloruro de lercanidipina en forma cristalina es nuevamente muy complejo. Después de la evaporación del solvente de la mezcla de reacción y la disolución del residuo obtenido de esta manera

en acetato de etilo, la solución se lava primero con salmuera, luego se lava cinco veces adicionales con una solución al 10% de carbonato de sodio, cinco veces con ácido clorhídrico 1N y eventualmente una vez más con salmuera.

Por lo tanto, existe la necesidad en el campo por un procedimiento para la preparación de clorhidrato de lercanidipina en forma cristalina que evite una o más de las desventajas de los procedimientos actualmente utilizados.

Además, se observó que la lercanidipina, producida por medio del segundo procedimiento descrito anteriormente, exhibió variabilidad de lote a lote a pesar de un control cuidadoso del procedimiento y aún la observación del punto de fusión que se creía era característico del producto sólido obtenido por medio del procedimiento del ejemplo 3 de la patente norteamericana No. 5,767,136 de 186-188°C. Esta variabilidad fue manifiesta en las diferencias que aparecen (y desaparecen) aparentemente imposibles de predecir en uno o más de la apariencia del producto (por ejemplo color), punto de fusión y solubilidad. Esto provocó problemas en cuanto a si se podrían dar garantías de pureza y/o reproducibilidad (por ejemplo a autoridades reguladoras) que el producto siempre fuera el mismo.

La investigación adicional por los presentes inventores reveló diferencias de lote a lote en la biodisponibilidad en animales y diferencias en el tamaño de

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los cristales. En el curso de la investigación de las causas del problema de variabilidad, los inventores descubrieron sorprendentemente nuevos polimorfos de clorhidrato de lercanidipina. Ellos también descubrieron procedimientos más adecuados para la preparación y aislamiento de productos cristalinos de clorhidrato de lercanidipina a partir de la mezcla de reacción. Se determinó sorprendentemente que el clorhidrato de lercanidipina muestra características polimórficas y se cristaliza en diferentes formas cristalinas dependiendo del proceso seguido y de los solventes utilizados. Además, se ha vuelto posible el aislamiento de cada polimorfo cristalino, individual, disminuyendo de esta manera la posibilidad de variabilidad de lote a lote de la lercanidipina, lo cual los presentes inventores determinaron que fue debido a que estaban presentes mezclas de diferentes formas sólidas en el mismo lote y a que tales mezclas de diferente composición tenían puntos de fusión dentro del mismo rango reducido como las formas individuales. Como resultado, fueron necesarios lotes más reproducibles de lercanidipina que fueron más adecuados para la producción a gran escala y el control de calidad.

BREVE DESCRIPCIÓN DE LA INVENCIÓN

La presente invención proporciona nuevas formas crudas y formas cristalinas de clorhidrato de lercanidipina y

procedimientos para su preparación.

En una modalidad, la invención proporciona la nueva forma cruda (A) de clorhidrato de lercanidipina, la cual tiene un punto de fusión de aproximadamente 150-152°C (pico de DSC) y comprende aproximadamente 3-4% (p/p) de acetato de etilo.

En otra modalidad, la invención proporciona la nueva forma cruda (B) de clorhidrato de lercanidipina, la cual tiene un punto de fusión de aproximadamente 131-135°C (pico de DSC) y comprende aproximadamente 0.3-0.7% (p/p) de acetato de etilo.

Se proporcionan métodos para la síntesis independiente de la forma cruda (A) de clorhidrato de lercanidipina y la forma cruda (B) de clorhidrato de lercanidipina, que hacen posible se obtenga cada forma cruda en forma aislada.

En una modalidad adicional, se proporciona una forma cristalina aislada (I) de clorhidrato de lercanidipina, la cual tiene el siguiente patrón de difracción de rayos X, a la longitud de onda Kα, en donde las distancias entre los picos (D en X), relaciones de intensidad relativa (I/Io) y ángulos de los picos significantes (2θ) son:

D(X)	Intensidad Relativa (I/Io)	ángulo 2θ
16.3	83	5.4

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6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

La forma cristalina (I) de clorhidrato de lercanidipina tiene un punto de fusión de aproximadamente 197-201°C, cuando el punto de fusión se determina como el pico de DSC.

En una modalidad alternativa, se proporciona la forma cristalina, aislada (II) de clorhidrato de lercanidipina, la cual tiene el siguiente patrón de difracción de rayos X, a la longitud de onda Kα, como se muestra en donde las distancias, relaciones (I/Io) y ángulos 2θ de los picos significantes son:

D(X)	Intensidad Relativa (I/Io)	ángulo 2θ
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8
3.81	41	23.4
3.77	100	23.6

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3.58	44	24.8
3.54	29	25.2

La forma cristalina (II) de clorhidrato de lercanidipina tiene un punto de fusión de aproximadamente 207-211°C, cuando el punto de fusión se determina como el pico de DSC.

De esta manera, la presente invención permite obtener mezclas de la forma I y la forma II que tienen un contenido predeterminado y reproducible de cada forma y, opcionalmente, también otras formas de lercanidipina, tales como amorfos.

También se proporcionan métodos de síntesis en los cuales cada forma cristalina, aislada (I) y (II) de clorhidrato de lercanidipina se puede obtener, independientemente, del material de inicio de la forma cruda (A) o la forma cruda (B) de clorhidrato de lercanidipina.

También se proporcionan composiciones farmacéuticas que comprenden (1) clorhidrato de lercanidipina cristalino y opcionalmente otras formas de lercanidipina, tales como amorfos, en donde el clorhidrato de lercanidipina cristalino se selecciona del grupo que consiste de la forma cristalina (I) de clorhidrato de lercanidipina, forma cristalina (II) de clorhidrato de lercanidipina y combinaciones de las mismas que comprenden un contenido predeterminado de cada forma

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cristalina y (2) al menos un componente seleccionado del grupo que consiste de un portador o diluyente farmacéuticamente aceptable, un saborizante, edulcorante, conservador, tinte, sustancia aglutinante, agente de suspensión, agente de dispersión, colorante, desintegrante, excipiente, lubricante, plastificante y aceite comestible.

En ciertas modalidades, las composiciones farmacéuticas mencionadas anteriormente se proporcionan como una forma de dosificación que comprende la forma cristalina (I) o (II) de clorhidrato de lercanidipina o una combinación de las mismas que tiene una formulación predeterminada de cada forma cristalina.

En las modalidades adicionales, la invención también proporciona métodos para el tratamiento de un sujeto con hipertensión arterial, el método comprende administrar una cantidad terapéuticamente efectiva de la forma cristalina (I) de clorhidrato de lercanidipina, forma cristalina (II) de clorhidrato de lercanidipina o combinaciones de las mismas que comprende un contenido predeterminado de cada forma a un sujeto en necesidad de tal tratamiento.

En otras modalidades, se proporciona un método para tratar o prevenir lesiones ateroscleróticas en las arterias de un sujeto, el método comprende administrar a una cantidad terapéuticamente efectiva de la forma cristalina (I) de clorhidrato de lercanidipina, forma cristalina (II) de

clorhidrato de lercanidipina o combinaciones de las mismas que comprende una cantidad predeterminada de cada forma, a un sujeto en necesidad de tal tratamiento. En un aspecto preferido, un sujeto en necesidad del tratamiento es un mamifero. Más preferiblemente, el sujeto en necesidad del tratamiento es un humano.

Estos y otros aspectos de la presente invención serán aparentes para aquellas personas de experiencia ordinaria en el campo en vista de la presente descripción, reivindicaciones y figuras.

BREVE DESCRIPCIÓN DE LAS FIGURAS

La figura 1 es una gráfica del análisis DSC llevado a cabo sobre la forma cristalina (I), de acuerdo con las condiciones de trabajo descritas en el ejemplo 12. La ordenada indica el flujo de calor en mW y la abscisa indica la temperatura en °C.

La figura 2 es una gráfica del análisis DSC llevado a cabo sobre la forma cristalina (II), de acuerdo con las condiciones de trabajo descritas en el ejemplo 12. La ordenada indica el flujo de calor en mW y la abscisa indica la temperatura en °C.

La figura 3 es una gráfica de los resultados de las pruebas termogravimétricas llevadas a cabo sobre la forma (I) y la forma (II), respectivamente, como se describe en el

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ejemplo 13. La abscisa indica la temperatura en °C y la ordenada indica el porcentaje de variación de masas.

La figura 4 es una gráfica de solubilidad a 25°C de las formas (I) y (II) en etanol en concentraciones de agua crecientes. Los experimentos se describen en el ejemplo 15. La ordenada indica el % de solubilidad expresado como p/p y la abscisa indica el % en peso de agua en etanol (cuyo título es "solubilidad en agua-etanol").

La figura 5 es una gráfica de solubilidad a 40°C de las formas (I) y (II) en etanol en concentraciones de agua crecientes. Las pruebas se describen en el ejemplo 15. La ordenada indica el % de solubilidad expresado como p/p y la abscisa indica el % en peso de agua en etanol.

La figura 6 muestra los espectros de RMN ^{13}C en la fase sólida de la forma cristalina (I). Las señales y atributos de los átomos de carbono correspondientes se pueden encontrar en la tabla 4.

La figura 7 muestra los espectros de RMN ^{13}C en la fase sólida de la forma cristalina (II). Las señales y atributos de los átomos de carbono correspondientes se pueden encontrar en la tabla 5.

La figura 8 muestra los espectros IR de la forma (I). Las señales y atributos correspondientes se pueden encontrar en la tabla 6 (cuyo título es "Espectro IR de la sustancia del fármaco en la forma I").

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La figura 9 muestra los espectros IR de la forma (II). Las señales y atributos correspondientes se pueden encontrar en la tabla 7 (cuyo titulo es "Espectro IR de la sustancia del fármaco en la forma II").

La figura 10 representa el porcentaje de concentración promedio del clorhidrato de lercanidipina en el plasma de perros después de la administración de la forma cristalina (I) y la forma cristalina (II) en una cantidad de 3 mg/kg, en la forma de una cápsula de gelatina dura. La ordenada indica el valor promedio de la concentración en el plasma y la abscisa incida el tiempo (en minutos).

Las figuras 11 y 12 muestran los espectros de difracción de rayos X, en la longitud de onda Kα, de las formas cristalinas (I) y (II), respectivamente. Las distancias (d) en X, las relaciones (I/Io) y los valores de los ángulos 2θ de la mayoria de picos significantes se pueden encontrar en las tablas 1 y 2 posteriormente. La ordenada indica el número de conteos/segundo y la abscisa muestra los valores de los ángulos 2θ.

Las figuras 13 y 14 son diagramas del porcentaje de cambio de masas como una función de tiempo en las pruebas de higroscopicidad llevadas a cabo sobre las formas (I) y (II) de clorhidrato de lercanidipina, respectivamente. La ordenada a la izquierda indica el porcentaje de cambio de masas y la ordenada a la derecha representa el porcentaje de humedad

relativa; la abscisa indica el tiempo en minutos. El protocolo para las pruebas de higroscopicidad se describe en el ejemplo 14.

Las figuras 15 y 16 muestran los espectros de difracción de rayos X, a la longitud de onda Kα, de la forma cruda (A) de clorhidrato de lercanidipina y de la forma cruda (B) de clorhidrato de lercanidipina, respectivamente.

Las figuras 17 y 18 muestran los espectros Raman de la forma cruda (A) de clorhidrato de lercanidipina y de la forma cruda (B) de clorhidrato de lercanidipina, respectivamente, donde la ordena representa las unidades Raman y la abscisa representa el número de onda (cm^{-1}).

Las figuras 19 y 20 muestran los resultados del análisis termogravimétrico llevado a cabo sobre la forma cruda (A) de clorhidrato de lercanidipina y sobre la forma cruda (B) de clorhidrato de lercanidipina, respectivamente. En estas figuras, la abscisa indica la temperatura (en °C) y la ordenada indica el porcentaje de variación de masas.

DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

La presente invención describe las nuevas formas crudas y formas cristalinas del clorhidrato de lercanidipina y procedimientos para prepararlas. Los solicitantes han determinado que el clorhidrato de lercanidipina exhibe polimorfismo y se cristaliza en diferentes formas dependiendo

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del procedimiento seguido y de los solventes utilizados, especialmente para la cristalización. Adicionalmente, las diversas formas novedosas tienen distintas propiedades químicas y físicas y perfiles de biodisponibilidad en animales, inclusive el hombre, como se describe en este texto.

Los métodos novedosos de preparación de la forma cruda de clorhidrato de lercanidipina son adecuados para la producción a escala comercial altamente reproducible de las composiciones sólidas, reproducibles de clorhidrato de lercanidipina. Los métodos producen de manera ventajosa las nuevas formas crudas (A) y (B) del clorhidrato de lercanidipina que también exhiben características deseables para aplicaciones industriales. Las formas crudas (A) y (B), por ejemplo, exhiben solubilidad superior y velocidades de secado más rápidas en comparación con otras formas crudas de clorhidrato de lercanidipina que han sido reportadas previamente. Las forma crudas (A) y (B) permiten además que se utilicen procedimientos de cristalización simplificados para la producción de las nuevas formas cristalinas, aisladas de clorhidrato de lercanidipina.

Las nuevas formas cristalinas, aisladas de clorhidrato de lercanidipina de la presente invención se pueden obtener a partir de las formas crudas (A) y (B) de clorhidrato de lercanidipina y se denominan la forma

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cristalina (I) y forma cristalina (II) de clorhidrato de lercanidipina. Cualquiera de la forma aislada (I) o la forma aislada (II) se puede obtener de manera reproducible a partir de los productos intermediarios (A) y (B) al variar las condiciones de cristalización descritas posteriormente. Las formas (I) y (II) también se pueden obtener utilizando otros materiales de inicio. Ambas formas (I) y (II) se pueden obtener utilizando, por ejemplo, la forma cruda (C) de lercanidipina como material de inicio, como se describe en este texto. La forma (II) también se puede obtener utilizando la forma (I) como material de inicio, como se describe en este texto.

Ambas formas cristalinas (I) y (II) de clorhidrato de lercanidipina exhiben buena estabilidad. La forma (I) está caracterizada por un color amarillo más pálido, tamaño de cristal más pequeño, solubilidad superior en medios acuosos (todo comparado con la forma (II)), y un punto de fusión (pico de DSC) dentro del rango de aproximadamente 197°C a aproximadamente 201°C, más específicamente, alrededor de 198.7°C y el patrón de difracción de rayos X expuesto, supra.

La forma (II) está caracterizada por un color amarillo más pronunciado, tamaño de cristal más grande, solubilidad ligeramente inferior en medios acuosos (todo comparado con la forma (I)), y un punto de fusión (pico de DSC) dentro del rango de aproximadamente 207-211°C, más

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especificamente alrededor de 209.3°C.

Tanto la forma (I) como la forma (II) son estables. La forma (II) exhibió biodisponibilidad superior en el perro y tampoco fue un equivalente para la forma I en el hombre, mostrando una concentración más alta en el plasma (AUCo-t) y un tiempo retardado de concentración máxima (tmax), en comparación con la forma (I).

Los métodos previamente conocidos para producir el clorhidrato de lercanidipina cristalino fueron inconsistentes en la producción del clorhidrato de lercanidipina con características físicas y químicas predecibles. Por lo tanto, los métodos previamente conocidos tuvieron la propiedad indeseable de producir clorhidrato de lercanidipina que varió, por ejemplo, en las propiedades fisicoquímicas, de lote a lote, aún entre los lotes producidos por medio del mismo procedimiento y bajo las mismas condiciones. Los presentes inventores han descubierto que la fuente de la inconsistencia exhibida por los métodos previamente conocidos para producir clorhidrato de lercanidipina es la presencia de cantidades variantes e impredecibles de la forma cristalina (II) de clorhidrato de lercanidipina. En contraste a los métodos previamente conocidos para la producción de clorhidrato de lercanidipina, la invención proporciona las nuevas formas cristalinas (I) y (II) que representan las formas cristalinas de clorhidrato de lercanidipina de una

pureza y uniformidad que no se ha obtenido con las formas sólidas previamente logradas de clorhidrato de lercanidipina.

La pureza y uniformidad de las formas (I) y (II) permite una facilidad incrementada en la producción de formas de dosificación de lercanidipina, debido a, por ejemplo, características fisicoquímicas definidas más precisamente, tales como por ejemplo uniformidad incrementada del tamaño de partícula después de la micronización y solubilidad más reproducible. Las formas (I) y (II) también proporcionan formas de dosificación con características farmacológicas definidas más precisamente, por ejemplo biodisponibilidad, en comparación con las formas de dosificación logradas previamente que variaron de lote a lote en sus características fisicoquímicas.

En un estudio humano en el hombre, donde los niveles en el plasma de lercanidipina fueron valorados después de la administración de una dosis individual de ya sea la forma (I) o (II) de clorhidrato de lercanidipina, la forma (I) tuvo un tiempo más corto en la obtención de la concentración máxima en el plasma, con relación a la forma (II). Por lo tanto, la forma (I) es más adecuada para las formulaciones y formas de dosificación de liberación inmediata. A partir del mismo estudio, la forma (II) mostró una biodisponibilidad superior, con relación a la forma (I), y de esta manera es adecuada para el uso en las formulaciones

y formas de dosificación de liberación controlada. Por consiguiente, la disponibilidad de las formas puras (I) y (II) proporciona la capacidad de mezclar los dos polimorfos en formas de dosificación con nuevas características controladas, por ejemplo una forma de dosificación con tanto un comienzo rápido como una acción biológica sostenida.

Como se utiliza en este texto, el término "forma cruda" se refiere a formas sólidas, precipitadas que comprenden cristales de un compuesto que no han sido lavadas y/o recristalizadas para eliminar las impurezas (que incluye, pero no está limitado a, un solvente) que pudieran estar presentes. En la presente especificación, las formas crudas son referidas como las formas (A) y (B) del clorhidrato de lercanidipina.

Como se utiliza en este texto, el término "forma cristalina" se refiere a cristales de un compuesto que han sido lavados y recristalizados para remover las impurezas. En la presente invención, el término formas cristalinas se refiere a las formas (I) y (II) del clorhidrato de lercanidipina. Estas formas cristalinas tienen una pureza proporcionada por la CLAR $\geq$ 99.5% y un contenido de solventes residuales de <3000 ppm.

Como se utiliza en este texto, el término "polimorfismo" se refiere a una propiedad de un compuesto para cristalizarse en dos o más formas con estructuras

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distintas. Las diferentes formas cristalinas se pueden detectar directamente por medio de técnicas cristalográficas o indirectamente por medio de la valoración de las diferencias en las propiedades físicas y/o químicas asociadas con cada polimorfo particular.

Como se utiliza en este texto, un "sujeto en necesidad del tratamiento" es un sujeto mamífero (por ejemplo un humano) que sufre o está en riesgo de desarrollar la condición particular que es tratada, por ejemplo, hipertensión esencial, hipertensión secundaria, hipertensión sistólica aislada, enfermedad coronaria del corazón (por ejemplo, angina estable crónica, infarto al miocardio), insuficiencia cardiaca congestiva. Un sujeto en necesidad del tratamiento para la hipertensión arterial puede ser identificado utilizando métodos bien conocidos en el campo tales como, por ejemplo, por medio de la medición directa de la presión sanguínea utilizando, por ejemplo, un esfigmomanómetro manual, dispositivos automáticos/electrónicos o el monitoreo ambulante de la presión sanguínea.

La presente invención contempla cualquier método que se pueda utilizar para producir las nuevas formas crudas de clorhidrato de lercanidipina descritas en este texto. Estas formas tienen diferentes propiedades fisicoquímicas, por ejemplo, puntos de fusión (los cuales se pueden

determinar por medio del análisis DSC), que la forma cruda del clorhidrato de lercanidipina producida por otros métodos conocidos, por ejemplo, por el método descrito en la patente norteamericana No. 5,912,351; denominada forma (C). La forma (A) tiene un punto de fusión de aproximadamente 150°C a aproximadamente 152°C (pico de DSC), la forma (B) tiene un punto de fusión de aproximadamente 131°C a aproximadamente 135°C (pico de DSC) y la forma (C) tiene un punto de fusión de aproximadamente 186°C a aproximadamente 192°C (pico de DSC). Adicionalmente, los estudios termogravimétricos muestran que la forma (A) comprende 3 - 4% de acetato de etilo residual y la forma (B) comprende 0.3-0.7% de acetato de etilo residual, en peso. Comparativamente, se ha determinado que el solvente residual presente en la forma (C) es 0-0.1%.

Los aspectos de la invención se dirigen a procedimientos para la preparación de clorhidrato de lercanidipina, cada uno da por resultado una forma cruda diferente del producto. Los primeros dos pasos en la producción de cualquier forma cruda son idénticos y son:

a) hacer reaccionar el ácido 2,6-dimetil-5-metoxicarbonil-4-(3-nitrofenil)-1,4-dihidropiridin-3-carboxílico (preparado como se describe en la patente alemana DE 2847 237) con cloruro de tionilo o cloruro de oxalilo en una mezcla de un solvente dipolar, aprótico y de un solvente

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polar, aprótico para producir un compuesto de cloruro, y

(b) la reacción in situ del cloruro obtenido del paso anterior con el alcohol de 2,N-dimetil-N-(3,3-difenilpropil)-1-amino-2-propilo, a una temperatura preferiblemente entre -5 y +5°C, en una mezcla de un solvente dipolar, aprótico y de un solvente polar, aprótico.

En una modalidad preferida, la mezcla de un solvente dipolar, aprótico y de un solvente polar, aprótico es acetato de etilo y dimetilformamida utilizados en una relación de 4:1.

Después de la reacción in situ, el clorhidrato de lercanidipina es aislado y recuperado de la mezcla. El método de aislamiento utilizado determina la forma cruda del clorhidrato de lercanidipina que se obtiene. Siguiendo el protocolo posterior (protocolo α) se produce la forma (A):

i) lavar la mezcla del paso (b), preferiblemente con agua,

ii) remover el agua de la mezcla de reacción del paso i), preferiblemente por medio de la destilación azeotrópica bajo vacio a 200-300 mmHg a una temperatura inferior a aproximadamente 60°C (preferiblemente a 40-50°C).

iii) concentrar la mezcla del paso ii) preferiblemente a aproximadamente 1/3 del volumen inicial a la misma temperatura y presión como en el paso (ii), adicionar solvente nuevo (por ejemplo, acetato de etilo) para

obtener preferiblemente el volumen inicial, obteniendo de esta manera una suspensión con un contenido de agua, determinado de acuerdo con Karl Fischer (Farmacopea norteamericana 25, Método 921), preferiblemente entre 0.10 y 0.15%;

iv) enfriar la suspensión del paso iii) preferiblemente a 0-5°C;

v) filtrar el sólido del paso iv);

vi) resuspender el sólido del paso v) preferiblemente en acetato de etilo y agitar preferiblemente a 60-65°C durante aproximadamente 1 hora; y

vii) enfriar a 5-10°C, filtrar y secar el sólido obtenido (por ejemplo, en un horno a aproximadamente 70°C).

El segundo proceso (protocolo β; utilizado para preparar la forma (B)) se realiza utilizando los siguientes pasos:

i') lavar la mezcla del paso (b) preferiblemente con agua,

ii') remover el agua del paso i') preferiblemente mediante el calentamiento a reflujo de manera azeotrópica del producto del paso i') con un aparato Dean Stark hasta que se obtiene un contenido de agua de aproximadamente 2%, medido de acuerdo con Karl Fischer;

iii') concentrar la mezcla del paso ii') preferiblemente a $^{3}/_{4}$ del volumen inicial y adicionar

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solvente nuevo (acetato de etilo) a la mezcla preferiblemente hasta que (1) se obtenga el volumen inicial y (2) se obtenga un contenido de agua, medido de acuerdo con Karl Fischer, entre 0.9 y 1.1%;

iv') enfriar la solución del paso iii') preferiblemente a 0-5°C para obtener un sólido;

v') filtrar el sólido del paso iv');

vi') resuspender el sólido del paso v') preferiblemente en acetato de etilo y agitar preferiblemente a 60-65°C durante aproximadamente 1 hora; y

vii') enfriar la suspensión del paso vi') preferiblemente a 5-10°C, filtrar y secar el sólido obtenido, preferiblemente en un horno a aproximadamente 70°C.

La temperatura del paso vii') debe ser controlada cuidadosamente a 5-10°C para maximizar el rendimiento.

Estas nuevas formas crudas de clorhidrato de lercanidipina presentan la ventaja de solubilidad superior y velocidad de secado más rápida en comparación con la forma (C) y hacen posible un procedimiento de cristalización adicional, simplificado (el cual se puede utilizar de manera ventajosa para preparar la forma (I) o forma (II)). Comparado con la forma cruda producida por el método de la patente norteamericana No. 5,912,351, estas formas permiten el uso de menos solvente para recristalizar el compuesto. Esto también incrementa el rendimiento al reducir la pérdida de compuesto.

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Adicionalmente, los métodos utilizados para producir estas formas crudas son más adaptables para el uso en un entorno a gran escala y un entorno comercial.

Se ha descubierto sorprendentemente que cada una de la forma cruda (A) y la forma cruda (B) del clorhidrato de lercanidipina, cuando se someten a diferentes tratamientos de purificación, dan por resultado dos formas cristalinas nuevas y diferentes del clorhidrato de lercanidipina. Los estudios indican que estas nuevas formas cristalinas tienen diferentes propiedades físicas y químicas. El análisis DSC de la forma cristalina (I) indica que tiene un pico de fusión de aproximadamente 197°C a aproximadamente 201°C, específicamente alrededor de 198.7°C. El análisis DSC de la forma cristalina (II) indica que tiene un pico de fusión de aproximadamente 207°C a aproximadamente 211°C, específicamente alrededor de 209.3°C.

Un procedimiento de purificación (procedimiento γ), que conduce a la formación de una de las nuevas formas cristalinas (forma I), comprende los siguientes pasos:

Procedimiento para Elaborar la Forma (I)

d) adicionar isopropanol al clorhidrato de lercanidipina crudo (forma (A) o forma (B)) y calentar bajo reflujo con agitación para producir una solución (si la solución no es clara, se debe filtrar en caliente);

e) enfriar la solución del paso d) preferiblemente a una temperatura entre 30 y 40°C, y agitar durante un periodo de tiempo preferiblemente entre 12 y 48 horas para producir un sólido; y

f) filtrar el sólido obtenido del paso e), lavar el sólido con isopropanol, filtrar nuevamente el sólido y secar el sólido (por ejemplo, en un horno) preferiblemente a 70°C durante un periodo de tiempo preferiblemente entre 12-48 horas.

La forma cruda (C) también puede utilizarse como material de inicio en el paso d). En este caso, sin embargo, existe el riesgo de un rendimiento disminuido del producto debido a que la solución debe ser filtrada en caliente, dando por resultado una pérdida incrementada del clorhidrato de lercanidipina en el paso d). En el paso e), la cristalización se considera completa cuando el contenido de la solución es $\leq$2% de HCl de lercanidipina. También se pueden utilizar otros alcoholes como el solvente en el paso d). Un solvente alternativamente preferido es un alcohol de 1 a 5 átomos de carbono que contiene un máximo de 5% de agua, por ejemplo etanol anhidro. La forma cristalina (I) puede adicionarse en el paso (e) como semillas para promover adicionalmente la formación de cristales.

Procedimiento Alternativo para Elaborar la Forma (I)

La presente solicitud también contempla un método

alternativo para producir la forma cristalina (I) que tiene clorhidrato de lercanidipina que comprende los pasos de:

d') adicionar etanol al clorhidrato de lercanidipina crudo, preferiblemente a una relación de peso/volumen de clorhidrato de lercanidipina solvente de 1:4 a 1:6, más preferiblemente 1:4, calentar a reflujo bajo agitación a fin de obtener una solución (si la solución no es clara, se debe filtrar en caliente de preferencia), enfriar bajo agitación, preferiblemente a 22°C, y adicionar semillas cristalinas de la forma (I);

e') enfriar la mezcla sembrada del paso d'), preferiblemente a una temperatura entre 10 y 15°C, y agitar a esta temperatura durante un periodo de tiempo preferiblemente entre 24 y 96 horas para formar un sólido; y

f') filtrar y secar el sólido del paso e'), preferiblemente en un horno a preferiblemente 70°C para obtener la forma (I) de clorhidrato de lercanidipina.

En el paso e'), la cristalización se considera completa cuando el contenido de la solución es $\leq$2% de clorhidrato de lercanidipina. Las semillas cristalinas de la forma (I) también se pueden adicionar a los pasos e') para promover adicionalmente la formación de cristales.

Procedimiento para Elaborar la Forma (II)

El segundo procedimiento de purificación

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(procedimiento δ), el cual produce la forma cristalina (II), comprende los pasos de:

d") adicionar acetonitrilo al clorhidrato de lercanidipina crudo (forma (A) o forma (B)) y calentar la mezcla bajo reflujo y agitación,

e") enfriar la mezcla del paso d") a temperatura ambiente y agitar preferiblemente durante 24 horas para formar un sólido,

f") filtrar el sólido obtenido del paso e") y secarlo preferiblemente en un horno.

En el paso e"), la cristalización se considera completa cuando el contenido de la solución es $\leq$2% de HCl de lercanidipina.

La presente solicitud también contempla dos métodos adicionales para producir la forma (II).

Primer Procedimiento Alternativo para Elaborar la Forma (II)

El primer método alternativo comprende los pasos de:

d"') adicionar isopropanol o etanol, preferiblemente etanol con un contenido de agua preferiblemente entre 5 a 10% en peso a clorhidrato de lercanidipina, calentar a reflujo con agitación para producir una solución;

e"') enfriar la mezcla a una temperatura

preferiblemente entre 20 y 40°C y agitar durante un periodo preferiblemente entre 24 y 96 horas para formar un sólido;

f"') filtrar el sólido y secar (por ejemplo en un horno) a preferiblemente 70°C durante 12-18 horas para producir la forma (II) de clorhidrato de lercanidipina.

En el paso e"'), la cristalización se considera completa cuando el contenido de la solución es $\leq$2% de HCl de lercanidipina.

Segundo Método Alternativo para la Elaboración de la Forma (II)

El segundo método alternativo para obtener el polimorfo de la forma (II) comprende los pasos de:

d"") disolver el clorhidrato de lercanidipina crudo o su forma cristalina (I) en solventes polares, próticos o dipolares, apróticos preferiblemente que contienen hasta 50% en peso de agua a una temperatura preferiblemente entre 20 y 70°C para producir una solución;

e"") agitar la solución del paso d"") a una temperatura preferiblemente entre 20 y 25°C para producir una solución;

f"") filtrar el sólido del paso e"") y secar (por ejemplo, en un horno) a preferiblemente 70°C durante preferiblemente 12-18 horas.

El segundo método alternativo puede comprender

opcionalmente el paso de adicionar hasta 60% de agua a la solución del paso d"") antes del paso e""). El segundo método alternativo puede comprender además irradiar con ultrasonido y/o adicionar preferiblemente semillas cristalinas, autenticas de la forma (II) al paso e""). En el paso e""), la cristalización se considera completa cuando el contenido de la solución es ≤2% de HCl de lercanidipina. En una modalidad preferida, el solvente polar, prótico es un solvente alcohólico tal como, pero no limitado a, metanol, etanol, n-propanol e isopropanol. En otra modalidad preferida, el solvente dipolar, aprótico es N-metilpirrolidona.

El procedimiento preferido para preparar la forma (I) es el procedimiento γ y el procedimiento preferido para preparar la forma (II) es el procedimiento δ. Los solicitantes han determinado que la forma (I) puede obtenerse cuantitativamente por medio del uso de un alcohol anhidro de 1 a 5 átomos de carbono (preferiblemente etanol o isopropanol anhidro) o un alcohol de 1 a 5 átomos de carbono que contiene hasta 5% de agua bajo condiciones controladas d'-f'). De hecho, los procedimientos anteriores, especialmente los procedimientos γ y δ, se pueden utilizar para producir el polimorfo deseado de manera reproducible y consistente.

Además de las diferencias en el punto de fusión, las dos formas cristalinas exhiben diferencias en la estructura de rayos X, solubilidad y biodisponibilidad. Los

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estudios de solubilidad muestran que la forma (I) es más soluble que la forma (II) en agua, etanol y mezclas de los mismos (véase las tablas 2 y 3). Los estudio de biodisponibilidad en perros y humanos indican que la forma (II) es más biodisponible que la forma (I). Sin embargo, el estudio en humanos también indica que la forma (I) tiene un tiempo más corto para la concentración máxima obtenible y de esta manera es adecuado para el uso en las formulaciones y formas de dosificación de liberación inmediata. Finalmente, los estudios de difracción de rayos X muestran que estas dos formas tienen diferentes patrones de difracción (véase las figuras 11 y 12 y el ejemplo 20). La forma I tiene un tamaño de cristal, y por lo tanto un tamaño de particula, más pequeño antes de la micronización y de esta manera es más fácil y más rápido para procesar que la forma II, lo cual presenta cristales más grandes.

La presente solicitud además describe formulaciones farmacéuticas y formas de dosificación unitaria que comprenden uno de los polimorfos aislados de la presente invención o una mezcla de los mismos del contenido de polimorfos predeterminado.

La presente invención también se dirige a un método para tratar a un sujeto con hipertensión (por ejemplo, hipertensión esencial, hipertensión secundaria o hipertensión sistólica aislada), enfermedad coronaria del corazón (por

ejemplo, angina estable crónica, infarto al miocardio) o insuficiencia cardiaca congestiva, el método comprende administrar una cantidad terapéuticamente efectiva de la forma cristalina, aislada (I) del clorhidrato de lercanidipina, la forma cristalina (II) de clorhidrato de lercanidipina o combinaciones de las mismas con un contenido de polimorfos predeterminado (opcionalmente con otra forma de lercanidipina, tal como la forma amorfa) a un sujeto en necesidad de tal tratamiento.

La invención también contempla un método para tratar y prevenir lesiones ateroscleróticas en arterias de un sujeto, el método comprende administrar una cantidad terapéuticamente efectiva de la forma cristalina, aislada (I) de clorhidrato de lercanidipina, la forma cristalina, aislada (II) de clorhidrato de lercanidipina o combinaciones de las mismas a un sujeto en necesidad de tal tratamiento.

Composiciones farmacéuticas

Los compuestos y polimorfos de la presente invención se pueden formular en una composición farmacéutica. Las composiciones farmacéuticas de acuerdo con la presente invención pueden comprender clorhidrato de lercanidipina (I), (II) o una mezcla de los mismos. Cuando los compuestos contienen una mezcla de las formas cristalinas, la relación del peso de la forma (I):(II) está comprendida

preferiblemente entre 1:9 y 9:1, las modalidades más preferidas de las composiciones farmacéuticas son aquellas en donde la relación del peso de (I):(II) se selecciona de: 9:1, 7:3, 1:1, 3:7 y 1:9. La composición farmacéutica también puede incluir aditivos opcionales, tales como un portador o diluyente farmacéuticamente aceptable, un saborizante, edulcorante, conservador, tinte, sustancia aglutinante, agente de suspensión, agente de dispersión, colorante, desintegrante, excipiente, agente formador de película, lubricante, plastificante, aceite comestible o cualquier combinación de dos o más de los anteriores.

Ambas formas cristalinas pueden someterse a la micronización, utilizando cualquier método conocido en el campo. El tamaño promedio de la partícula producida por este método es preferiblemente D(50%)2-8 µm, D(90%)<15 µm.

Los portadores o diluyentes farmacéuticamente aceptables, adecuados incluyen, pero no están limitados a, etanol; agua; glicerol; propilenglicol; gel de aloe vera; alantoina; glicerina; aceites de vitamina A y E; aceite mineral; propionato de miristilo PPG2; carbonato de magnesio; fosfato de potasio; aceite vegetal; aceite animal; y solketal™.

Las sustancias aglutinantes adecuadas incluyen, pero no están limitadas a, almidón; gelatina; azucares naturales, tales como glucosa, sacarosa y lactosa;

edulcorantes de maíz; gomas naturales y sintéticas, tales como acacia, tragacanto, goma vegetal y arginato de sodio; carboximetilcelulosa; hidroxipropilmetilcelulosa; polietilenglicol; povidona; ceras; y similares.

Los desintegrantes adecuados incluyen, pero no están limitados a, almidón, por ejemplo almidón de maíz, metilcelulosa, agar, bentonita, goma de xantano, glicolato de almidón sódico, crospovidona y similares.

Los lubricantes adecuados incluyen, pero no están limitados a, oleato de sodio, estereato de sodio, estearil-fumarato de sodio, estearato de magnesio, benzoato de sodio, acetato de sodio, cloruro de sodio y similares.

Un agente de suspensión adecuado es, pero no está limitado a, bentonita, alcoholes isoestearílicos etoxilados, polioxietilen-sorbitol y ésteres de sorbitan, celulosa microcristalina, metahidróxido de aluminio, agar-agar y tragacanto, o mezclas de dos o más de estas sustancias y similares.

Los agentes de dispersión y suspensión adecuados incluyen, pero no están limitados a, gomas sintéticas y naturales, tales como goma vegetal, tragacanto, acacia, alginato, dextrano, carboximetilcelulosa de sodio, metilcelulosa, polivinilpirrolidona y gelatina.

Los agentes formadores de película adecuados incluyen, pero no están limitadas a,

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hidroxipropilmetilcelulosa, etilcelulosa y polimetacrilatos.

Los plastificantes adecuados incluyen, pero no están limitados a, polietilenglicoles de diferentes pesos moleculares (por ejemplo 200-8000 Da) y propilenglicol.

Los colorantes adecuados incluyen, pero no están limitados a, óxido(s) férrico(s), dióxido de titanio y lacas naturales y sintéticas.

Los aceites comestibles adecuados incluyen, pero no están limitados a, aceite de semilla de algodón, aceite de ajonjolí, aceite de coco y aceite de cacahuate.

Los ejemplos de aditivos adicionales incluyen, pero no están limitados a, sorbitol, talco, ácido esteárico, fosfato dicálcico y polidextrosa.

Formas de Dosificación Unitaria

Las composiciones farmacéuticas se pueden formular como formas de dosificación unitaria, tales como tabletas, píldoras, cápsulas, comprimidos oblongos, bolos, polvos, gránulos, soluciones parenterales estériles, suspensiones parenterales estériles, emulsiones parenterales estériles, elixires, tinturas, aerosoles medidos o pulverizaciones líquidas, gotas, ampolletas, dispositivos autoinyectores o supositorios. Las formas de dosificación unitaria se pueden utilizar para la administración oral, parenteral, intranasal, sublingual o rectal o para la administración por medio de la

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inhalación o insuflación, parches transdérmicos y una composición liofilizada. En general, se puede utilizar cualquier suministro de ingredientes activos que de por resultado la disponibilidad sistémica de los mismos. Preferiblemente, la forma de dosificación unitaria es una forma de dosificación oral, más preferiblemente una forma de dosificación oral, sólida, por lo tanto, las formas de dosificación preferidas son las tabletas, píldoras, comprimidos oblongos y cápsulas. También se prefieren las preparaciones parenterales (por ejemplo, preparaciones inyectables y preparaciones para sistemas de administración de polvo por chorro).

Las formas de dosificación unitaria, sólidas se pueden preparar al mezclar un agente activo de la presente invención con un portador farmacéuticamente aceptable y cualquier otro aditivo deseado como se describiera anteriormente. La mezcla es combinada típicamente hasta que se forma una mezcla homogénea de los agentes activos de la presente invención y el portador y cualquier otro aditivo deseado, es decir, hasta que el agente activo es dispersado uniformemente por toda la composición. En este caso, las composiciones pueden formarse como gránulos secos o húmedos.

Las formas de dosificación con cantidades predeterminadas de clorhidrato de lercanidipina se pueden formular iniciando con composiciones con cantidades conocidas

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de clorhidrato de lercanidipina utilizando métodos bien conocidos en el campo. En una modalidad preferida, una forma de dosificación se obtiene al mezclar composiciones que comprenden cantidades conocidas de clorhidrato de lercanidipina cristalino, por ejemplo la forma (I) o (II), que incluye opcionalmente clorhidrato de lercanidipina no cristalino. Se prefiere adicionalmente donde una forma de dosificación con cantidades predeterminadas de clorhidrato de lercanidipina cristalino es formulada al mezclar composiciones que comprenden clorhidrato de lercanidipina cristalino, esencialmente puro, se mezclan para dar formas de dosificación que comprenden una relación predeterminada de las forma cristalinas (I) y (II).

Las tabletas o píldoras pueden ser revestidas o de otra manera combinadas para dar una forma de dosificación unitaria, la cual tiene acción retardada y/o prolongada, tal como formas de dosificación unitaria de liberación cronometrada y de liberación sostenida. Por ejemplo, la tableta o píldora puede comprender una dosificación interior y un componente de dosificación exterior, el último está en la forma de una capa o envoltura sobre la primera. Los dos componentes se pueden separar por medio de una capa entérica, la cual sirve para resistir la desintegración en el estómago y permite que el componente interior pase intacto dentro del duodeno o que sea retardado en su liberación.

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Los polímeros biodegradables para controlar la liberación de los agentes activos incluyen, pero no están limitados a, ácido poliláctico, poliepsilon-caprolactona, ácido polihidroxi-butírico, poliortoésteres, poliacetales, polihidro-piranos, policianoacrilatos y copolímeros de bloque reticulados o anfipáticos de hidrogeles.

Para las formas de dosificación líquidas, las sustancias activas o sus sales fisiológicamente aceptables se ponen en solución, suspensión o emulsión, opcionalmente con las sustancias usualmente empleadas tales como solubilizadores, emulsionantes u otros auxiliares. Los solventes para las combinaciones activas y las sales fisiológicamente aceptables, correspondientes pueden incluir agua, soluciones de sales fisiológicas o alcoholes, por ejemplo etanol, propanodiol o glicerol. Adicionalmente, se pueden utilizar soluciones de azúcar tales como soluciones de glucosa o manitol. Además, una mezcla de los diversos solventes mencionados se puede utilizar en la presente invención.

Una forma de dosificación transdérmica también está contemplada por la presente invención. Las formas transdérmicas pueden ser un sistema transdérmico impulsado por la difusión (parche transdérmico) utilizando ya sea un recipiente de fluido o un sistema de matriz de fármaco en adhesivo. Otras formas de dosificación transdérmicas

incluyen, pero no están limitadas a, geles tópicos, lociones, ungüentos, sistemas y dispositivos transmucosa y sistemas de suministro iontoforéticos (difusión eléctrica). Las formas de dosificación transdérmica se pueden utilizar para la liberación cronometrada y la liberación sostenida de los agentes activos de la presente invención.

Las composiciones farmacéuticas y las formas de dosificación unitaria de la presente invención para la administración de manera parenteral, y en particular por medio de inyección, incluyen típicamente un portador farmacéuticamente aceptable, como se describiera anteriormente. Un portador liquido preferido es un aceite vegetal. La inyección puede ser, por ejemplo, intravenosa, intratecal, intramuscular, intrarruminal, intratraqueal o subcutánea.

El agente activo también puede administrarse en la forma de sistemas de suministro de liposomas, tales como vesiculas unilaminares pequeñas, vesiculas unilaminares grandes y vesiculas multilaminares. Los liposomas se pueden formar a partir de una variedad de fosfolipidos, tales como colesterol, estearilamina o fosfatidilcolinas.

Los polimorfos de la presente invención también se pueden acoplar con polímeros solubles como portadores de fármacos que se pueden fijar como objetivo. Estos poliméros incluyen, pero no están limitados a, polivinil-pirrolidona,

copolímero de pirano, polihidroxipropilmetacril-amidofenol, polihidroxi-etilaspartamidofenol y polietil-enoxidopolilisina sustituida con residuos de palmitoilo.

Administración

La composición farmacéutica o las formas de dosificación unitaria de la presente invención se pueden administrar por medio de una variedad de rutas tales como intravenosa, intratraqueal, subcutánea, oral, mucosa, parenteral, bucal, sublingual, oftálmica, pulmonar, transmucosa, transdérmica e intramuscular. Las formas de dosificación unitaria también se pueden administrar en forma intranasal por medio del uso tópico de vehículos intranasales adecuados o por medio de rutas transdérmicas, utilizando aquellas formas de parches transdérmicos para la piel conocidos para aquellas personas expertas ordinarias en el campo. Se prefiere la administración oral.

La composición farmacéutica o las formas de dosificación unitaria de la presente invención pueden administrase a un animal, preferiblemente un ser humano, en necesidad del tratamiento antihipertensivo. La composición farmacéutica o la forma de dosificación unitaria de la presente invención se pueden administrar de acuerdo con un régimen de dosificación y administración definido por medio de una prueba de rutina en vista de las guias dadas

anteriormente a fin de obtener la actividad antihipertensiva óptima y una disminución en la presión sanguínea mientras que se minimiza la toxicidad o efectos secundarios para un paciente particular. Sin embargo, este giro cuidadoso del régimen terapéutico es una rutina en vista de las guías dadas en este texto.

La dosificación de los polimorfos que contienen la composición o mezclas de la presente invención pueden variar de acuerdo con una variedad de factores tales como el estado de enfermedad subyacente, la condición del individuo, peso, sexo y edad y el modo de administración. Para la administración oral, las composiciones farmacéuticas se pueden proporcionar en las formas de dosificación unitaria sólidas, marcadas o no marcadas.

Una composición farmacéutica que comprende (1) clorhidrato de lercanidipina, donde el clorhidrato de lercanidipina se selecciona del grupo que consiste de la forma cristalina, aislada (I) de clorhidrato de lercanidipina, la forma cristalina, aislada (II) de clorhidrato de lercanidipina o combinaciones de las mismas de la composición de polimorfos predeterminada; y (2) al menos un componente seleccionado del grupo que consiste de un portador o diluyente farmacéuticamente aceptable, un saborizante, edulcorante, conservador, tinte, sustancia aglutinante, agente de suspensión, agente de dispersión,

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colorante, desintegrante, excipiente, diluyente, lubricante, plastificante y aceite comestible. En una modalidad preferida, la composición farmacéutica o forma de dosificación comprende 0.1 a 400 mg de clorhidrato de lercanidipina. Preferiblemente, la composición o forma de dosificación comprende 1 a 200 mg de clorhidrato de lercanidipina. Más preferiblemente, la composición o forma de dosificación comprende 5 a 40 mg de clorhidrato de lercanidipina.

La composición farmacéutica o forma de dosificación unitaria se puede administrar en una dosis individual diaria o la dosis total diaria puede ser administrada en dosis divididas. Además, la coadministración o administración secuencial de otros agentes activos puede ser deseable. Los polimorfos y mezclas de los mismos de la invención se pueden combinar con cualquier terapia de fármacos conocida, preferiblemente para el tratamiento de la hipertensión. Por ejemplo, la terapia bimodal que involucra además un diurético, bloqueador de receptores β, inhibidor de ACE o antagonista de receptores de angiotensina II está contemplada por la presente invención (véase, por ejemplo, la solicitud de patente provisional norteamericana No. 60/344,601, presentada el 23 de octubre de 2001 y la solicitud italiana No. MI 2001 A 002136 presentada el 16 de octubre de 2001).

Para la terapia en combinación, los compuestos

pueden proporcionarse inicialmente como formas de dosificación separadas hasta que se logra una combinación de dosificación y un régimen de administración óptimos. Por lo tanto, el paciente puede ser titulado a dosificaciones apropiadas para su condición hipertensa particular. Después de que se determina la dosificación apropiada de cada uno de los compuestos para lograr un descenso de la presión sanguínea sin efectos secundarios indeseados, el paciente entonces puede ser cambiado a una forma de dosificación individual que contiene las dosificaciones apropiadas de cada uno de los agentes activos o puede continuar con una forma de dosificación doble.

La dosificación exacta y el régimen de administración que utilizan la terapia de combinación de la presente invención se seleccionan de acuerdo con una variedad de factores que incluyen el tipo, especie, edad, peso, sexo y condición médica del paciente; la gravedad y etiología de la hipertensión a tratarse; la ruta de administración; la función renal y hepática del paciente; la historia del tratamiento del paciente; y la respuesta del paciente. La precisión óptima en el logro de concentraciones de los compuestos dentro del rango que producen eficacia sin toxicidad requiere un régimen basado en la cinética de la disponibilidad del fármaco para los sitios objetivo. Esto involucra una consideración de la adsorción, distribución,

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metabolismo, excreción de un fármaco y respuesta del paciente al régimen de dosificación. Sin embargo, este giro cuidadoso del régimen terapéutico es una rutina en vista de las guías dadas en este texto.

Una composición farmacéutica para la administración parenteral contiene no menos de 0.1%, preferiblemente de aproximadamente 0.5% a aproximadamente 30%, en peso de un polimorfo o mezcla de la presente invención, en base al peso total de la composición farmacéutica. Los polimorfos aislados, individuales son preferidos para la administración parenteral.

Generalmente, las formas de dosificación transdérmicas contienen de aproximadamente 0.01% a aproximadamente 100% en peso de los agentes activos, en base al 100% del peso total de la dosificación.

En una modalidad preferida de la presente invención, la composición es administrada diariamente al paciente. Preferiblemente en la modalidad, la composición farmacéutica tiene una forma de dosificación que contiene de 0.1 a 400 mg de clorhidrato de lercanidipina. Más preferiblemente, la composición o forma de dosificación comprende 1 a 200 mg de clorhidrato de lercanidipina. Aún más preferiblemente, la composición o forma de dosificación comprende 5 a 40 mg de clorhidrato de lercanidipina.

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EJEMPLOS

Los siguientes ejemplos de preparación de las formas curdas (A) y (B) y las formas cristalinas (I) y (II) de clorhidrato de lercanidipina ahora se describen para propósitos ilustrativos, no limitantes, junto con los resultados del análisis DSC y las pruebas de solubilidad, estabilidad e higroscopicidad; las pruebas de biodisponibilidad para las nuevas formas cristalinas también se describen.

EJEMPLO 1 Preparación inicial

El cloruro de tionilo (36 g) diluido en acetato de etilo (25 g) se adicionó lentamente a una solución del ácido 2,6-dimetil-5-metoxicarbonil-4-(3-nitrofenil)-1,4-dihidropiridin-3-carboxílico (90 g) en dimetilformamida (115 g) y acetato de etilo (396 g), manteniendo la temperatura entre -1 y +1°C. Una solución de 2,N-dimetil-N-(3,3-difenilpropil)-1-amino-2-propanol (84 g) en acetato de etilo (72 g) se adicionó lentamente a la mezcla obtenida de esta manera. El conjunto se mantuvo bajo agitación a la misma temperatura durante 3 horas. La mezcla luego se calentó a 20-25°C y se mantuvo bajo agitación durante 12 horas. Luego se adicionó agua (340 ml), el total se agitó durante 30 minutos y, después del asentamiento, se desechó la fase acuosa. La fase orgánica se lavó nuevamente con agua (340 ml).

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EJEMPLO 2 Forma Cruda (A) de clorhidrato de lercanidipina

La fase orgánica obtenida en el ejemplo 1 luego se sujetó a la destilación azeotrópica bajo vacío a aproximadamente 250 mmHg, sin superar una temperatura de 60°C. Después de la remoción de aproximadamente 50 ml de agua, la solución se concentró a aproximadamente 1/3 del volumen inicial en las mismas condiciones de temperatura y presión y luego se llevó a su volumen inicial con acetato de etilo nuevo hasta que el valor K.F. (valor Karl Fisher) fue de aproximadamente 0.10-0.15%. La suspensión final se enfrió a 0-5°C. El sólido se filtró, se suspendió en acetato de etilo (350 g) y se agitó a 60-65°C durante 1 hora. En conjunto se enfrió a 5-10°C y luego se filtró. El sólido se secó en un horno a 70°C. Se obtuvieron 133 g de la forma cruda, anhidra (A) de clorhidrato de lercanidipina (rendimiento del 75%), pico de DSC 150-152°C.

EJEMPLO 3 Forma Cruda (B) de clorhidrato de lercanidipina

La fase orgánica obtenida al final del ejemplo 1 se calentó bajo reflujo (70-75°C) y el agua contenida en la solución se removió con un aparato Dean Stark (Spaziani Rolando, Nettuno, Roma, Italia) hasta que se obtuvo un valor K.F. de aproximadamente 2%. El conjunto entonces se destiló a presión atmosférica para alcanzar $^3/_4$ del volumen inicial. La solución se llevó a su volumen inicial al adicionar acetato

de etilo nuevo. El valor K.F. al final de esta operación fue 0.9-1.1%. La solución final se enfrió a 0-5°C. Se precipitó lentamente un sólido, el cual se filtró. El sólido obtenido de esta manera se suspendió en acetato de etilo (350 g) y se agitó a 60-65°C durante 1 hora. El conjunto se enfrió a 5-10°C, luego se filtró y secó en un horno a 70°C, para obtener de esta manera 133 g de la forma cruda (B) de clorhidrato de lercanidipina, pico de DSC 131-135°C, rendimiento del 75%.

EJEMPLO 3A Forma Cruda (B) de clorhidrato de lercanidipina

La fase orgánica obtenida al final del ejemplo 1 se calentó bajo reflujo (70-75°C) y el agua contenida en la solución se removió con un aparato Dean Stark hasta que se obtuvo un valor K.F. de aproximadamente 2%. El conjunto entonces se destiló a presión atmosférica para alcanzar $^3/_4$ del volumen inicial. La solución se llevó a su volumen inicial al adicionar acetato de etilo nuevo. El valor K.F. al final de esta operación fue 0.9-1.1%. La solución final se enfrió a 20°C, se sembró con 0.1% de la forma cruda (B) de clorhidrato de lercanidipina y se enfrió a 0-5°C. Un sólido se precipitó lentamente y luego se filtró. El sólido obtenido de esta manera se suspendió en acetato de etilo (350 g) y se agitó a 60-65°C durante 1 hora. El conjunto se enfrió a 5-10°C, luego se filtró y secó en un horno a 70°C durante 24 horas, para obtener de esta manera 133 g de la forma cruda

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(B) de clorhidrato de lercanidipina, pico de DSC 131-135°C, rendimiento del 75%.

EJEMPLO 4 Preparación de la forma cristalina (I) de clorhidrato de lercanidipina

En experimentos representativos separados, se cargaron 100 g de la forma cruda (A), (B) o (C) de clorhidrato de lercanidipina en un reactor seguido por 400 ml de 2-propanol. La mezcla se calentó bajo reflujo fuerte y bajo agitación, para obtener de esta manera una disolución casi completa de la sustancia cruda. La mezcla luego se filtró en caliente para eliminar una ligera opalescencia y la solución clara mantenida bajo agitación se enfrió a 40°C. La temperatura luego se estableció a 35°C. El conjunto se mantuvo durante 24 horas bajo agitación a 35°C, luego la temperatura se estableció a 30°C y la agitación continuó a esa temperatura durante otras 24 horas. El sólido se filtró a 30°C y se lavó con 50 ml de 2-propanol, luego se secó en un horno a 70°C bajo vacío durante 24 horas. El peso del producto seco en cada caso fue 90 g (HCl de lercanidipina (I)) (pureza del producto en la forma (I) mediante la CLAR >99.5%).

EJEMPLO 4A Preparación de la forma cristalina (I) de clorhidrato de lercanidipina

En experimentos representativos separados, se

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cargaron 100 g de la forma cruda (A), (B) o (C) de clorhidrato de lercanidipina en un reactor, seguido por 400 ml de 2-propanol. La mezcla se calentó bajo reflujo fuerte y bajo agitación, para obtener de esta manera una disolución casi completa de la sustancia cruda. La mezcla se filtró en caliente para eliminar una ligera opalescencia y la solución clara mantenida bajo agitación se enfrió lentamente a 40°C. Luego, la precipitación fue provocada con 100 mg de la forma (I) de clorhidrato de lercanidipina y temperatura se estableció a 35°C, manteniendo la mezcla bajo agitación. El conjunto se mantuvo durante 24 horas bajo agitación a 35°C, luego la temperatura se estableció a 30°C, manteniendo bajo agitación a esa temperatura durante otras 24 horas. El sólido se filtró a 30°C y se lavó con 50 ml de 2-propanol, luego se secó en un horno a 70°C bajo vacío durante 24 horas. El peso del producto seco (HCl de lercanidipina (I)) fue 90 g (pureza del producto en la forma (I) mediante la CLAR >99.5%).

EJEMPLO 5 Preparación de la forma cristalina (I) de clorhidrato de lercanidipina

En preparaciones independientes, se cargaron 25 kg de la forma cruda (A) o (B) de clorhidrato de lercanidipina y luego 100 mL de etanol al 95% y se llevaron al reflujo fuerte bajo agitación. La solución se enfrió bajo agitación a 20°C y luego se sembró con la forma cristalina (I). El conjunto

luego se enfrió a una temperatura entre 10 y 15°C, manteniendo la mezcla de reacción bajo agitación durante 4 dias. El sólido obtenido de esta manera se filtró y se lavó con etanol al 95%, el producto precipitado se filtró y secó en un horno bajo vacio a 70°C durante 24 horas. Se obtuvieron 20.2 kg del producto, correspondiente a un rendimiento del 81%; pureza en la forma (I) mediante la CLAR >99.5%. Los resultados comparables se obtienen con la forma (C) como material de inicio.

EJEMPLO 6 Preparación de la forma cristalina (II) de clorhidrato de lercanidipina

Se cargaron en un reactor 100 g de la forma cruda (C) de clorhidrato de lercanidipina y luego 200 ml de acetonitrilo. La mezcla se calentó bajo fuerte reflujo y bajo agitación, para obtener de esta manera una disolución completa. La mezcla se llevó a 20-30°C bajo agitación ligera y se mantuvo a esa temperatura durante 24 horas. El producto precipitado se filtró y secó en un horno a 70°C durante 24 horas. Se obtuvieron 95 g del producto seco, correspondiente a un rendimiento del 95%; pureza mediante la CLAR >99.5% en la forma (II) de clorhidrato de lercanidipina. Los resultados comparables se obtienen cuando la forma (A) o (B) de clorhidrato de lercanidipina se utiliza como material de inicio.

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EJEMPLO 7 Preparación de la forma cristalina (II) de clorhidrato de lercanidipina

En experimentos representativos separados, se cargaron en un reactor 100 g de la forma cruda (A), (B) o (C) de clorhidrato de lercanidipina en 200 ml de etanol al 95%, la mezcla obtenida de esta manera se calentó bajo agitación y bajo reflujo fuerte y luego se enfrió a 25°C, siempre bajo agitación. La solución se mantuvo a esa temperatura durante 24 horas bajo agitación. El producto precipitado obtenido de esta manera entonces se filtró y secó en un horno a 70°C durante 24 horas. Se obtuvieron 90 g de la forma (II), pureza mediante la CLAR >99.5%).

EJEMPLO 7A Preparación de la forma cristalina (II) de clorhidrato de lercanidipina

25 g de la sustancia cruda de HCl de lercanidipina o la forma (C) se disolvieron a 60°C en 100 ml de una mezcla de etanol-H_2O (8:2). El conjunto se filtró por medio de la gravedad para eliminar la posible porción insoluble y se diluyó con 100 ml de H_2O. La solución obtenida de esta manera se agitó a 25°C como tal, o se adicionó con 0.1 g de la forma (II) de clorhidrato de lercanidipina o se sonicó durante 6 segundos 20 kHz y 100 Vatios, siempre a 25°C. Cualquiera que sea la selección, después de 48 horas bajo agitación, el producto precipitado formado de esta manera se colectó y secó

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en un horno a 70°C durante 24 horas, para obtener un rendimiento de 80-85% de la forma (II). Los resultados comparables se obtienen utilizando las formas crudas (A) o (B) o la forma cristalina (I) de clorhidrato de lercanidipina como material de inicio.

Como una alternativa, la solución clara inicial se diluye con 100 ml de etanol y se siembra con la forma (II) de clorhidrato de lercanidipina (0.1 g). Después de 48 horas con agitación a 25°C, se obtiene un rendimiento del 80% con respecto a la forma estequiométrica (II) de clorhidrato de lercanidipina.

EJEMPLO 8 Preparación de la forma cristalina (II) de clorhidrato de lercanidipina en metanol acuoso

En los ejemplos independientes, representativos, se disolvieron 40 g de la forma cruda (C) o la forma cristalina (I) de clorhidrato de lercanidipina en 100 ml de metanol a 30°C. El conjunto se filtró por medio de la gravedad para eliminar la posible porción insoluble y se adicionaron 25 ml de agua. La solución obtenida de esta manera se agitó a 25°C como tal, o se mezcló con 0.1 g de la forma (II) de clorhidrato de lercanidipina, o se sonicó durante 6 segundos a 20 kHz y 100 Vatios, siempre a 25°C. Cualquiera que sea la selección, después de 48 horas bajo agitación, el producto precipitado formado de esta manera se colectó y secó, con

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rendimientos de 80-85% con respecto a la forma estequiométrica (II) de clorhidrato de lercanidipina. Los resultados comparables se obtienen utilizando la forma cruda (A) o (B).

EJEMPLO 9 Preparación de la forma cristalina (II) de clorhidrato de lercanidipina en 1-propanol acuoso

60 g de la forma cruda (C) de HCl de lercanidipina se disolvieron a 60°C en 100 ml de 1-propanol-H_2O (8:2). Después de la filtración por medio de la gravedad de la posible porción insoluble, la solución se enfrió en 2 horas a 25°C y se agitó durante 120 horas a esa temperatura, con o sin sonicación durante 6 segundos a 20 kHz y 100 Vatios. El producto precipitado formado de esta manera se colectó, para obtener un rendimiento del 90% con respecto a la forma estequiométrica (II) de clorhidrato de lercanidipina después de un paso de secado. Los resultados comparables se obtienen utilizando las formas crudas (A) o (B) o la forma cristalina (I) de clorhidrato de lercanidipina como material de inicio.

EJEMPLO 10 Preparación de la forma cristalina (II) de clorhidrato de lercanidipina en 2-propanol acuoso

30 g de la forma cruda (C) de clorhidrato de lercanidipina se disolvieron a 60°C en 100 ml de 2-

propanol-H_2O (8:2). Después de la filtración por medio de la gravedad de la posible porción insoluble, la solución se enfrió en dos horas a 25°C y se agitó durante 72 horas a esa temperatura, con o sin sonicación durante 6 segundos a 20 kHz y 100 Vatios. El producto precipitado formado de esta manera se colectó, para obtener un rendimiento del 85% con respecto a la forma estequiométrica (II) de clorhidrato de lercanidipina después de un paso de secado. El mismo resultado se obtiene mediante la agitación durante 168 horas a 10°C. Los resultados comparables se obtienen utilizando las formas crudas (A) o (B) o la forma cristalina (I) de clorhidrato de lercanidipina como material de inicio.

EJEMPLO 11 Preparación de la forma cristalina (II) de clorhidrato de lercanidipina en N-metilpirrolidona acuosa

Una suspensión de 50 g de la forma cruda (C) de clorhidrato de lercanidipina en 30 ml de N-metilpirrolidona/agua (1:1) se agitó a 20-25°C durante 12 días. El sólido formado de esta manera se colectó mediante la filtración y secó, para producir 40 g de la forma (II) de clorhidrato de lercanidipina. Los resultados comparables se obtienen utilizando las formas crudas (A) o (B) o la forma cristalina (I) de clorhidrato de lercanidipina, como material de inicio.

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EJEMPLO 12 Análisis DSC de las formas cristalinas (I) y (II) de clorhidrato de lercanidipina

El análisis DSC mide los cambios que ocurren en una muestra dada con el calentamiento, en donde los cambios identifican las fases de transición. Las variaciones en la entalpia que toman lugar en una fase de transición se calculan en base al área bajo la curva. Las fases de transición más comunes son la fusión y la sublimación. La temperatura a la cual inicia la transición, comienzo T, se proporciona por el punto en el cual la curva inicia a desviarse de la linea de base (punto de flexión).

DSC de la forma (I): 3.8 mg de la forma (I) se colocaron en una cacerola de oro del aparato Perkin Elmer DSC7. La velocidad de calentamiento durante la prueba fue 10°C/minuto.

DSC de la forma (II): 4.6 mg de la forma (II) se colocaron en una cacerola de oro del aparato Perkin Elmer DSC7. La velocidad de calentamiento durante la prueba fue 10°C/minuto.

Los datos se muestran en las figuras 1 y 2 y los puntos característicos de las figuras son resumidos brevemente en la siguiente tabla 1.

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Tabla 1.

Compuesto	T de fusión (T pico) [°C]	T de inicio [°C]
Forma (I)	198.7	179.8
Forma (II)	209.3	169.0

Inmediatamente después de la fusión de la forma (I) o (II), se puede observar un evento exotérmico debido a la descomposición de sales.

EJEMPLO 13 Termogravimetría

Un análisis gravimétrico asociado con un análisis IR se llevó a cabo en ambas formas cristalinas (I) y (II), y también sobre la forma cruda (A) de clorhidrato de lercanidipina y sobre la forma cruda (B) de clorhidrato de lercanidipina, utilizando un aparato Netsch Thermomicrobalance 209 en combinación con un espectrómetro FTIR Bruker Vector 22.

Las pruebas se llevaron a cabo de acuerdo con las siguientes condiciones de trabajo: se calentaron 2-5 mg de la muestra en un crisol de acero en una atmósfera de nitrógeno, con una velocidad de calentamiento de 10°C/minuto. Los resultados obtenidos con las formas cristalinas (I) y (II) se muestran en la figura 3, a partir de los cuales se puede concluir que en ambas formas cristalinas no se pudo observar

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pérdida de peso hasta su punto de fusión (es decir, hasta aproximadamente 190-200°C).

Durante la degradación, la cual toma lugar como se indica anteriormente después de la fusión, se puede observar una pérdida de CO_2.

Los resultados obtenidos con la forma cruda (A) de clorhidrato de lercanidipina se muestran en la figura 19, donde se puede observar una pérdida de peso de 3.4% en el rango de temperatura de 25-153°C. El compuesto volátil ha sido identificado por su espectro IR correspondiente y es acetato de etilo. Durante la degradación (T >170°C) se pudo observar una pequeña cantidad de acetato de etilo en su fase gaseosa.

Los resultados obtenidos con la forma cruda (B) de clorhidrato de lercanidipina se muestran en la figura 20, donde se puede observar una pérdida de peso de 0.5% en el rango de temperatura de 25-153°C. El compuesto volátil identificado con su espectro IR correspondiente es acetato de etilo (0.4%) y agua (0.1%). Durante la degradación (T >170°C) se puede observar una pequeña cantidad de acetato de etilo en su fase gaseosa.

EJEMPLO 14 Higroscopicidad de las formas cristalinas (I) y (II)

La higroscopicidad de ambas formas cristalinas (I)

y (II) se midió con análisis DVS por medio de un analizador de absorción de agua (SURFACE MEASUREMENT SYSTEM, Marion, Buckinghamshire, UK) de acuerdo con las siguientes condiciones de trabajo:

10-15 mg de la forma (I) y (II) respectivamente se colocaron en un soporte para muestras de cuarzo, colocado a su vez sobre una microbalanza, y la muestra se sometió a ciclos de humedad entre 0 y 95%, iniciando de 50% de humedad relativa (25°C, humedad relativa (HR): 50-95-0-95-0-50% a HR/h:5%).

Los resultados de las pruebas se muestran en los diagramas de las figuras 13 y 14.

14-1 Resultados obtenidos con la forma cristalina (I)

La exposición de la forma (I) a la humedad en el analizador DVS da por resultado un cambio de masas de +0.15% a 95% de HR y de -0.3% a 0% de HR, casi sin histéresis durante el incremento y pérdida de masa. Estas ligeras variaciones son probablemente debido a una absorción reversible de agua por la superficie.

14-2 Resultados obtenidos con la forma cristalina (II)

La exposición de la forma (II) a la humedad en DVS causa una variación de masas insignificante (<0.05%) en el rango de HR total sometido a prueba.

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EJEMPLO 15 Solubilidad de las Formas cristalinas (I) y (II)

15.1 Solubilidad en agua y en etanol a temperatura ambiente

La solubilidad a 23°C de ambas formas cristalinas (I) y (II) se evaluó por medio de la espectroscopia con luz UV visible en agua bidestilada (al valor de pH alcanzado espontáneamente por el sistema) y en etanol puro. La absorbencia molar ha sido determinada previamente en acetonitrilo. La misma absorbencia molar se consideró para la determinación en agua y etanol. La solubilidad en agua depende ciertamente del pH. El sólido residual obtenido por la filtración de la suspensión se analizó inmediatamente con la espectroscopia Raman. Los resultados se muestran en las siguientes tablas 2 y 3.

TABLA 2. Solubilidad en agua (aproximadamente 40 mg/ml como condición inicial).

Material de inicio	Tiempo [min]	Solubilidad [mg/ml]	Material residual
Forma (I)	5/25/45/990	0.4/0.5/0.5/0.5	Forma (I)
Forma (II)	5/25/45/990	0.2/0.2/0.3/0.3	Forma (II)

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TABLA 3. Solubilidad en etanol (100 mg/ml como condición inicial).

Material de inicio	Tiempo (min)	Solubilidad (mg/ml)	Material residual
Forma (I)	15/45/120	28/27/27	Forma (I)
Forma (II)	15/45/120	11/12/12	Forma (II)

La forma (II) es menos soluble que la forma (I) en ambos solventes.

15.2 Solubilidad en mezclas de agua-etanol a 25°C y a 40°C, con concentraciones crecientes de agua

Las figuras 4 y 5 muestran la solubilidad en agua-etanol a 25°C y a 40°C de la forma (I) y de la forma (II). La solubilidad máxima se alcanza para ambas formas, a ambas temperaturas, cuando la concentración de agua es de 20%. También en este caso, la solubilidad de la forma cristalina (I) es mayor que aquella de la forma cristalina (II).

EJEMPLO 16 Estudios de RMN ^{13}C de la fase sólida

Los espectros de la fase sólida de RMN ^{13}C de alta resolución se llevaron a cabo con el instrumento ASX300 Bruker equipado con un Accesorio de rotor de 7 mm, utilizando varias técnicas combinadas:

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Giro en ángulo mágico (MAS, por sus siglas en inglés). Aproximadamente 300 mg de la muestra se colocaron en el rotor que giraba a 4.3 kHz alrededor de un eje orientado en el ángulo mágico (54°70') al campo magnético para superar el ampliamiento dipolar causado por la Anisotropia de Desplazamiento Químico (CSA, por sus siglas en inglés). Los experimentos se condujeron a temperatura ambiente.

Acoplamiento Dipolar. Puesto que mucho del ampliamiento de las lineas en los espectros ^{13}C de los sólidos orgánicos es debido al acoplamiento a los protones, se removió por el desacoplamiento heteronuclear (el nivel de potencia de desacoplamiento fue casi un 1 Kilovatio).

Polarización Cruzada (CP, por sus siglas en inglés). La polarización cruzada permitió la magnetización de carbono a partir de la magnetización de protones más grandes por medio del acoplamiento dipolar para incrementar la intensidad de señales.

Supresión Total de Bandas Laterales (TOSS, por sus siglas en inglés). La TOSS se realizó utilizando ecos del espin sincronizados con la rotación de la muestra para causar la alteración de fases de las bandas laterales giratorias, dando por resultado la cancelación cuando los espectros sucesivos se adicionaron conjuntamente.

Las formas cristalinas (I) y (II) muestran diferentes espectros de RMN ^{13}C en la fase sólida. Las

señales (desplazamiento químico) y la atribución de los átomos de carbono correspondientes (numerados en la fórmula del clorhidrato de lercanidipina mostrada a continuación) son representadas en las siguientes tablas 4 y 5, respectivamente.

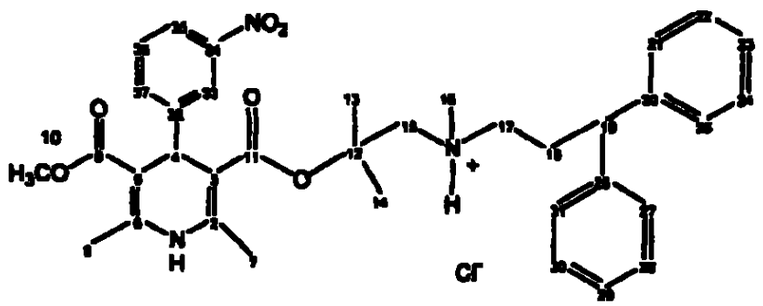


Tabla 4. Forma cristalina (I) de clorhidrato de lercanidipina

Desplazamiento químico (,ppm)	Atribución de átomos de carbono
168.7; 167.7	9; 11 o 11; 9
150.1 a 120.4	2; 6 y 20 a 37
104.3; 100.9	3; 5 o 5; 3
79.7	12
63.0; 60.1 (débil)	15; 17 o 17; 15
48.6	10
47.7	16
45.4	19
41.1	4
31.6	18

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27.7; 26.4	13; 14 o 14; 13
19.6; 18.0	7; 8 u 8; 7

Tabla 5. Forma cristalina (II) de clorhidrato de lercanidipina

Desplazamiento químico (,ppm)	Atribución de átomos de carbono
168.1; 166.6	9; 11 o 11; 9
151.9 a 121.9	2; 6 y de 20 a 37
104.0; 102.8	3; 5 o 5; 3
79.0	12
66.0; 58.0 (débil)	15; 17 o 17; 15
49.7	10
48.8	16
44.3	19
40.5	4
29.8	18
27.6; 23.5	13; 14 o 14; 13
19.6; 18.3	7; 8 u 8; 7

EJEMPLO 17 Estudios IR

Los espectros infrarrojos (IR) se registraron en polvo de KBr mediante la Técnica de Reflectancia Difusa utilizando un instrumento Spectrum-one de Perkin Elmer. Los espectros IR, cuyas longitudes de onda y atribución

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correspondientes se muestran en las siguientes tablas 6 y 7, son claramente diferentes para las nuevas formas (I) y (II).

Tabla 6. Espectro IR en polvo de KBr de la forma (I) de clorhidrato de lercanidipina

Longitud de onda (cm^{-1})	Atribución
3186	Alargamiento de NH
3100-2800	Alargamiento de alquilo y fenilo
2565	Alargamiento de N^+H
1673	Alargamiento de C=O
1525; 1348	Alargamiento asimétrico y simétrico del grupo NO_2
1405; 1386	Plegamiento de los grupos metilo geminales
785-685	Plegamiento fuera de plano de los hidrógenos adyacentes 5 y 3 en anillos aromáticos

Tabla 7. Espectro IR en polvo de KBr de la forma (II) de clorhidrato de lercanidipina

Longitud de onda (cm^{-1})	Atribución
3183	Alargamiento de NH
3100-2800	Alargamiento de alquilo y

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	fenilo
2684	Alargamiento de N°H
1705; 1675	Alargamiento de C=O
1526; 1350	Alargamiento asimétrico y simétrico del grupo NO_2
1402; 1380	Plegamiento de los grupos metilo geminales
800-680	Plegamiento fuera de plano de los hidrógenos adyacentes 5 y 3 en anillos aromáticos

EJEMPLO 18: Espectros Raman

Un espectrofotómetro RFS100 de Bruker FT-Raman se utilizó bajo las siguientes condiciones típicas: aproximadamente 100 mg de muestra (sin ningún tratamiento previo), 64 exploraciones a una resolución de 2 cm^{-1}, potencia del rayo láser 100 mW, detector Ge.

Las siguientes tablas 8 y 9 muestran los picos más significantes de los espectros Raman de la forma (I) y la forma (II), respectivamente.

Tabla 8. Espectro Raman de la forma cristalina (I)

Número de ondas (cm^{-1})	Intensidad pico*
3054	M
3040	M

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2981	M
2941	M
1675	S
1646	M
1583	M
1489	M
1349	Vs
1236	M
1005	S
821	M
174	M
98	S
73	Vs

*M= moderado; S= fuerte, Vs = muy fuerte

Tabla 9. Espectro Raman de la forma cristalina (II)

Número de onda (cm^{-1})	Intensidad pico*
3074	M
3064	M
3055	M
3048	M
3030	M
2973	M

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2940	M
1675	S
1647	S
1630	M
1584	M
1489	M
1351	Vs
1005	M
995	M
103	Vs
85	S

*M= moderado; S= fuerte, Vs = muy fuerte

EJEMPLO 19 Biodisponibilidad de las formas cristalinas (I) y (II)

Ejemplo 19a-Perro

Un estudio se llevó a cabo en seis perros Beagle para evaluar la biodisponibilidad de las formas cristalinas (I) y (II).

Los productos, en forma micronizada, se administraron por via oral mediante cápsulas de gelatina dura rellenadas con el agente activo, forma (I) y (II), a una dosificación de 3 mg/kg, administradas una vez en la mañana del dia del experimento.

Las muestras sanguineas se tomaron en tiempos dados

y las concentraciones en el plasma de lercanidipina se determinaron con un método analítico, estereoselectivo CLAR-EM/EM, de acuerdo con las siguientes condiciones de trabajo;

La lercanidipina se extrajo del plasma del perro por medio una extracción de líquido-líquido con una mezcla de n-hexano y éter etílico. El residuo seco de la fase orgánica se tomó con una mezcla de metanol y agua y se llevó a cabo una separación cromatográfica de fase líquida (LC, por sus siglas en inglés); los dos enantiómeros de lercanidipina se separaron en una columna CHIROBIOTIC V (Vancomycin) (tamaño de particula 5 m, tamaño de columna 150 x 4.6 mm (ASTEX, NJ, EUA)) y se detectaron con un espectrómetro de masas (EM/EM) al utilizar una técnica de electropulverización.

El método analítico se validó en un rango de concentración entre 0.1 y 20 ng/ml de plasma para ambos enantiómeros. El método ha mostrado que es específico con una precisión de 15%. Las concentraciones promedio de lercanidipina en las tablas representan la suma de ambos enantiómeros.

Los perfiles que se refieren a las concentraciones promedio de lercanidipina para ambas formas se muestran en la figura 10. Las siguientes tablas 10 y 11 muestran los valores individuales que se refieren AUC, Tmax, Cmax y a las concentraciones en el plasma.

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TABLA 10. Valores promedio (n=5) de AUC_{0-t}, C_{max}, T_{max} de la forma cristalina (I) y la forma cristalina (II) de clorhidrato de lercanidipina (S+R), en perros, después de la administración oral en una dosificación de 3 mg/kg.

Forma (I)

Parámetro	Perro 1	Perro 2*	Perro 3	Perro 4	Perro 5	Perro 6	Promedio	SD
AUC_{0-t} ng/h/ml	15.41	263.63	27.544	46.57	70.39	28.72	37.73	19.12
T_{max} (h)	2.00	4.00	6.00	3.00	3.00	6.00	4.00	1.67
C_{max} (ng/ml)	8.29	128.67	11.62	27.17	22.68	17.83	17.60	6.91

Forma (II)

Parámetro	Perro 1	Perro 2*	Perro 3	Perro 4	Perro 5	Perro 6	Promedio	SD
AUC_{0-t} ng/h/ml	54.59	119.77	75.62	173.82	142.34	61.91	104.68	43.99
T_{max} (h)	3.00	1.50	1.50	4.00	2.00	6.00	3.00	1.61
C_{max} (ng/ml)	18.46	62.19	19.78	62.64	55.38	18.66	36.17	17.27

* no incluido en el cálculo del valor promedio

Tabla 11. Concentración promedio en el plasma de la forma cristalina (I) y la forma cristalina (II) de clorhidrato de lercanidipina (S+R), en perros, después de la administración

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oral en una dosificación de 3 mg/kg.

Forma (I)

Tiempo (h)	Perro 1	Perro 2*	Perro 3	Perro 4	Perro 5	Perro 6	Promedio	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.1	0.20	0.00	0.00	0.00	0.00	0.00	0.02
1	0.59	0.29	0.00	0.00	0.00	0.00	0.12	0.22
1.5	1.83	1.06	0.32	0.00	1.33	0.00	0.70	0.73
2	8.29	8.94	0.94	0.35	17.11	0.28	5.39	6.34
3	4.44	36.39	0.92	27.17	22.58	1.29	11.28	11.11
4	1.81	128.87	9.42	11.07	16.39	6.26	8.99	5.66
6	0.80	26.65	11.62	2.63	9.73	17.83	8.50	6.50

Forma (II)

Tiempo (h)	Perro 1	Perro 2*	Perro 3	Perro 4	Perro 5	Perro 6	Promedio	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.00	22.67	6.99	0.00	0.00	0.00	1.40	2.81
1	0.00	52.13	16.61	5.50	3.28	0.00	5.08	5.91
1.5	0.23	52.19	19.78	35.43	32.69	3.49	18.32	14.66
2	7.63	35.45	17.81	38.10	55.39	10.19	25.82	19.23
3	18.48	17.43	15.80	28.36	40.57	14.10	23.46	12.56
4	14.83	5.17	14.10	52.64	23.68	13.24	23.69	16.28
6	8.05	4.50	3.62	17.46	6.76	18.56	10.88	6.82

* no incluido en el cálculo del valor promedio

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La formulación que contiene la forma (II) es más biodisponible que aquella que contiene la forma cristalina (I) en 5 animales de 6.

Para simplificar la comparación, el perro 2 se excluyó de la evaluación, puesto que después de la administración de la forma (I) el perro 2 mostró una AUC en el plasma de 264 ng/h/ml contra un valor promedio de 38 ± 19 (SD) de los otros 5 perros. Por otra parte, su AUC después de la administración de la forma (I) fue similar a aquella de los otros animales, el valor es 120 contra 105 ± 44 ng/h/ml.

La biodisponibilidad del clorhidrato de lercanidipina (forma (II)), expresada como un incremento en la AUC de lercanidipina (R+S) obtenida después de la administración de la forma (II), es aproximadamente 3 veces más alta que aquella obtenida con la forma (I). El perfil promedio de las concentraciones en el plasma para ambas formas cristalinas se muestra en la figura 10.

El análisis de estos resultados muestra que la cantidad de lercanidipina (S+R) adsorbida después de la administración de la forma cristalina (II) es 3 veces más alta que de la forma (I), mientras que la velocidad de absorción expresada como T_{max}, permanece prácticamente sin cambios.

Las concentraciones en el plasma 6 horas después de la administración (último momento de la toma de muestras) son

similares, las concentraciones son 8.5 ± 6.5 ng/ml después de la administración de la forma (I) y de 10.9 ± 6.8 ng/ml después de la administración de la forma (II).

EJEMPLO 19b-Hombre

Un estudio se llevó a cabo en 16 voluntarios saludables para valorar la biodisponibilidad relativa de la forma (I) y la forma (II) de clorhidrato de lercanidipina. La forma (I) fue representada por una tableta de Zanedip® correspondiente a 10 mg de clorhidrato de lercanidipina (Referencia-R). La forma (II) fue administrada en la forma de una tableta de 10 mg preparada exactamente de la misma manera y con la misma composición de Zanedip® 10 mg, iniciando a partir de la forma (II) micronizada que tiene el mismo tamaño de particula de la forma I (Prueba-T)- Las muestras sanguíneas se tomaron en 15 puntos desde el momento 0 a 24 h después de la dosificación y las concentraciones en el plasma de lercanidipina se determinaron con un método analítico, estereoselectivo CLAR-EM/EM. Los parámetros farmacocinéticos obtenidos se proporcionan en la siguiente tabla:

	Promedio mínimo-cuadrático, geom. de la forma (I)	Promedio mínimo-cuadrático, geom. de la forma (R)	Estimado de Puntos (T/R)	90% de C.I.
AUC_{0-t} (ng.h/mL)	8.82	10.38	1.17	0.93 - 1.48

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C_{max} (ng/mL)	3.18	3.22	1.01	0.73 - 1.42
t_{max} (h)	1.50*	2.50*	0.75**	0.00 - 1.25
C_{max}/AUC	0.388^	0.329^	0.85	0.69 - 1.02

* medio

** diferencia media

^ promedio mínimo-cuadrático

Los resultados obtenidos indican que la forma (II) de clorhidrato de lercanidipina no fue bioequivalente a la forma I, porque se obtienen niveles más altos en el plasma de la forma (II), ya que la forma I de clorhidrato de lercanidipina tiene un t_{max} que es más corto que aquel de la forma (II), lo que sugiere su uso en formulaciones de liberación inmediata.

EJEMPLO 20 Estudios de difracción de rayos X

El difractómetro PW 1710 de Philips y X pert PW 3040 de Philips (radiación Kα del Cobre) se utilizó bajo las siguientes condiciones típicas: aproximadamente 5-70 mg de la muestra (sin ningún tratamiento previo) con la aplicación de una ligera presión para obtener una superficie plana. Atmósfera de aire ambiente. 2θ gradual de 0.02°, paso-1 de 2 segundos, 2θ de 2-50.

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Los espectros obtenidos se proporcionan en las figuras 11 y 12 y los picos promedio correspondientes se describen en las tablas 12 y 13. Los datos son claramente diferentes para las muevas formas aisladas (I) y (II).

Tabla 12. Espectro XRD de la forma (I) de clorhidrato de lercanidipina

D(Δ)	Intensidad Relativa (I/Io)	Ángulo 2θ
16.3	83	5.4
6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

Tabla 13. Espectro XRD de la forma (II) de clorhidrato de lercanidipina

D(Δ)	Intensidad Relativa (I/Io)	Ángulo 2θ
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8

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3.81	41	23.4
3.77	100	23.6
3.58	44	24.8
3.54	29	25.2

EJEMPLO 21 Determinación del punto de fusión de varias mezclas de las formas cristalinas (I) y (II) de clorhidrato de lercanidipina

Los puntos de fusión de las composiciones que consisten de las relaciones conocidas de las formas cristalinas (I) y (II) de clorhidrato de lercanidipina se determinaron manualmente. Las condiciones consistieron del uso de un punto de referencia de 177°C y la introducción del tubo capilar en el instrumento (Aparato de Punto de Fusión modelo 535, Büchi Labortechnik AG, Flawil, Suiza) a aproximadamente 5°C abajo del punto de fusión. Los resultados se muestran en la tabla 14.

Tabla 14. Puntos de fusión de las composiciones que consisten de relaciones conocidas de las formas cristalinas (I) y (II) de clorhidrato de lercanidipina. Las muestras en la serie A y la serie B se calentaron a un gradiente de 1°C/minuto y 0.5°C/minuto, respectivamente. Los resultados se presentan en °C.

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Muestra	Forma Pura (I)	Relación de la forma cristalina (I):forma cristalina (II) de clorhidrato de lercanidipina					Forma Pura (II)
		9:1	7:3	1:1	3:7	1:9	
Serie A	186.8	188.0	189.5	190.0	192.2	194.2	194.3
Serie B	185.9-186.8	184.4-186.1	184.5-187.0	186.7-187.4	188.5-189.4	188.7-190.5	190.6-192.9

La patente norteamericana No. 5,767,136 describe el clorhidrato de lercanidipina cristalino que tiene un punto de fusión de 186-188°C. La tabla 14 muestra que este punto de fusión es exhibido por las mezclas de la forma (I) y la forma (II) en las cuales la relación de la forma (I):forma (II) varia entre 9:1 a 3:7. Bianchi y colaboradores, (Drugs of the Future, 1987, 12:1113-1115) reportan un punto de fusión de 186-188°C (sin DSC) para un producto de lercanidipina que ellos caracterizan como "cristales". Por lo tanto, el punto de fusión de una preparación de clorhidrato de lercanidipina no es suficiente por si mismo para distinguir la forma o formas particulares que están presentes en la misma, y muchas mezclas de diferentes composiciones tienen el mismo rango de punto de fusión.

EJEMPLO 22. Micronización del clorhidrato de lercanidipina.

La micronización se lleva a cabo por medio de un

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procedimiento de molino por chorro utilizando un aparato MICRONETTE M300 de la firma NUOVA GUSEO (Villanova sull'Arda -PC- Italia). Los parámetros son como sigue: presión de inyección, 5 Kg/cmq; presión de micronización, 9 Kg/cmq; y presión del ciclón, 2.5 Kg/cmq. La capacidad de micronización es 16 Kg/h. El tamaño de particula se determina por medio de la difracción de luz láser utilizando un instrumento de rayos láser GALAI CIS I (GALAI, Haifa, Israel). La micronización se realiza para obtener un tamaño de particula promedio de D (50%) 2-8 µm y D (90%)<15 µm.

* * *

La presente invención no se debe limitar en cuanto al alcance por las modalidades especificas descritas en este texto. En realidad, varias modificaciones de la invención, además de aquellas descritas en este texto, llegarán a ser aparentes para aquellas personas expertas en el campo a partir de la descripción anterior y las figuras que la acompañan. Se propone que estas modificaciones se encuentren dentro del alcance de las reivindicaciones anexas.

Por toda esta solicitud se citan patentes, solicitudes de patente, publicaciones, procedimientos y similares, las descripciones de las cuales se incorporan en este texto a manera de referencia en su totalidad.

Se hace constar que con relación a esta fecha, el mejor método conocido por la solicitante para llevar a la práctica la citada invención, es el que resulta claro de la presente descripción de la invención.

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REIVINDICACIONES

Habiéndose descrito la invención como antecede, se reclama como propiedad lo contenido en las siguientes reivindicaciones:

1. Una forma sólida, cruda (A) de clorhidrato de lercanidipina, caracterizada porque tiene un punto de fusión de aproximadamente 150-152°C (pico de DSC) y que comprende aproximadamente 3-4% (p/p) de acetato de etilo.

2. La forma sólida, cruda (B) de clorhidrato de lercanidipina, caracterizada porque tiene un punto de fusión de aproximadamente 131-135°C (pico de DSC) y que comprende aproximadamente 0.3-0.7% (p/p) de acetato de etilo.

3. Un método para producir la forma cruda de clorhidrato de lercanidipina de conformidad con la reivindicación 1, caracterizado porque comprende los pasos de:

a) hacer reaccionar el ácido 2,6-dimetil-5-metoxicarbonil-4-(3-nitrofenil)-1,4-dihidropiridin-3-carboxílico con un cloruro seleccionado del grupo que consiste de cloruro de tionilo y cloruro de oxalilo en un solvente dipolar, aprótico y un solvente polar, aprótico para producir el cloruro de carbonilo correspondiente;

(b) hacer reaccionar, in situ, el cloruro del paso a) con el alcohol de 2,N-dimetil-N-(3,3-difenilpropil)-1-amino-2-propilo para formar el clorhidrato de lercanidipina

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crudo; y

c) aislar el clorhidrato de lercanidipina crudo del paso b) y recuperar la forma cruda (A) de clorhidrato de lercanidipina,

en donde el paso (c) consiste esencialmente de los siguientes pasos:

i. lavar el clorhidrato de lercanidipina crudo del paso (b) con agua;

ii. remover el agua del paso i), para producir una mezcla;

iii. concentrar la mezcla del paso ii) y adicionar un solvente para producir una suspensión que tiene aproximadamente el mismo volumen como el volumen inicial de la mezcla del paso ii) y un contenido de agua, de acuerdo con Karl Fischer, de entre 0.10 y 0.15%;

iv. enfriar la suspensión obtenida en el paso iii) para obtener un sólido;

v. filtrar el sólido del paso iv);

vi. resuspender el sólido del paso v) en acetato de etilo;

vii. enfriar la suspensión del paso vi); y

viii. filtrar y secar el producto precipitado del paso vii) para producir la forma cruda (A) de clorhidrato de lercanidipina.

4. El método de conformidad con la reivindicación 3, caracterizado porque el paso de reacción b) se conduce a una temperatura entre -5 y +5°C.

5. El método de conformidad con la reivindicación 3, caracterizado porque el cloruro en el paso a) es cloruro de tionilo.

6. El método de conformidad con la reivindicación 3, caracterizado porque el paso c) ii) comprende la remoción del agua del paso c) i) por medio de la destilación azeotrópica bajo vacío dentro del rango de 200-300 mmHg, a una temperatura no mayor de 60°C, para producir una mezcla.

7. El método de conformidad con la reivindicación 3, caracterizado porque el paso de resuspensión c) vi) comprende la agitación a 60-65°C durante aproximadamente 1 hora.

8. El método de conformidad con la reivindicación 3, caracterizado porque el secado en el paso c) viii) es un horno a 70°C.

9. El método de conformidad con la reivindicación 3, caracterizado porque el paso de lavado c) i) es con agua; la mezcla en el paso c) iii) es concentrada a 1/3 de su volumen inicial y el solvente se adiciona para producir una suspensión que tiene aproximadamente el mismo volumen como el volumen inicial de la mezcla; y el contenido de agua de la suspensión, de acuerdo con Karl Fischer, es entre 0.1 y

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0.15%.

10. El método de conformidad con la reivindicación 3, caracterizado porque el enfriamiento en el paso c) iv) se lleva a cabo a una temperatura dentro del rango de 0-5°C.

11. El método de conformidad con la reivindicación 3, caracterizado porque el enfriamiento en el paso vii) se lleva a cabo a una temperatura dentro del rango de 5-10°C.

12. Un método para producir la forma cruda (B) de clorhidrato de lercanidipina de conformidad con la reivindicación 2, caracterizado porque comprende los pasos de:

a) hacer reaccionar el ácido 2,6-dimetil-5-metoxicarbonil-4-(3-nitrofenil)-1,4-dihidropiridin-3-carboxilico con un cloruro seleccionado del grupo que consiste de cloruro de tionilo y cloruro de oxalilo en un solvente dipolar, aprótico y un solvente polar, aprótico para producir el cloruro de carbonilo correspondiente;

(b) hacer reaccionar, in situ, el cloruro del paso a) con el alcohol de 2,N-dimetil-N-(3,3-difenilpropil)-1-amino-2-propilo para producir el clorhidrato de lercanidipina crudo; y

c) aislar el clorhidrato de lercanidipina crudo del paso b) y recuperar la forma cruda (B) de clorhidrato de lercanidipina, en donde el paso (c) consiste esencialmente de los siguientes pasos:

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i') lavar el clorhidrato de lercanidipina crudo del paso (b) con agua;

ii') remover el agua del paso i'), para producir una mezcla que tiene un contenido de agua de aproximadamente 2%, medido de acuerdo con Karl Fischer;

iii') concentrar la mezcla del paso ii') y adicionar un solvente para producir una solución que tiene aproximadamente el mismo volumen como el volumen inicial de la mezcla del paso ii') y un contenido de agua, de acuerdo con Karl Fischer, de entre 0.9 y 1.1%;

iv') enfriar la solución del paso iii') para obtener un sólido;

v') filtrar el sólido del paso iv');

vi') resuspender el sólido del paso v') en un solvente;

vii') enfriar la suspensión del paso vi'); y

viii') filtrar y secar el sólido obtenido para producir la forma cruda (B) de clorhidrato de lercanidipina.

13. El método de conformidad con la reivindicación 12, caracterizado porque el paso de reacción b) es conducido a una temperatura entre -5 y +5°C.

14. El método de conformidad con la reivindicación

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12, caracterizado porque el cloruro es cloruro de tionilo.

15. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) ii') comprende la remoción de agua del paso i') mediante el calentamiento a reflujo azeotrópico para producir la mezcla.

16. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) iii') comprende la concentración de la mezcla a 3/4 de su volumen inicial.

17. El método de conformidad con la reivindicación 12, caracterizado porque el solvente en los pasos c) iii') y vi') es acetato de etilo.

18. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) iv') comprende el enfriamiento de la solución a una temperatura dentro del rango de 0-5°C.

19. El método de conformidad con la reivindicación 12, caracterizado porque el paso c) vi') además comprende agitar la suspensión a 60-65°C durante aproximadamente 1 hora.

20. El método de conformidad con la reivindicación 18, caracterizado porque el paso c) vii') además comprende el enfriamiento del sólido a una temperatura entre 5 y 10°C.

21. El método de conformidad con la reivindicación 12, caracterizado porque el secado en el paso viii') es en un horno a aproximadamente 70°C.

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22. El método de conformidad con cualquiera de las reivindicaciones 3-21, caracterizado porque el solvente dipolar, aprótico es dimetilformamida y el solvente polar, aprótico es acetato de etilo.

23. La forma cristalina, aislada (I) de clorhidrato de lercanidipina, caracterizada porque tiene el patrón de difracción de rayos X, a una longitud de onda Kα, como se muestra en la figura 11.

24. La forma cristalina de lercanidipina de conformidad con la reivindicación 23, caracterizada porque las distancias, relaciones (I/Io), y ángulos 2θ de los picos significantes en la figura 11 son:

D(X)	Intensidad Relativa (I/Io)	ángulo 2θ
16.3	83	5.4
6.2	47	14.2
4.78	29	18.6
4.10	63	21.7
4.06	36	21.9
3.90	100	22.8

25. La forma cristalina, aislada (II) de clorhidrato de lercanidipina, caracterizada porque tiene un patrón de difracción de rayos X, a la longitud de onda Kα, como se muestra en la figura 12.

26. La forma cristalina de lercanidipina de

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conformidad con la reivindicación 25, caracterizada porque las distancias, relaciones (I/Io), y ángulos 2θ de los picos significantes en la figura 12 son:

D(X)	Intensidad Relativa (I/Io)	ángulo 2θ
9.3	35	9.5
6.0	45	14.7
5.49	65	16.1
4.65	52	19.1
4.27	74	20.8
3.81	41	23.4
3.77	100	23.6
3.58	44	24.8
3.54	29	25.2

27. Un método para producir la forma cristalina (I) de clorhidrato de lercanidipina de conformidad con cualquiera de las reivindicaciones 23 y 24, caracterizado porque comprende:

d) adicionar un solvente de alcohol de 1 a 5 átomos de carbono que contiene un máximo de 5% de agua (v/v) a una forma cruda de clorhidrato de lercanidipina y calentar bajo reflujo y con agitación producir una solución clara;

e) enfriar la solución del paso d) y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

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f) recuperar el sólido obtenido del paso e), y secar el sólido para producir la forma cristalina (I) de clorhidrato de lercanidipina.

28. El método de conformidad con la reivindicación 27, caracterizado porque el paso f) comprende filtrar el sólido obtenido del paso e), lavar el sólido con isopropanol y filtrar nuevamente el sólido antes del secado.

29. El método de conformidad con la reivindicación 27, caracterizado porque el alcohol del paso d) se selecciona del grupo que consiste de isopropanol, etanol y etanol anhidro.

30. El método de conformidad con la reivindicación 27, caracterizado porque la forma cruda es la forma cruda (A) de clorhidrato de lercanidipina, la forma cruda (B) de clorhidrato de lercanidipina o la forma cruda (C) de lercanidipina.

31. El método de conformidad con la reivindicación 27, caracterizado porque el paso d) además comprende filtrar la solución calentada.

32. El método de conformidad con la reivindicación 29, caracterizado porque cuando el alcohol de 1 a 5 átomos de carbono es isopropanol, el paso e) comprende enfriar la solución a una temperatura entre 30 y 40°C.

33. El método de conformidad con la reivindicación 32, caracterizado porque el paso e) además comprende agitar

durante un periodo de tiempo de 12-48 horas.

34. El método de conformidad con la reivindicación 27, caracterizado porque el secado en el paso f) toma lugar en un horno.

35. El método de conformidad con la reivindicación 29, caracterizado porque cuando el alcohol de 1 a 5 átomos de carbono es etanol, éste comprende los siguientes pasos

d') proporcionar una mezcla de etanol y clorhidrato de lercanidipina, calentar a reflujo bajo agitación y enfriar a 20°C y adicionar las semillas cristalinas de la forma (I);

e') enfriar adicionalmente la mezcla sembrada del paso d') y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f') recuperar el sólido del paso e') para formar la forma (I) de clorhidrato de lercanidipina.

36. El método de conformidad con la reivindicación 35, caracterizado porque la relación de clorhidrato de lercanidipina con el volumen del solvente en el paso d') en una relación de volumen en peso está dentro del rango de aproximadamente 1:4 a 1:6.

37. El método de conformidad con la reivindicación 35, caracterizado porque la relación de 1:4.

38. El método de conformidad con la reivindicación

35, caracterizado porque d') además comprende filtrar la solución calentada.

39. El método de conformidad con la reivindicación 35, caracterizado porque el enfriamiento en el paso e') se lleva a cabo a una temperatura entre 10 y 15°C.

40. El método de conformidad con la reivindicación 35, caracterizado porque el secado en el paso f') toma lugar en un horno a 70°C.

41. El método de conformidad con la reivindicación 35, caracterizado porque las semillas auténticas de la forma (I) de lercanidipina se adicionan al final del enfriamiento en los pasos e') y d').

42. Un método para producir la forma cristalina (II) de clorhidrato de lercanidipina, que tiene un patrón de difracción de rayos X, en la longitud de onda Kα, como se muestra en la figura 12, de conformidad con cualquiera de las reivindicaciones 25 y 26, el método está caracterizado porque comprende los pasos de:

d") adicionar acetonitrilo al clorhidrato de lercanidipina y calentar la mezcla obtenida de esta manera para formar una solución;

e") enfriar la solución del paso d") y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f") recuperar el sólido del paso e") y secar el

sólido para producir la forma (II) de clorhidrato de lercanidipina.

43. El método de conformidad con la reivindicación 42, caracterizado porque el paso d") comprende calentar la mezcla bajo reflujo con agitación.

44. El método de conformidad con la reivindicación 42, caracterizado porque el paso e") comprende enfriar la solución a temperatura ambiente.

45. El método de conformidad con la reivindicación 42, caracterizado porque el paso e") comprende agitar la solución a temperatura ambiente durante 24 horas.

46. El método de conformidad con la reivindicación 42, caracterizado porque el paso de secado f") toma lugar en un horno.

47. El método de conformidad con la reivindicación 42, caracterizado porque la forma cruda es la forma cruda (A) de clorhidrato de lercanidipina, la forma cruda (B) de clorhidrato de lercanidipina o la forma cruda (C) de lercanidipina.

48. Un método para producir la forma cristalina (II) de clorhidrato de lercanidipina, que tiene un patrón de difracción de rayos X, en la longitud de onda Kα, como se muestra en la figura 12, de conformidad con cualquiera de las reivindicaciones 25 y 26, caracterizado porque comprende:

d"') adicionar etanol o isopropanol con un

contenido de agua inferior a 10% en peso al clorhidrato de lercanidipina y calentar a reflujo para producir una solución;

e"') enfriar la solución a una temperatura entre 20 y 40°C y agitar hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f"') recuperar el sólido producido del paso e") para producir la forma (II) de clorhidrato de lercanidipina.

49. El método de conformidad con la reivindicación 48, caracterizado porque el etanol se adiciona en el paso d"').

50. El método de conformidad con la reivindicación 48, caracterizado porque el contenido de agua del solvente en el paso d"') es entre 5 y 10%.

51. El método de conformidad con la reivindicación 48, caracterizado porque el paso f"') comprende filtrar el sólido y secar en un horno.

52. Un método para producir la forma cristalina (II) de clorhidrato de lercanidipina, que tiene un patrón de difracción de rayos X, en la longitud de onda Kα, como se muestra en la figura 12, de conformidad con cualquiera de las reivindicaciones 25 y 26, caracterizado porque comprende:

d"") disolver el clorhidrato de lercanidipina crudo o la forma cristalina (I) de clorhidrato de lercanidipina en

un solvente polar, prótico o dipolar aprótico que contiene hasta 50% en peso de agua para producir una solución;

e"") agitar la solución del paso d"") hasta que la concentración de clorhidrato de lercanidipina disuelto en el solvente de cristalización es ≤ 2%; y

f"") recuperar el sólido del paso e"") para producir la forma (II) de lercanidipina.

53. El método de conformidad con la reivindicación 52, caracterizado porque además comprende irradiar con ultrasonido y/o adicionar semillas cristalinas de la forma (II) al paso e"").

54. El método de conformidad con la reivindicación 52, caracterizado porque además comprende adicionar hasta 60% de agua a la solución del paso d"").

55. El método de conformidad con la reivindicación 52, caracterizado porque el solvente polar, prótico es un solvente de alcohol.

56. El método de conformidad con la reivindicación 52, caracterizado porque el solvente de alcohol se selecciona del grupo que consiste de metanol, etanol, n-propanol e isopropanol.

57. El método de conformidad con la reivindicación 52, caracterizado porque el solvente dipolar, aprótico es N-metilpirrolidona.

58. El método de conformidad con la reivindicación

52, caracterizado porque la temperatura del paso d"") es entre 20 y 70°C.

59. El método de conformidad con la reivindicación 52, caracterizado porque la agitación en el paso e"") toma lugar a una temperatura entre 20 y 25°C.

60. El método de conformidad con la reivindicación 52, caracterizado porque el secado en el paso f"") toma lugar a 70°C.

61. Una composición farmacéutica antihipertensiva, caracterizada porque comprende (1) clorhidrato de lercanidipina cristalino y opcionalmente otras formas de lercanidipina, en donde el clorhidrato de lercanidipina cristalino se selecciona del grupo que consiste de la forma cristalina (I) de clorhidrato de lercanidipina, la forma cristalina (II) de clorhidrato de lercanidipina y combinaciones de las mismas que comprenden un contenido predeterminado de cada forma cristalina y (2) al menos un componente seleccionado del grupo que consiste de un portador o diluyente farmacéuticamente aceptable, un saborizante, edulcorante, conservador, tinte, sustancia aglutinante, agente de suspensión, agente de dispersión, colorante, desintegrante, excipiente, lubricante, plastificante y aceite comestible.

62. Una forma de dosificación unitaria, caracterizada porque comprende la composición farmacéutica,

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antihipertensiva de conformidad con la reivindicación 61.

63. La forma de dosificación unitaria de conformidad con la reivindicación 62, caracterizada porque la forma de dosificación es una forma de dosificación de liberación inmediata de lercanidipina.

64. La forma de dosificación unitaria de conformidad con la reivindicación 62, caracterizada porque la forma de dosificación es una forma de dosificación de liberación sostenida de lercanidipina.

65. La forma de dosificación unitaria de conformidad con la reivindicación 64, caracterizada porque la forma de dosificación comprende una fase de liberación inmediata de lercanidipina y una fase de liberación sostenida de lercanidipina.

66. La forma de dosificación unitaria de conformidad con la reivindicación 65, caracterizada porque la composición comprende 0.1 a 400 mg de clorhidrato de lercanidipina.

67. La forma de dosificación unitaria de conformidad con la reivindicación 66, caracterizada porque la composición comprende 1 a 200 mg de clorhidrato de lercanidipina.

68. La forma de dosificación unitaria de conformidad con la reivindicación 67, caracterizada porque la composición comprende 5 a 40 mg de clorhidrato de

lercanidipina.

69. Una composición antihipertensiva, caracterizada porque comprende cantidades predeterminadas de la forma cristalina (I) de clorhidrato de lercanidipina y la forma cristalina (II) de clorhidrato de lercanidipina.

70. La composición antihipertensiva de conformidad con la reivindicación 69, caracterizada porque la forma cristalina (I) de clorhidrato de lercanidipina tiene un punto de fusión de aproximadamente 197-201°C y la forma cristalina (II) de clorhidrato de lercanidipina tiene un punto de fusión de aproximadamente 207-211°C, cuando los puntos de fusión son determinados como los picos de DSC.

71. La composición antihipertensiva de conformidad con las reivindicaciones 69 y 70, caracterizada porque la relación de la forma (I) con la forma (II) es entre 1:9 y 9:1.

72. La composición antihipertensiva de conformidad con la reivindicación 71, caracterizada porque la relación de la forma (I):forma (II) se selecciona del grupo que consiste de 9:1, 7:3, 1:1, 3:7 y 1:9.

73. La forma cristalina, aislada de lercanidipina de conformidad con cualquiera de las reivindicaciones 23, 24, 25 o 26, caracterizada porque comprende un tamaño de partícula promedio de D (50%) 2-8 µm y D (90%) <15 µm.

74. La composición farmacéutica, antihipertensiva

de conformidad con la reivindicación 62, caracterizada porque las formas cristalinas (I) y (II) de clorhidrato de lercanidipina tienen cada una un tamaño de partícula promedio de D (50%) 2-8 µm y D (90%) < 15 µm.

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RESUMEN DE LA INVENCIÓN

La invención describe las nuevas formas crudas (A) y (B) de lercadinipina, las nuevas formas cristalinas (I) y (II) de clorhidrato de lercanidipina obtenidas a partir de las formas crudas, composiciones antihipertensivas, farmacéuticas que contienen como agente activo al menos una de las formas cristalinas (I) y (II) de clorhidrato de lercanidipina y métodos de uso de las mismas.

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RADICACION Y CONSULTA Folios: 29/1
Radicacion : 04009119 0000000
Fecha (AMD): 2004-02-06 11:12:21
Tramite : 011 PCI-PATENT 1/9 PAGE MACI 411 PRESENTA
Dependencia: 2020 DIVISION DE MARCAS CARACTERES

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REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCIÓN **MODELO DE UTILIDAD**	( )	(X)
- Indicación de que se solicita la concesión de una patente.	( )	(X)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	( )	(X)
- Descripción de la invención.	( )	(X)
- Dibujos de ser estos pertinentes.	( )	(X)
- Comprobante de Pago de las tasas establecidas.		
COMPLETA ( ) **INCOMPLETA**	( )	( )
DISEÑO INDUSTRIAL ( )		
- Indicación de que se solicita el registro de Diseño Industrial	( )	( )
- Datos de identificación del solicitante o de la persona que presenta	( )	( )
- la solicitud.		
- Representación gráfica y fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.	( )	( )
Comprobante de pago de las tasas establecidas	( )	( )
COMPLETA ( ) INCOMPLETA	( )	( )
ESQUEMA DE TRAZADO ( )		
- Indicación de que solicita el registro de un Esquema de trazado.	( )	( )
- Datos de identificación del solcitante o de la persona que presenta La solicitud.	( )	( )
Representación gráfica del esquema de trazado	( )	( )
Comprobante de pago de las tasas establecidas.	( )	( )
COMPLETA ( ) INCOMPLETA	( )	( )

Firma Responsable:

Edgar Narvaez

Fecha: 06-02-2004

2020
Bogotá, D.C., 299

Oficio N° 02167

Doctora:
Dora María Gómez Díaz.
Apoderada
Recordati Ireland Limited.

ASUNTO:	Radicación N°	04-9319
	Solicitud internacional	PCT/EP02/08699
	Fecha inicio fase nacional	06/03/2004
	Trámite	011
	Evento	379
	Actuación	430
	Folios	001

Como resultado del examen de forma, realizado con fundamento el el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

- La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486).

- La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL.
Jefe de la División de Nuevas Creaciones.

27 FEB. 2004

El anterior requerimiento fue notificado por fijación en lista de fecha_____

202061

Industria y Comercio
SUPERINTENDENCIA

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2020
Bogotá, D.C.,

Doctora: **Oficio N°** 02167
Dora María Gómez Díaz.
Apoderada
Recordati Ireland Limited.

ASUNTO:		
	Radicación N°	04-9319
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