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SEÑOR

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: Expediente 06-106-511

TÍTULO SOLICITADO: SAL DE CLOPIDOGREL Y SUS FORMAS
POLIMORFAS.

PUBLICACIÓN: Gaceta 583 de 18/12/2007, N° de publicación 918

PRIORIDAD: US 60/563,795 de 20/04/2004

SOLICITANTE: SANOFI-AVENTIS.

APODERADO: EMILIO FERRERO

TRÁMITE: SUSTENTACION DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUÍMICAS S.A.**, dentro del trámite de la referencia, en ejercicio de lo dispuesto por el artículo 42 inciso 2° de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada en los siguientes términos:

I. COMPLEMENTO DE INFORMACIÓN.

La solicitud colombiana 06-106-511 en trámite, que se refiere a la obtención de las formas polimórficas A, B, C, D, E y F de metil(+)-(S)- α _(2-clorofenil)-6,7-dihidrotieno[3,2-c]piridina-5(4H)-acetato Naftaleno-,1,5-disulfonato o uno de sus hidratos y/o solvatos, con un perfil característico de cristalografía de difracción de rayos X, una formulación farmacéutica que contiene dichos polimorfos y un

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método de inhibición de la agregación plaquetaria útil en el tratamiento de episodios ateroscleróticos; carece de los requerimientos fundamentales para otorgársele el beneficio de patente de invención, tal y como se puede evidenciar a través de los siguientes argumentos:

- La solicitud colombiana en trámite, específicamente en sus reivindicaciones 1 a 39, se refiere a la formas polimórficas A, B, C, D, E y F de naftaleno-1,5-disulfonato de (+)-(S)- α -(2-clorofenil)-6,7-dihidrotieno[3,2-c]piridina-5(4H)-acetato de metilo o uno de sus hidratos y/o solvatos. como es de amplio reconocimiento por este despacho, el polimorfismo, en los términos de la Farmacia Práctica de Remington¹, es "la capacidad de cualquier elemento o compuesto de cristalizar en más de un sistema cristalino".

Los sólidos pueden existir, de acuerdo con su estructura u ordenamiento interno, en formas cristalinas o amorfas. En las formas amorfas las partículas componentes no presentan una disposición interna ordenada, sino que se encuentran distribuidas al azar. Por el contrario, en las formas cristalinas se presenta un arreglo interno ordenado y regular de las partículas, que implica la apreciación de formas geométricas definidas. Es importante resaltar que dicha apariencia cristalina o amorfa depende de factores manipulables como son temperatura, presión, velocidad de cristalización, concentración de saturación, e inclusive, la influencia de sustancias extrañas y campos de fuerza exteriores al cristal²

Como el tema del polimorfismo es un tema ampliamente divulgado y discutido, es necesario recordar que, el descubrimiento del polimorfismo como propiedad de las entidades químicas se atribuye al farmacéutico Martín Enrique Klaproth en 1798, así mismo, la tesis según la cual los mismos elementos químicos pueden adoptar formas sólidas diversas – polimorfos - y mostrar propiedades físicas diferentes, se atribuye a Louis Jacques Thénard (1777-1857) y Jean Baptiste Biot (1774-1862)³, lo que indiscutiblemente demuestra que el encontrar o el atribuir a un compuesto "nuevas" formas polimórficas no es algo

¹ Farmacia Práctica de Remington, VII – Preparados farmacéuticos y su elaboración, Robert E. King, Páginas 1912 y 1913

² J. Garrido. Forma y estructura de los Cristales, (obra citada), capítulo V, página 204

³ A. R. Verna, P. Krishna – "Polytypism in crystals". John Willey and Son Inc., New York 1966.

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que se conozca recientemente, es una propiedad inherente de la molécula y que está influenciada por el medio y las condiciones en el que se encuentre.

De igual manera, la caracterización de dicha forma cristalina (polimorfo), dada por los patrones de difracción de rayos X en polvo y como consta en algunas de las reivindicaciones de la solicitud colombiana 06-106-506, carece de nivel inventivo, pues es bien sabido que la difracción de rayos X corresponde a una técnica analítica útil y rutinaria en la caracterización de formas cristalinas. En otras palabras, la caracterización hecha de las formas cristalinas por el solicitante, en función de sus patrones de difracción de RX (método de identificación que data de 1912⁴) permite a cualquier persona versada en la materia identificar y caracterizar diversas variaciones en la estructura cristalina de una molécula. Los resultados de un procedimiento de caracterización por difracción de RX son una manifestación del comportamiento natural de una molécula, en función de las condiciones ambientales y demás variables a que esta sea sometida, incluyendo desde luego la orientación e intensidad de los rayos, sin que dichos resultados constituyan de manera alguna aporte inventivo de quien realiza el procedimiento de identificación⁵.

En conclusión, el reporte de las formas polimórficas A, B, C, D, E y F de metil (+)-S- α -(2-clorofenil)-6,7-dihidrotieno[3,2-C]piridina-5(4H) acetato, naftaleno, 1,5-disulfonato es un hallazgo previsible y no una invención, en el sentido que es una propiedad inherente a la materia en su estado sólido y dicho reporte es el simple encuentro de características fisicoquímicas que ya se sabía podían ser halladas y que hacen parte de la práctica rutinaria en el campo farmacéutico.

Ahora bien, si tenemos en cuenta que la sal de naftaleno 1,5-disulfonato no había sido revelada con anterioridad para este principio activo, no lo es menos que ésta no es más que un tipo de sal, o una sal más que se puede formar del compuesto Clopidogrel bajo condiciones típicas de formación de sales, es decir, la reacción de un ácido con una base en solución en un solvente

⁴ Ver "Remington - The Science and Practice of Pharmacy", 20th Edition, Philadelphia, PA, USA, 2000, Páginas 172-178.

⁵ Confirma lo anterior lo dicho por Skoog, Holler y Mieman "For analytical diffraction studies, the crystalline sample is ground to a fine homogeneous powder in such a form the enormous number of small crystallites are oriented in every possible direction; this when a X-ray beam traverses the material, a significant number of the particles can be expected to be oriented in such ways as to fulfill the bragg condition for reflection from every possible interplanar spacing"⁵.

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adecuado y posterior precipitación espontánea o luego de la adición de un no-solvente y que cualquier persona medianamente versada en la materia sería capaz de obtener.)

En el mismo sentido hago notar que los hidratos son una forma de presentarse las sustancias cuando éstas cristalizan en el agua y retienen moléculas de dicho disolvente en la red de cristalización, y solvatos cuando el solvente es diferente del agua; y es por ello que se debe considerar aplicable al caso la misma carencia de nivel inventivo que envuelve la caracterización fisicoquímica de la estructura organizacional de sustancias químicas en función de su medio de cristalización, tanto a lo que refiere a su forma de presentación polimórfica como a sus posibles solvataciones.

Ahora bien, para lograr una mayor comprensión y evidente revelación de sales y/o polimorfos ya existentes para el compuesto Clopidogrel, y desvirtuar así mismo el nivel inventivo de la solicitud colombiana en trámite por el hecho de sugerir ahora la sal naftaleno 1,5-disulfonato y sus formas polimórficas, me permito citar lo revelado en:

- ✓ Si bien es cierto, la patente US 4,529,596 de título "Thieno [3,2-c] pyridine Derivatives and their therapeutic application" y publicación 16-06-1985, describe mezclas racémicas de derivados de tieno [3,2-c] piridina tal como el compuesto methyl α -(4,5,6,7-tetrahydro-thieno (3,2-c) 5-pyridyl)-o.chlorophenyl- acetate, se debe tener en cuenta no solo que Clopidogrel posee un carbono asimétrico en su estructura, y que por tanto puede existir en forma de dos enantiómeros (dextro y levorrotatorio), sino que en dicho documento también se incluye la posibilidad de formar sales de adición de ácidos orgánicos o minerales farmacéuticamente aceptables, por tal razón podría pensarse que la sal de naftaleno 1,5-disulfonato pueda estar contenida dentro de ésta generalidad. ✓
- ✓ La patente americana US 4,847,265, titulada "Dextro-rotatory enantiomer of methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thieno pyridyl) (2-chlorophenyl)-acetate and the pharmaceutical compositions containing it" y publicada el 11 de Julio de 1989; en este documento se revelan las formas enantioméricas dextrorrotatorias de Clopidogrel y de sus sales, ✓

cuya actividad farmacológica es la inhibición de la agregación plaquetaria; más específicamente se refiere a las sales hidrogenosulfato, taurocolato e **bromhidrato** de las formas enantioméricas dextrorrotatorias de methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thieno pyridyl) (2-chlorophenyl)-acetate, obtenidas de manera convencional.

Así mismo, en la columna 1 líneas 55 a 62, se evidencia la gran cantidad de sales que se sugieren y que pueden ser formadas para la forma dextrorrotatoria de Clopidogrel con ácidos de distintos tipos, veamos:

Thus, the salts of carboxylic acid

and sulfonic acids classically used in pharmacy have been prepared, acids such as acetic, benzoic, fumaric, maleic, citric, tartaric, gentisic, methane-sulfonic, ethanesulfonic, benzenesulfonic and laurylsulfonic acids as well as the salts of dobesilic acid (m.p.=70° C.) and para-toluenesulfonic acid (m.p.=51° C.), the purification of which proved to be difficult.

- ✓ La publicación internacional WO 99/65915 de título "Polymorphic Clopidogrel Hydrogenesulphate Form" y publicada el 23 de diciembre de 1999, revela formas polimorfas de la sal hidrogenosulfato de Clopidogrel designadas como formas 1 y 2, útiles como antitrombóticos.
- ✓ La publicación internacional WO 03/051362, titulada "Polymorphs of clopidogrel hidrogensulfate" y publicada el 26 de junio de 2003 revela formas cristalinas de la sal hidrogenosulfato de Clopidogrel designadas como formas III a IV.

En cuanto a la existencia de las sales 1,5-naftalenodisulfonato me permito citar la patente americana US 4,558,053 de título "Naphtalene-2,5-disulfonate salts of dimeric indole-dihydroindole alkaloids" y publicada en 10-12-1985, en donde se revela:

EXAMPLE 1

Preparation of vinzolidine 1,5-naphthalenedisulfonate (...)

Naphthalene-1,5-disulfonate salts were also prepared from vinblastine, vincristine and vindesine. The salts were stable, white solids sparingly soluble in water. (...)

We claim:

1. A 1,5-naphthalenedisulfonate salt of an indole-dihydroindole alkaloid of the formula

2. A compound according to claim 1, said compound being the 1,5-naphthalenedisulfonate salt of vinzolidine.

3. A compound according to claim 1, said compound being vincristine 1,5-naphthalenedisulfonate.

4. A compound according to claim 1, said compound being vinblastine 1,5-naphthalenedisulfonate.

5. A compound according to claim 1, said compound being vindesine 1,5-naphthalenedisulfonate.

6. A pharmaceutical formulation for oral administration in dosage unit form containing per dosage unit an oncolytic amount of vinzolidine 1,5-naphthalenedisulfonate plus an inert pharmaceutical excipient.

7. A polymorphic form of compound according to claim 2, said polymorphic form being vinzolidine 1,5-naphthalenedisulfonate Form II.

8. A polymorphic form of a compound according to claim 2, said polymorphic form being vinzolidine 1,5-naphthalenedisulfonate Form III.

Si bien es cierto no se refiere a clopidogrel, si lo hace para otro tipo de fármacos que se encuentran en forma de sales 1,5-naftalendisulfonato y en formas polimórficas, los cuales también tienen su aplicabilidad en farmacología (anticancerígenos) y demuestran sin más que este tipo de sales (formadas a partir del ácido 1,5-naftalendisulfónico) existen y pertenecen al estado del arte, y proveen compuestos estables.

Así mismo, es importante resaltar que la solicitud en trámite no resalta el efecto inesperado o sobresaliente de tales polimorfos dentro de una formulación con respecto a las formas ya existentes en el estado del arte, y si así lo hiciera, es del caso hacer notar que, para cualquier persona medianamente versada en la materia y aquella que se dedica a la fabricación de medicamentos, la etapa de preformulación es un estadio crítico del desarrollo de un medicamento ya que en esta etapa se definen las propiedades fisicoquímicas del fármaco frente a los excipientes y al proceso de fabricación, por tanto, el control o el conocimiento de la naturaleza cristalina del fármaco es fundamental ya que como se sabe de manera amplia en la literatura científica, los polimorfos suelen presentar diferencias en sus parámetros fisicoquímicos y, evidentemente, la elección de la forma cristalina o polimórfica deberá estar dada por la biodisponibilidad y por aquella que termodinámicamente sea más

estable para que ni durante las etapas del proceso ni durante el almacenamiento se den cambios no deseables.

- En adición a los argumentos anteriores, podemos decir que la obtención de dicha sal de clopidogrel, tampoco representa de ninguna manera un motivo suficiente como para solicitar protección ante la ley de patentes, ya que la formación de sales es un recurso que es ampliamente reconocido en la industria farmacéutica, y que se aplica sobre diversos compuestos, ya sea para modificar sus características de estabilidad durante el proceso de producción, de estabilidad en la formulación, de solubilidad, de biodisponibilidad o de cualquier otro tipo de consideraciones biofarmacéuticas que deban ser tenidas en cuentas para asegurar la efectividad y seguridad del medicamento.)

En otras palabras, la solicitud colombiana en trámite puede reducirse a la formulación de una sal de clopidogrel, para aplicarse en la prevención de eventos ateroscleróticos, argumento de la invención que también puede desvirtuarse si, como cualquier persona medianamente versada en la materia reconoce, debido a su actividad antiagregaria sobre las plaquetas (debido a su mecanismo de acción que corresponde a un bloqueo irreversible del receptor ADP en las membranas celulares de las plaquetas), el clopidogrel es utilizado y formulado con bastante anterioridad, para este y otros tipos de trastornos cardiovasculares relacionados: Prueba de esto es la amplia cantidad de información de carácter científico⁶ que demuestra la aplicación de clopidogrel en este tipo de trastorno, veamos una pequeña muestra:

❖ Clopidogrel Antiplatelet Activity Is Independent Of Age And Presence Of Atherosclerosis⁷.

Abstract: The pharmacodynamic and pharmacokinetic effects of clopidogrel 75 mg taken once daily in the morning before breakfast for 10 days were compared among three groups: 12 healthy young subjects, 10 healthy elderly subjects (>65 years) and 10 otherwise healthy elderly subjects with atherosclerosis, manifested by intermittent claudication. Platelet aggregation induced by 5 microM of ADP was measured in plasma samples taken at screening, 2 hours after dosing on day 1 to day 9; 2, 5, and 24 hours after dosing on day 10; and on alternate days until day 24. The inhibition of platelet aggregation was expressed as the percent reduction in maximum platelet

⁶ <http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer>

⁷ Denninger MH, Necciari J, Serre-Lacroix E, Sissmann J. Semin Thromb Hemost. 1999;25 Suppl 2:41-5.

aggregation with respect to baseline. The bleeding time was measured at screening, 5 hours after dosing on day 10, and on day 18. Plasma concentrations of SR26334, the main circulating metabolite of clopidogrel, were determined before dosing on day 1 to day 10 and at regular intervals over 72 hours after dosing on day 10. Inhibition of platelet aggregation appeared 2 hours after the first dose, became significant after the second dose, and progressed to a steady-state value of 55 to 57% by day 7 in the three groups, with no statistically significant difference between groups. A moderate, statistically significant prolongation of bleeding time of similar extent (prolongation factor of 1.5 to 1.6) was found on day 10 in the three groups. The pharmacodynamic parameters generally returned to baseline within 8 days after treatment. Based on AUC(0-24h) values, drug exposures were very similar for the two groups of elderly subjects but approximately twice that for the young group. The pharmacodynamic effects of clopidogrel were comparable in all three groups.

❖ Acute Antithrombotic Effect Of A Front-Loaded Regimen Of Clopidogrel In Patients With Atherosclerosis On Aspirin⁸.

Abstract: There is a need for a rapid antithrombotic effect after the administration of antiplatelet drugs in the setting of acute coronary syndromes and percutaneous interventions. Clopidogrel, a new thienopyridine derivative, is an efficient antiplatelet agent. However, the standard regimen of clopidogrel (75 mg/d) requires 2 to 3 days before significant antithrombotic effects. Patients with stable arterial disease on chronic aspirin therapy (n520) were treated with clopidogrel either with a front-loaded regimen, 300 mg the first day and 75 mg/d the next 7 days, or with a standard regimen, 75 mg/d for 8 days. Blood thrombogenicity was assessed by quantification of platelet-thrombus formation in an ex vivo perfusion chamber, by ADP-induced platelet aggregation, and by ADP-induced fibrinogen binding. At 2 hours, mean total thrombus area with the standard regimen was not significantly reduced. In contrast, at 2 hours, the mean total thrombus area with the front-loaded regimen was significantly decreased by 23.168.5% versus baseline ($P, 0.05$). ADP-induced platelet aggregation (with 5 and 10 mmol/L) was also significantly ($P, 0.05$) reduced with the front-loaded regimen at 2 hours, with the mean platelet aggregation being 82.264.4% and 81.864.5%, respectively, versus baseline. Similarly, flow cytometry demonstrated a significant decrease ($P, 0.05$) in the ADP-induced fibrinogen binding (with 0.12 and 0.6 mmol/L) at 2 hours in this front-loaded regimen group (36.162.0% and 53.269.3%). With the standard regimen, platelet activity was not significantly reduced at 2 hours. Our data suggest that a front-loaded regimen of clopidogrel added to aspirin achieves a significant antithrombotic effect at 2 hours in patients with known atherosclerotic disease on chronic aspirin therapy. This provides a rationale for using front-loaded clopidogrel in combination with aspirin in percutaneous coronary interventions.

❖ Clopidogrel: Hemorheological Effects In Subjects With Subclinical Atherosclerosis⁹.

⁸ Gérard Helft, Julio I. Osende, Stephen G. Worthley, Azfar G. Zaman, Oswaldo J. Rodriguez, Eli I. Lev, Michael E. Farkouh, Valentin Fuster, Juan J. Badimon and James H. Chesebro. *Arterioscler Thromb. Arterioscler Thromb Vasc Biol.* 2000;20:2316-2321.

⁹ Giovanni Ciuffetti¹, Rita Lombardini¹, Matteo Pirro¹, Graziana Lupattelli¹, Elmo Mandarino. *Journal Clinical Hemorheology and Microcirculation* Publisher IOS Press ISSN 1386-0291 (Print) 1875-8622 (Online) Issue Volume 25, Number 1/2001 Pages 31-39.

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Abstract: The usefulness of Clopidogrel as inhibitor of platelet aggregation has been demonstrated, but its effect on hemoreological parameters, such as whole blood viscosity at low and high shear rate, red cell aggregation and deformability indexes, filterability rate and deformability of red blood cells has not been studied. This study revealed that, in subjects with impaired blood rheology and ultrasound evidence of atherosclerosis, 3-weeks treatment with clopidogrel (75 mg daily) improved blood viscosity at high shear rate and other hemorheological parameters, including red cell filterability rates and dynamic red cell deformability index ($p < 0.01$ for all), without any unwanted side effect. Blood viscosity at low shear rate and red cell aggregation index improved after only 1-week treatment, and the reductions were maintained after two and three weeks ($p < 0.01$). These results indicate that, similarly to another thienopyridine such as ticlopidine, Clopidogrel may have a positive influence on several hemorheological parameters, thus exerting its protection not only through inhibition of platelet function, but also through changes in the hemorheological profile.

- Para terminar, la solicitud colombiana en trámite reivindica, específicamente en sus reivindicaciones 41 y 42, un método para inhibir la agregación plaquetaria que comprende la administración a un paciente necesitado de esta de una cantidad eficaz de uno de los compuestos descritos en reivindicaciones anteriores (polimorfos A, B, C, D, E y F de naftaleno-1,5-disulfonato de (+)-(S)- α -(2-clorofenil)-6,7-dihidrotieno[3,2-c]piridina-5(4H)-acetato de metilo o uno de sus hidratos y/o solvatos, para reducir los episodios ateroscleróticos; con lo cual, dicha solicitud, incurre directamente en una improcedencia de patentabilidad, ya que como lo expone claramente el literal d) del artículo 20 del capítulo 1 del título II "No serán patentables (...) d) los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales".

Así las cosas, y con fundamento en los argumentos expuestos a lo largo del presente escrito, respetuosamente reitero la solicitud para que su despacho se abstenga de conceder el privilegio de patente solicitado, y ordene en consecuencia el archivo del expediente respectivo.

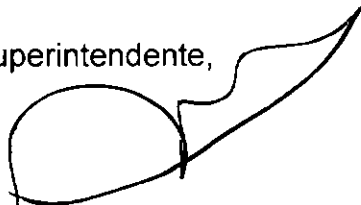
III. ANEXOS

- Abstracts de los artículos científicos mencionados en el acápite de Nuevos Argumentos.
- La patente US 4,529,596 de título "Thieno [3,2c] pyridine Derivatives and their therapeutic application" y publicación 16-06-1985. Documento completo.
- La patente americana US 4,847,265, titulada "Dextro-rotatory enantiomer of methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thieno pyridyl) (2-chlorophenyl)-" por 1 y 2

acetate and the pharmaceutical compositions containing it" y publicada el 11 de Julio de 1989. Portada.

- La publicación internacional WO 99/65915 de título "Polymorphic Clopidogrel Hydrogenesulphate Form" y publicada el 23 de diciembre de 1999. Portada.
- La publicación internacional WO 03/051362, titulada "Polymorphs of clopidogrel hidrogensulfate" y publicada el 26 de junio de 2003 .Portada. *oport L-2*
- La patente americana US 4,558,053 de título "Naphtalene-2,5-disulfonate salts of dimeric indole-dihydroindole añaloids" y publicada en 10-12-1985. Documento completo.
- Copia de los resultados arrojados por el meta-buscador de <http://www.sinab.unal.edu.co:2080/muse/servlet/MusePeer> relacionados a Clopidogrel y aterosclerosis.

Señor Superintendente,



JOSE LUIS REYES VILLAMIZAR

C.C. 79.152.473 de Usaquén

T.P. 44655 del C. S de la J.

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Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis.

Denninger MH, Necciari J, Serre-Lacroix E, Sissmann J.

Department of Hematology, Hôpital Beaujon, Clichy, France.

The pharmacodynamic and pharmacokinetic effects of clopidogrel 75 mg taken once daily in the morning before breakfast for 10 days were compared among three groups: 12 healthy young subjects, 10 healthy elderly subjects (>65 years) and 10 otherwise healthy elderly subjects with atherosclerosis, manifested by intermittent claudication. Platelet aggregation induced by 5 microM of ADP was measured in plasma samples taken at screening, 2 hours after dosing on day 1 to day 9; 2, 5, and 24 hours after dosing on day 10; and on alternate days until day 24. The inhibition of platelet aggregation was expressed as the percent reduction in maximum platelet aggregation with respect to baseline. The bleeding time was measured at screening, 5 hours after dosing on day 10, and on day 18. Plasma concentrations of SR26334, the main circulating metabolite of clopidogrel, were determined before dosing on day 1 to day 10 and at regular intervals over 72 hours after dosing on day 10. Inhibition of platelet aggregation appeared 2 hours after the first dose, became significant after the second dose, and progressed to a steady-state value of 55 to 57% by day 7 in the three groups, with no statistically significant difference between groups. A moderate, statistically significant prolongation of bleeding time of similar extent (prolongation factor of 1.5 to 1.6) was found on day 10 in the three groups. The pharmacodynamic parameters generally returned to baseline within 8 days after treatment. Based on AUC(0-24h) values, drug exposures were very similar for the two groups of elderly subjects but approximately twice that for the young group. The pharmacodynamic effects of clopidogrel were comparable in all three groups.

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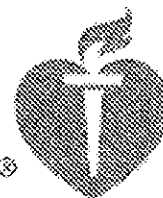
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Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on Aspirin

Gérard Helft, Julio I. Osende, Stephen G. Worthley, Azfar G. Zaman, Oswaldo J. Rodriguez, Eli I. Lev, Michael E. Farkouh, Valentin Fuster, Juan J. Badimon and
James H. Chesebro

Arterioscler. Thromb. Vasc. Biol. 2000;20:2316-2321

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Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on Aspirin

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Abstract—There is a need for a rapid antithrombotic effect after the administration of antiplatelet drugs in the setting of acute coronary syndromes and percutaneous interventions. Clopidogrel, a new thienopyridine derivative, is an efficient antiplatelet agent. However, the standard regimen of clopidogrel (75 mg/d) requires 2 to 3 days before significant antithrombotic effects. Patients with stable arterial disease on chronic aspirin therapy (n=20) were treated with clopidogrel either with a front-loaded regimen, 300 mg the first day and 75 mg/d the next 7 days, or with a standard regimen, 75 mg/d for 8 days. Blood thrombogenicity was assessed by quantification of platelet-thrombus formation in an ex vivo perfusion chamber, by ADP-induced platelet aggregation, and by ADP-induced fibrinogen binding. At 2 hours, mean total thrombus area with the standard regimen was not significantly reduced. In contrast, at 2 hours, the mean total thrombus area with the front-loaded regimen was significantly decreased by $23.1 \pm 8.5\%$ versus baseline ($P < 0.05$). ADP-induced platelet aggregation (with 5 and 10 $\mu\text{mol/L}$) was also significantly ($P < 0.05$) reduced with the front-loaded regimen at 2 hours, with the mean platelet aggregation being $82.2 \pm 4.4\%$ and $81.8 \pm 4.5\%$, respectively, versus baseline. Similarly, flow cytometry demonstrated a significant decrease ($P < 0.05$) in the ADP-induced fibrinogen binding (with 0.12 and 0.6 $\mu\text{mol/L}$) at 2 hours in this front-loaded regimen group ($36.1 \pm 2.0\%$ and $53.2 \pm 9.3\%$). With the standard regimen, platelet activity was not significantly reduced at 2 hours. Our data suggest that a front-loaded regimen of clopidogrel added to aspirin achieves a significant antithrombotic effect at 2 hours in patients with known atherosclerotic disease on chronic aspirin therapy. This provides a rationale for using front-loaded clopidogrel in combination with aspirin in percutaneous coronary interventions. (*Arterioscler Thromb Vasc Biol.* 2000;20:2316-2321.)

Key Words: platelet aggregation inhibitors ■ thrombus ■ atherosclerosis

The central role of platelets in arterial thrombosis is well established.¹ Platelet activity and thrombin and thromboxane generation are markedly increased after myocardial infarction, thrombolysis, unstable angina, and percutaneous coronary interventions.^{1,2} Numerous studies have shown that antiplatelet agents like aspirin or ticlopidine are effective for secondary prevention of coronary and cerebrovascular events.³ Combined antiplatelet therapy with aspirin and ticlopidine improves clinical outcome after stent implantation compared with aspirin alone or aspirin plus full anticoagulation.⁴⁻⁷ New antiplatelet drugs, including glycoprotein IIb/IIIa antagonists and clopidogrel, a new ADP-receptor antagonist, also reduce coronary events.^{8,9}

Aspirin inhibits cyclooxygenase, whereas clopidogrel inhibits the binding of ADP to its platelet receptor, which leads to reduced binding of fibrinogen to glycoprotein IIb/IIIa receptors.^{10,11} Given the different mechanisms of action and proven safety of these combined drugs, combined therapy appears logical and has already given encouraging results in nonrandomized comparisons in patients undergoing coronary stenting.¹²⁻¹⁵

There is a need for rapid efficacy after the administration of antiplatelet drugs in the setting of percutaneous interventions and acute coronary syndromes. However, the standard regimen of clopidogrel (75 mg/d) requires 2 to 3 days before significant effects occur in platelet aggregation.¹⁶

The major objective of this study was to investigate whether a front-loaded regimen of clopidogrel added to aspirin could achieve a significant antithrombotic activity 2 hours after its administration. Antithrombotic activity was assessed by evaluating (1) the quantitative growth of thrombus in a well-validated ex vivo perfusion chamber, (2) ADP-induced platelet aggregation, and (3) ADP-induced fibrinogen binding by flow cytometry in patients with atherosclerosis receiving chronic aspirin therapy.¹⁷

No clinical or experimental study has assessed, in patients with atherosclerosis, the acute antithrombotic effect of the combination of clopidogrel and aspirin (load and no-load) compared with aspirin alone. Therefore, the secondary objective was to compare the antithrombotic effect at day 8 of the standard regimen of clopidogrel versus the antithrombotic effect of

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baseline aspirin therapy alone in atherosclerotic patients on chronic aspirin therapy.

Methods

Patient Population

The study population included patients with stable coronary or peripheral arterial disease ($n=20$). All patients were receiving chronic aspirin therapy (325 mg/d) for ≥ 3 months. The presence of coronary artery disease was documented by coronary angiography, history of previous myocardial infarction, or typical angina pectoris with a positive stress test. The existence of peripheral disease was documented by lower-extremity claudication, previous peripheral angioplasty, bypass grafting, or $>50\%$ stenosis in any artery distal to the aortic bifurcation by angiography or ultrasound. Exclusion criteria included hemoglobin <12 g%, history suggestive of recurrent blood loss, or poorly controlled hypertension ($>180/100$ mm Hg). The study was approved by the Institutional Review Board, and all patients signed informed consent to participate.

The selected patients were randomized to 1 of 2 treatment groups in a double-blind, placebo-controlled design. The front-loaded regimen group received a loading dose of 300 mg (four 75-mg capsules) of clopidogrel on the first day, followed by 75 mg/d for the next 7 days. The standard regimen group received a loading dose of 3 identical placebo capsules plus a 75-mg clopidogrel capsule on the first day followed by 75 mg/d of clopidogrel for the next 7 days. All patients continued taking 325 mg/d of aspirin for the duration of the study. The CAPRIE Trial used a dose of 75 mg/d clopidogrel, which is approved by the FDA.⁸ The effects of the 2 treatments were studied at serial time points throughout the study and were compared with their respective pretreatment values. This design allowed each patient to serve as his or her own control. The investigators performing the perfusion and platelet studies, described further below, were blinded as to treatment assignment.

Quantification of Platelet-Thrombus Formation

Quantification of platelet-thrombus formation was assessed at baseline, after 2 hours, and on day 8. The effect of clopidogrel on ex vivo arterial thrombus formation was assessed by measuring the area of thrombus formation in a perfusion chamber. The description, validity, and reliability of the Badimon perfusion system to study thrombus formation on defined thrombogenic substrates under stable rheological conditions were reported previously.¹⁷⁻¹⁹ In brief, a 19-gauge needle was carefully inserted into an antecubital vein. After the first 5 mL of blood had been discarded, the needle was connected to the perfusion chamber. Native, nonanticoagulated blood was directly perfused from the antecubital vein to the perfusion chambers at a constant flow rate with a peristaltic pump (Masterflex, Cole-Parmer Instruments) distal to the chambers. The Plexiglas perfusion chamber consists of a cylindrical flow channel (1-mm diameter, 2.5-cm length) that allows the blood to flow over the thrombogenic substrate. All perfusions were performed for a period of 5 minutes at a flow rate of 10 mL/min (calculated shear rate of 1690/s; Reynolds number of 60; average blood velocity 21.2 cm/s). The selected dynamic conditions model the local rheology associated with mildly stenosed coronary arteries. Previous work has demonstrated that these rheological conditions result in consistent levels of platelet deposition.¹⁸

To mimic the in vivo situation of severe arterial injury, porcine aortic tunica media was used as the substrate to trigger thrombus formation. Segments of porcine aorta were cut into segments (2.8×0.8 cm) and surgically prepared to expose the deeper components of the arterial wall as previously described.¹⁷

The histological assessment of thrombus formation was performed by histomorphometry as previously described.¹⁷⁻¹⁹ In brief, after blood perfusion, the segments were removed from the chambers, fixed in 4% paraformaldehyde, and embedded in paraffin. Sections (5 μ m) were cut and stained with a combined Masson's trichrome-chalcin to visualize the total thrombus formed on the exposed substrates. Morphometric analysis of thrombus was conducted at 400-fold magnification. Images were digitized with a Sony DKC-5000 camera using Adobe Photoshop 4.0 software on a Power-Macintosh 8500 computer. The thrombus area was measured from

measured by computerized planimetry using Image Pro-plus software (Media Cybernetics). Results are given as the average of the analyzed sections ($n=6$) per chamber and expressed as the percentage of thrombus area compared with baseline. The measurements were performed by a single investigator blinded to the assigned therapy.

Platelet Aggregation

Platelet aggregation was performed at baseline, 2 hours, 6 hours, day 2, and day 8. Blood (16 mL) was drawn from an antecubital vein through a 19-gauge needle into 2 mL of 110 mmol/L trisodium citrate solution. Platelet-rich plasma and platelet-poor plasma were prepared by differential centrifugation. Platelet concentration in platelet-rich plasma was adjusted to $2.5 \times 10^9/L$ by the addition of platelet-poor plasma. ADP (Chronolog) at concentrations of 5 and 10 μ mol/L served as an agonist to trigger platelet aggregation. The aggregation response was recorded for 6 minutes after addition of the agonist in an aggregometer (Chrono-log model 440, Chrono-log Corp). Platelet aggregation was expressed as the mean percentage of maximum platelet aggregation compared with baseline.

Flow Cytometry

Platelet activation was assessed at baseline, 2 hours, day 2, and day 8. Samples were prepared within 1 hour of collection. FITC-conjugated chicken anti-fibrinogen antibody (10 μ L; Wak-Chemie Medical GmbH) was added to 3 tubes containing 230 μ L HEPES buffer or HEPES-EDTA buffer for the negative control.²⁰ Thereafter, 20 μ L platelet-rich plasma was added and incubated for 10 minutes at room temperature. Twenty microliters of a solution of ADP in HEPES buffer (0, 0.12, and 0.6 μ mol/L) was added to each sample tube, and 20 μ L of HEPES-EDTA buffer was added to the negative control. After exactly 10 minutes of incubation, 2 mL of ice-cold HEPES buffer was added to each tube. Samples were analyzed within the next 3 hours in a Becton-Dickinson flow cytometric analyzer. Activated platelets are expressed as a percentage, and the results are expressed as the relative fibrinogen binding compared with baseline.

Blood Samples

Blood was collected at baseline and day 8 for complete blood examination. Blood (4.5 mL) was also collected in SCAT tubes (Hematologic Technologies Inc) containing PPACK, EDTA, and aprotinin. Repeated blood samples for prothrombin fragment 1+2, a marker of thrombin generation, were obtained from the 20 patients at baseline, 2 hours, and day 8. Aliquots of plasma were stored at -70°C until analysis with ELISA (Enzygnost, Dade Behring).

Statistical Analysis

As already mentioned, the study design allowed each patient to serve as his or her own control (pretreatment versus posttreatment). Continuous variables were expressed as percentage of baseline $\pm$ SEM and compared by a paired Student's *t* test (within-group comparisons). Statistical significance was considered as a 2-tailed probability <0.05 .

Results

Study Population and Hematological Parameters

Twenty patients with stable coronary or peripheral arterial disease were studied; all of them completed the platelet reactivity data and 16 of them the perfusion chamber data. Baseline characteristics of these patients are summarized in the Table. No statistically significant differences were observed between the groups. In particular, use of antianginal and hypolipidemic drugs did not differ between groups.

There were no adverse events related to the medication. All patients received aspirin (325 mg/d) with the clopidogrel. In particular, no bleeding, gastrointestinal upset or rash was observed. Hematologic parameters, including hemoglobin, cell count, and platelet count were

Baseline Characteristics in Patients Randomized to Standard (n=10) Versus Front-Loaded (n=10) Regimen of Clopidogrel

	Standard Regimen	Front-Loaded Regimen
Age, y, mean $\pm$ SEM	55.6 $\pm$ 2.4	57.4 $\pm$ 2.5
Men, n	9	8
Coronary artery disease, n	8	8
Diabetes, n	3	3
Hypertension, n	3	4
Current smoking, n	0	1
Hypolipidemic drugs, n	6	5
β -Blocker	5	4
Ca-channel blocker	1	2
Long-acting nitrate	1	1

measured in EDTA-blood at baseline and at day 8 and did not change significantly.

Effects of Clopidogrel Treatments on Thrombus Area

The effects of the 2 regimens on thrombus formation were assessed at 2 time points (2 hours and day 8) and compared with their respective baseline (aspirin-treated) values (Figure 1). No significant difference was observed in the mean thrombus formation between the 2 groups at baseline, indicating the homogeneity of the study groups. At 2 hours, mean thrombus area with the standard regimen was decreased by $9.9 \pm 7.9\%$ but was not significantly different from baseline (aspirin alone, $P=NS$). In contrast, at 2 hours, the mean thrombus area in patients receiving the front-loaded regimen was $23.1 \pm 8.5\%$ less than the mean pretreatment value ($P<0.05$). As expected, the mean thrombus areas were significantly ($P<0.05$) reduced at day 8 in both groups compared with the baseline ($22.2 \pm 6.9\%$ with the standard regimen and $29.1 \pm 9.1\%$ with the front-loaded regimen).

Effects of Clopidogrel Treatments on Platelet Aggregation

Mean maximum percentages of ADP-induced platelet aggregation at baseline were comparable in both groups (Figure 2A

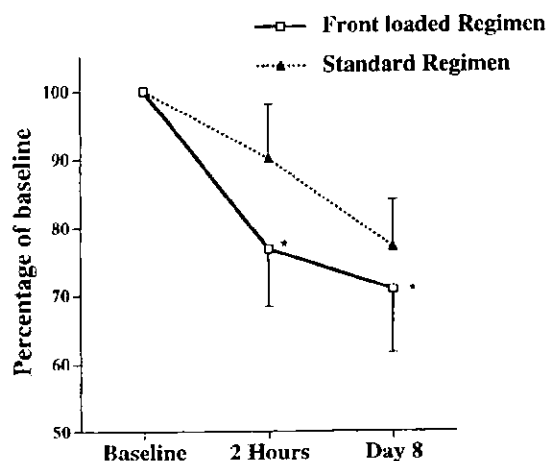


Figure 1. Effects of clopidogrel treatments on thrombus area. Results on thrombus formation at baseline, 2 hours, and day 8 with the respective treatments. Data are expressed as mean thrombus area $\pm$ SEM (μm^2). * $P<0.05$ vs baseline.

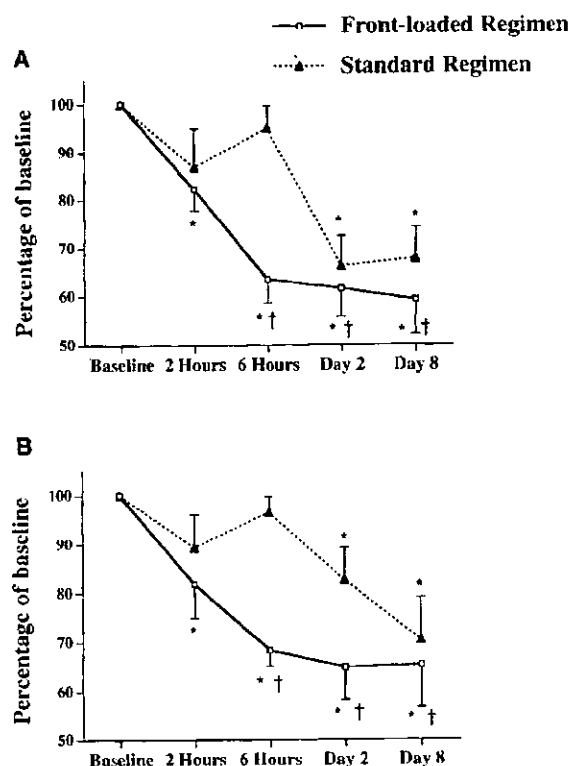


Figure 2. Effects of clopidogrel treatments on platelet aggregation to ADP 5 $\mu\text{mol/L}$ (A) and 10 $\mu\text{mol/L}$ (B). Results on maximal aggregation at baseline, 2 hours, 6 hours, day 2, and day 8 with the respective treatments. Data are expressed as mean percentage $\pm$ SEM. * $P<0.05$ vs baseline. † $P<0.05$ vs 2 hours.

and 2B). No significant inhibition of ADP-induced platelet aggregation was noted at 2 and 6 hours with ADP 5 and 10 $\mu\text{mol/L}$ with the standard regimen of clopidogrel (mean platelet aggregation: $86.9 \pm 8.0\%$, $95.0 \pm 4.7\%$ and $89.3 \pm 6.8\%$, $96.5 \pm 3.2\%$, respectively). At day 2, significant inhibition was achieved, the mean platelet aggregation with ADP 5 and 10 $\mu\text{mol/L}$ being $66.3 \pm 6.2\%$ and $82.6 \pm 6.7\%$, respectively.

In contrast, a significant inhibition of ADP-induced platelet aggregation was observed at 2 hours with the front-loaded regimen of clopidogrel. At this time, the mean platelet aggregation for ADP 5 and 10 $\mu\text{mol/L}$ was $82.2 \pm 4.4\%$ and $81.8 \pm 4.5\%$, respectively, compared with baseline (aspirin alone). Further significant inhibition ($P<0.05$) was observed at 6 hours, with a mean platelet aggregation of $63.5 \pm 4.9\%$ and $68.4 \pm 5.2\%$ for both concentrations of ADP used. At day 2 and day 8, no further significant inhibition of platelet aggregation was noted.

Effects of Clopidogrel Treatments on Fibrinogen Binding to ADP-Activated Platelets

There was no significant difference at baseline between the 2 groups (Figure 3A and 3B). With the front-loaded regimen, platelet activation, as determined by ADP-induced platelet-fibrinogen binding by flow cytometry, was significantly reduced ($P<0.05$) at 2 hours for ADP concentrations of 0.12 and 0.6 $\mu\text{mol/L}$, the fibrinogen binding being $36.1 \pm 2.0\%$ and $53.2 \pm 9.3\%$, respectively, compared with baseline. In contrast, fibrinogen binding with the standard regimen was not significantly reduced at 2

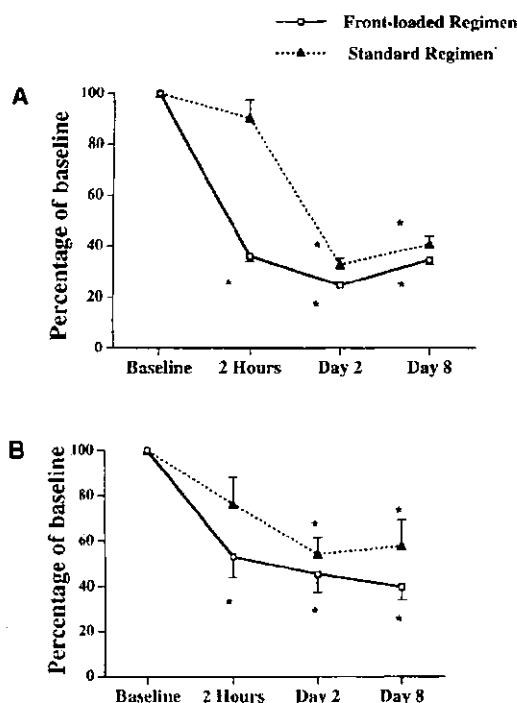


Figure 3. Effects of clopidogrel treatments on fibrinogen binding by flow cytometry induced by ADP 0.12 $\mu\text{mol/L}$ (A) and 0.6 $\mu\text{mol/L}$ (B). Results on activated platelets at baseline, 2 hours, day 2, and day 8 with the respective treatments. Data are expressed as the relative fibrinogen binding $\pm$ SEM compared with baseline. * $P < 0.05$ vs baseline.

hours in the standard regimen, the fibrinogen binding being $99.4 \pm 7.1\%$ and $74.6 \pm 11.8\%$, respectively.

Effect of Clopidogrel Treatments on Thrombin Generation

Baseline values of prothrombin fragment F1+2 plasma levels were similar in both groups at baseline on aspirin (0.79 ± 0.08 nmol/L with the front-loaded regimen and 0.91 ± 0.09 nmol/L with the standard regimen). These values remained unchanged when the patients were treated, at 2 hours and day 8 (0.89 ± 0.11 and 0.86 ± 0.06 nmol/L with the front-loaded regimen and 0.84 ± 0.09 and 0.86 ± 0.12 nmol/L with the standard regimen, respectively).

Discussion

We have shown that a front-loaded regimen of clopidogrel (300 mg) added to aspirin achieved a significant antithrombotic activity as early as 2 hours after its administration, as assessed by reduction in thrombus formation and platelet reactivity. We have also demonstrated that the antithrombotic effect at day 8 of a standard regimen of clopidogrel (75 mg/d) in patients with atherosclerosis receiving chronic aspirin therapy is greater than the antithrombotic effect of chronic aspirin therapy alone.

Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel

With a front-loaded regimen of clopidogrel (300 mg), it is possible to achieve a significant antithrombotic effect at 2 hours, an effect not obtained with the standard regimen, in patients with known atherosclerotic disease and daily receiving

aspirin. To the best of our knowledge, this is the first report of quantitative reduction in thrombus formation in a patient population with documented atherosclerotic disease receiving aspirin. Previous data in normal subjects have indicated that a dose of 300 to 400 mg produced a rapid onset of the pharmacodynamic activity of clopidogrel, with levels of platelet inhibition close to steady state observed within the first hours after dosing.²¹ Our findings confirm and extend these results in patients with atherosclerotic disease receiving chronic aspirin therapy. But more importantly, we have shown that the antithrombotic effect, assessed in a perfusion chamber mimicking an arterial injury atop a mild stenosis in a coronary artery, is achieved as early as 2 hours after front-loaded regimen administration. This provides evidence for the rapid onset of an antithrombotic effect with a loading dose (300 mg) of clopidogrel in the treatment of acute coronary syndromes and in coronary stent implantation. This is important because the median time from stent implantation to stent thrombosis in patients treated with ticlopidine and aspirin is ≈ 12 hours.²² To date, it appears that a standard regimen of ticlopidine or clopidogrel does not achieve a significant early antithrombotic effect.

We have also demonstrated that although a significant reduction in ADP-platelet aggregation is obtained with 300 mg of clopidogrel after 2 hours, a further significant reduction is achieved at 6 hours. This confirms recent findings showing that a loading regimen of clopidogrel induces maximal inhibition of platelet aggregation at 5 hours.²¹

The early antithrombotic effect on platelet activation we observed with a loading dose of clopidogrel, as assessed by flow cytometry, is consistent with the reduction in thrombus formation. The reduction in the fibrinogen binding obtained with a front-loaded regimen of clopidogrel is already significant at 2 hours, whereas with the standard regimen, a significant reduction was observed only at day 2. Similarly, an acceleration of platelet inhibition has been shown with a loading dose of a similar agent, ticlopidine (500 mg), in patients undergoing coronary angiography at 24 hours, but no data at 2 hours were provided.²³

Prothrombin fragment F1+2 plasma levels were similar throughout the study and between the groups. This is consistent with other published data^{24,25} showing that thrombin generation is not directly reduced with thienopyridines.

Antithrombotic Effect of Clopidogrel Administration in Atherosclerotic Patients Receiving Chronic Aspirin Therapy

We showed that the antithrombotic effect of treatment with clopidogrel for 8 days (75 mg/d) in patients with atherosclerosis receiving chronic aspirin therapy is greater than the antithrombotic effect of chronic aspirin therapy alone. In animal models, clopidogrel has antithrombotic effects in both arterial and venous thrombosis.²⁶⁻²⁹ In a porcine *ex vivo* model of high shear-induced stent thrombosis, intravenous clopidogrel produced a dose-dependent inhibition of stent thrombosis.³⁰ In nonhuman primates, clopidogrel reduced thrombosis in a baboon model of arterial thrombosis.³¹ To the best of our knowledge, only 1 study focused on the inhibition of thrombogenesis by clopidogrel in humans.²⁹ In this study, a standard regimen of clopidogrel significantly inhibited thrombus formation in healthy male subjects after 2 weeks of

a standard regimen of clopidogrel. We found that the inhibition was significant at day 8 in patients with atherosclerosis receiving chronic aspirin therapy when a standard regimen of clopidogrel was used.

Large-scale, randomized, placebo-controlled trials have clearly established that oral aspirin therapy decreases the risk of thrombotic events in patients with symptomatic atherosclerotic disease by 20% to 25%.³ Because aspirin is only a cyclooxygenase inhibitor,³² its effectiveness is limited, because it fails to block platelet activation by other important agonists such as shear stress, thrombin, collagen, and ADP. Clopidogrel is an ADP-receptor antagonist that is free of the hematological side effects of ticlopidine therapy.⁸ Clopidogrel acts by irreversibly inactivating platelet ADP receptor-initiated signaling in a dose-dependent manner.³³ In a large-scale, randomized clinical trial, clopidogrel was shown to be significantly more effective than and at least as safe as aspirin in decreasing arterial thrombo-occlusive episodes in patients with symptomatic atherosclerotic disease.⁸ However, aspirin and clopidogrel interfere with different pathways of platelet activation. Therefore, to improve the antithrombotic effect, the combination of these 2 drugs has been suggested. Additive antithrombotic effects of clopidogrel and aspirin have been demonstrated in experimental models in the rabbit.³⁴ Interestingly, clopidogrel and aspirin have produced additive antithrombotic effects as assessed by measurements of ¹¹¹In-labeled platelets and ¹²⁵I-labeled fibrin deposition in a porcine ex vivo model of high shear-induced stent thrombosis.²⁸ In our study, the combination of aspirin and clopidogrel significantly improved the antithrombotic effect of aspirin alone in patients receiving chronic aspirin therapy. The rheological conditions (1690/s) we used mimicked those typical of a mildly stenosed coronary artery. These rheological conditions were selected because ~70% of the plaques responsible for an acute myocardial infarction have <50% stenosis.³⁵

Limitations

Our data support the added benefit of combining clopidogrel with aspirin on thrombus formation without any bleeding complications. However, this is a pilot study, and large-scale clinical trials are needed to further clarify the efficacy and safety of the combination of aspirin and clopidogrel.

Conclusions

Our data suggest that a front-loaded regimen of clopidogrel 300 mg added to aspirin achieves a significant antithrombotic effect at 2 hours, an effect not achieved with the standard regimen, in patients with known atherosclerotic disease on chronic aspirin therapy. This supports the efficacy of a loading dose of clopidogrel in acute coronary syndromes and percutaneous coronary interventions.

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Clopidogrel: Hemorheological effects in subjects with subclinical atherosclerosis



Authors: Cluffeth G.; Lombardini R.; Pirro M.; Lupatelli G.; Maimarino E

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Abstract:

The usefulness of Clopidogrel as inhibitor of platelet aggregation has been demonstrated, but its effect on hemorheological parameters, such as whole blood viscosity at low and high shear rate, red cell aggregation and deformability indexes, filterability rate and and deformability of red blood cells has not been studied. This study revealed that, in subjects with impaired blood rheology and ultrasound evidence of atherosclerosis, 3-weeks treatment with clopidogrel (75 mg daily) improved blood viscosity at high shear rate and other hemorheological parameters, including red cell filterability rates and dynamic red cell deformability index ($p < 0.01$ for all), without any unwanted side effect. Blood viscosity at low shear rate and red cell aggregation index improved after only 1-week treatment, and the reductions were maintained after two and three weeks ($p < 0.01$). These results indicate that, similarly to another thienopyridine such as ticlopidine, Clopidogrel may have a positive influence on several hemorheological parameters, thus exerting its protection not only through inhibition of platelet function, but also through changes in the hemorheological profile.

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United States Patent [19]

Aubert et al.

[11] Patent Number: 4,529,596

[45] Date of Patent: Jul. 16, 1985

[54] THIENO [3,2-c] PYRIDINE DERIVATIVES AND THEIR THERAPEUTIC APPLICATION

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[22] Filed: Jul. 5, 1983

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[51] Int. Cl.³ A61K 31/435; C07D 495/04

[52] U.S. Cl. 514/231; 514/301;
544/127; 544/362; 546/114

[58] Field of Search 546/114; 544/362, 127;
424/248.51, 250, 256

[56] References Cited

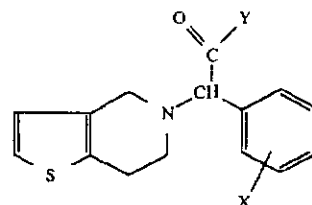
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Primary Examiner—Henry R. Jiles
Assistant Examiner—Bernard L. Dentz
Attorney, Agent, or Firm—Weiser & Stapler

[57] ABSTRACT

This invention relates to new thieno [3,2-c] pyridine derivatives having the formula:



in which:

Y represents the OH group or an OR group in which R is a straight or branched lower alkyl radical, or Y represents a group



in which R₁ and R₂ are each independently of each other, hydrogen or a straight or branched lower alkyl group; or R₁ and R₂ form together and with the nitrogen atom to which they are attached a heterocycle which may include a second heteroatom such as oxygen or nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may be substituted; and X represents hydrogen, a halogen or a lower alkyl radical; and their addition salts with pharmaceutically acceptable mineral or organic acids if Y represents the group OR or



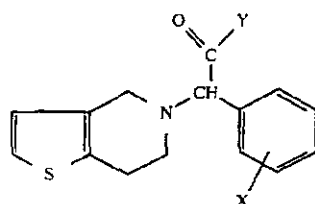
or with mineral bases if Y represents OH, as well as the two enantiomers or their mixture.

The invention also relates to the preparation process of new thieno [3,2-c] pyridine derivatives and to their therapeutic application as blood-platelet aggregation inhibiting agents and antithrombotics.

12 Claims, No Drawings

THIENO [3,2-c] PYRIDINE DERIVATIVES AND THEIR THERAPEUTIC APPLICATION

This invention relates to new thieno[3,2-c]pyridines, to a process for their preparation and to their therapeutic applications. The new derivatives of this invention have the following general formula:



in which:

Y represents the OH group or an OR group in which R is a straight or branched lower alkyl radical, or Y represents a group



in which R₁ and R₂ are each independently of each other hydrogen or a straight or branched lower alkyl group; or R₁ and R₂ form together and with the nitrogen to which they are attached a heterocycle which may include a second heteroatom such as oxygen or nitrogen, wherein the latter may be substituted by a lower alkyl or benzyl radical which may be substituted; and X represents hydrogen, a halogen or a lower alkyl radical.

These compounds having an asymmetrical carbon may exist in the form of two enantiomers. The invention relates both to each enantiomer and their mixture.

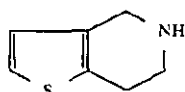
The invention also includes addition salts with pharmaceutically acceptable mineral or organic acids if Y represents the groups OR or



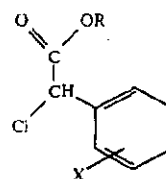
or with mineral bases if Y represents OH.

A lower alkyl radical is understood to mean a C₁-C₄ saturated hydrocarbon chain.

The invention also relates to a process for the preparation of compounds of the formula (I) as defined above, wherein the esters of the invention in which Y represents an OR group as defined above are prepared by condensation of 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine, having the following formula:



with an α-chlorophenyl acetate having the following formula



(III)

in which R and X have the above-defined meanings. Acids of formula (I), in which Y represents OH, are obtained by saponification.

To prepare the amides, in which Y represents a group



in which R₁ and R₂ have the above-defined meanings or even some esters of formula (I), the acid of formula (I)(R=OH) is reacted, after activation if required, either with the amine



or with the alcohol R-OH.

The α-halogenated esters of the formula (III) are prepared using known methods (E. L. ELIEL, M. T. FISK and T. PROSSER, Organic Syntheses, Coll. Vol. IV, J. WILEY and SONS, Inc. New York, 1963, p. 169).

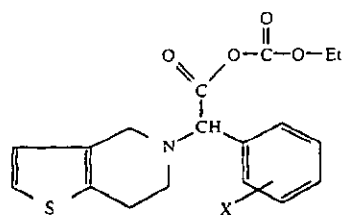
Although it is possible to obtain all the esters of formula (I) by the reaction between the compounds of formulae (II) and (III), for economic reasons it is preferable to prepare certain higher esters of the formula (I) from the acid of formula (I) and alcohol (R-OH).

The condensation of the tetrahydro-thieno-pyridine with the ester of formula (III) is effected in the presence of an alkali metal carbonate, such as potassium carbonate for example, in an inert solvent such as dimethylformamide, tetrahydrofuran or 1,2-dimethoxy ethane, at temperatures between 60° C. and the boiling point of the solvent.

The saponification of the ester of formula (I) in which R is methyl or ethyl is effected by an alkali metal hydroxide such as sodium or potassium hydroxide in a hydroalcoholic solvent, at temperatures between room temperature and the boiling point of the solvent.

Activation of the acid of formula (I) may be obtained by treatment with ethyl chloroformate in the presence of a slight excess of triethylamine at temperatures between -5° C. and 0° C. in an inert solvent such as chloroform, 1,2-dimethoxy ethane or tetrahydrofuran.

A mixed anhydride having the following formula:



is formed and if treated in situ with a slight excess either of alcohol or amine, at temperatures between 10° C. and room temperature, the esters or amides of the formula (I) will be formed respectively.

The activation of the acid of formula (I) may also be obtained in different ways: the amides of formula (I) were also thus prepared by condensing the acid (I) with the amine



in the presence of dicyclohexycarbodiimide in solution in 1,2-dichloro-ethane.

The esters of formula (I) may also be obtained in the conventional manner by condensing the corresponding acid and alcohol (ROH) in the presence of hydrochloric gas or thionyl chloride.

The following non-limiting Examples are given to illustrate the invention.

EXAMPLE 1

Methyl

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chlorophenylacetate ($R_1 = -CH_3$; $X = 2-Cl$) derivative No. 1.

3.47 g (0.144 mole) of methyl 2-chloro-o-chlorophenylacetate and 19.82 g (0.144 mole) of potassium carbonate are added to a 20 g (0.144 mole) solution of 4,5,6,7-tetrahydro thieno[3,2-c]pyridine in 200 ml of dimethylformamide. The solution is then heated for four hours at 90° C. The reaction mixture is cooled to room temperature, the mineral salts are filtered and the solvent is evaporated. The residue is taken up in water and then extracted with ethylic ether. The ether extracts are washed with water, dried over sodium sulphate and evaporated, to give a yellow oil which is purified by means of its hydrochloride. White crystals: M.p. = 130°-140° C. (ethylacetate, isopropanol). Yield: 45%.

EXAMPLE 2

Methyl

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]phenylacetate ($R_1 = -CH_3$; $X = H$) derivative No. 2.

This compound is prepared by the same procedure described in Example 1 by alkylation of the 4,5,6,7-tetrahydro thieno[3,2-c]pyridine with methyl 2-chlorophenylacetate. Hydrochloride; white crystals: softening point = 200° C. (ethanol). Yield: 50%.

EXAMPLE 3

Methyl

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-fluorophenylacetate ($R_1 = -CH_3$; $X = 2-F$) derivative No. 3.

This compound is prepared by the same procedure as described in Example 1 by alkylation of 4,5,6,7-tetrahydro thieno[3,2-c]pyridine with methyl 2-chloro-o-fluorophenylacetate. Hydrochloride; white crystals: M.p. = 100° C. Yield: 76.5%.

EXAMPLE 4

Ethyl

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-methylphenylacetate. ($R_1 = -CH_2-CH_3$; $X = 2-CH_3$) derivative No. 4.

This compound is prepared by the procedure described in Example 1 by alkylation of 4,5,6,7-tetrahydro thieno[3,2-c]pyridine with ethyl 2-chloro-o-methylphenylacetate. Bisulphate; white crystals: M.p. = 188°-190° C. (isopropanol). Yield: 54%.

EXAMPLE 5

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chlorophenylacetic acid. ($R_1 = H$; $X = 2-Cl$) derivative No. 5.

A mixture of 157.9 g of ethyl α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chlorophenylacetate and 100 ml of a 30% sodium hydroxide solution in 600 ml of ethanol is heated under reflux for 2 hours 30 minutes. After evaporation of the ethanol, the mixture is acidified with glacial acetic acid and extracted with methylene chloride. The organic phase is washed with water, dried over sodium sulphate and then evaporated. After recrystallization in water, the product is isolated in monohydrate form. White crystals; Softening point = 125° C. (water). Yield: 46%.

EXAMPLE 6

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetic acid. ($R_1 = H$; $X = H$) derivative No. 6.

This compound is prepared by the procedure described in Example 9 by saponification of ethyl α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-phenylacetate. The product is purified by means of its sodium salt.

White crystals: M.p. = 210°-215° C. (ethanol, methanol). Yield: 74%.

EXAMPLE 7

n-Propyl α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chlorophenylacetate. ($R_1 = -CH_2-CH_2-CH_3$; $X = 2-Cl$) derivative No. 7.

A stream of hydrochloric gas is bubbled through a 10 g (0.0306 mole) solution of α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chlorophenylacetic acid monohydrate (Example 5) for 12 hours in 100 ml of n-propanol which is refluxed. The mixture is evaporated and the residue is taken up in water, basified with sodium bicarbonate and extracted with ethylic ether. The ether extracts are washed with water, dried over sodium sulphate and evaporated to give a yellow oil which is purified by means of its bisulphate. White crystals: M.p. = 146° C. (crude) Yield: 78%.

EXAMPLE 8

n-Butyl

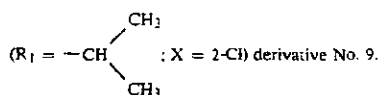
α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chloro-phenylacetate. ($R_1 = -CH_2-CH_2-CH_2-CH_3$; X = 2-Cl) derivative No. 8.

This compound is prepared by the procedure described in Example 5 by esterification of α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chloro-phenylacetic acid monohydrate (Example 5) with n-butanol. Purified using the bisulphate; white crystals; M.p. = 155° C. Yield: 79.5%.

EXAMPLE 9

Isopropyl

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chloro-phenylacetate.



2 ml of thionyl chloride are added dropwise to a 1 g (0.0031 mole) suspension of α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chloro-phenylacetic acid monohydrate (Example 5) in 20 ml of isopropanol cooled to -10° C. The reaction mixture is then refluxed for 6 hours. After evaporation, the residue is taken up in water, basified with sodium bicarbonate and extracted with methylene chloride. The organic phase is washed with water, dried over sodium sulphate and evaporated. A colourless resin is collected and purified using the bisulphate.

White crystals: softening point = 140°-150° C. Yield: 44%.

EXAMPLE 10

Ethyl

α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chloro-phenylacetate. ($R_1 = CH_2-CH_3$; X = 2-Cl) derivative No. 10.

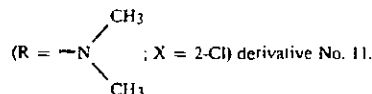
4.86 ml (0.051 mole) of ethylchloroformate are added dropwise to a 15 g (0.046 mole) solution of α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-phenylacetic acid monohydrate (Example 5) and 7.12 ml (0.051 mole) of triethylamine in 150 ml of chloroform, cooled to a temperature between -5° and 0° C. The mixture is then allowed to return to room temperature and is stirred for 30 minutes. The reaction mixture is then cooled to a temperature of around 10° C. and 30 ml of ethanol are added dropwise. The reaction mixture is stirred at room temperature for one night and is then washed with water. The organic phase, dried over sodium sulphate, is evaporated to give a colourless oil which is purified by means of its hydrobromide.

White crystals: M.p. = 180° C. Yield: 94%.

EXAMPLE 11

N,N-dimethyl

α -(4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl)-o-chloro-phenylacetamide.



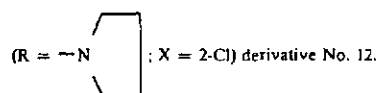
9.72 ml (0.102 mole) of ethyl chloroformate are added dropwise to a 30 g (0.092 mole) solution of α -(4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl)-o-chloro-phenylacetic acid monohydrate and 14.24 ml (0.102 mole) of triethylamine in 300 ml of chloroform, cooled to a temperature between -5° and 0° C. The mixture is then allowed to return to room temperature and is stirred for 30 minutes. The reaction mixture is then cooled to a temperature of around 10° C. and 4.57 ml (0.102 mole) of dimethylamine are added dropwise to 60 ml of chloroform and the mixture is stirred at room temperature for one night. Water is added, the mixture is decanted, and the organic phase is dried over sodium sulphate and evaporated. A colourless resin is collected, which then crystallizes.

White crystals: M.p. = 95°-100° C. (isopropyl ether).

Yield: 49%.

EXAMPLE 12

1-[(2-chloro-phenyl)-(4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl)-acetyl]-pyrrolidine.

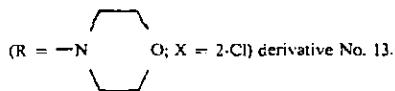


This compound is prepared by the procedure described in Example 11 by condensing α -(4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl)-o-chloro-phenylacetic acid monohydrate with pyrrolidine.

White crystals: M.p. = 130° C. (isopropyl ether). Yield: 61.5%.

EXAMPLE 13

1-[(2-chloro-phenyl)-(4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl)-acetyl]-morpholine.



2.67 g (0.031 mole) of morpholine are added to a 10 g (0.031 mole) solution of α -[4,5,6,7-tetrahydro-thieno[3,2-c]-5-pyridyl]-o-chloro-phenylacetic acid monohydrate and 13.3 g (0.064 mole) of dicyclohexylcarbodiimide in 100 ml of 1,2-dichloro ethane and the mixture is stirred at room temperature for one night. The mixture is evaporated then taken up with 2N hydrochloric acid and ethyl ether. After filtering the dicyclohexylurea formed, the filtrate is decanted and the aqueous phase is basified with 2N sodium hydroxide then extracted with methylene chloride. The organic

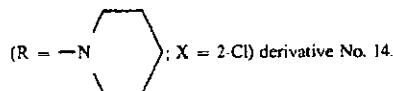
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phase is washed with water, dried over sodium sulphate and evaporated to give a yellow resin which is purified by means of its hydrochloride hemihydrate.

White crystals: M.p. = 215°-255° C. (isopropanol).
Yield: 71%

EXAMPLE 14

1-[(2-chloro
phenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-
acetyl]-piperidine.



This compound is prepared by the procedure described in Example 13 by condensing α -[4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl]-o-chloro-phenylacetic acid monohydrate with piperidine.

White crystals: M.p. = 139° C. (isopropanol).
Yield: 51.5%.

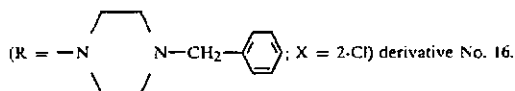
The following compounds were prepared by the process described in Example 11:

{ α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o-chlorophenylacetamide. (R = -NH₂; X = 2-Cl). derivative No. 15.

White crystals; M.p. = 126°-128° C. (isopropyl ether-isopropanol).

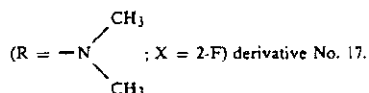
Yield: 46%.

4-benzyl-1-[(2-chloro-phenyl)-(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)acetyl]-piperazine.



oxalate: white crystals: M.p. = 178° C. (ethanol).
Yield: 82.5%.

N,N-dimethyl[α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.fluoro-phenylacetamide.



Slightly yellowish powder; M.p. = 125° C. (isopropyl ether-isopropanol).

Yield: 41%.

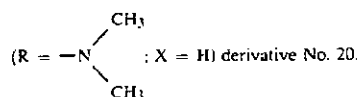
N-methyl-[α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chloro-phenylacetamide. (R = NH-CH₃; X = 2-Cl)—derivative No. 18.

White crystals: M.p. = 137° C. (isopropanol).
Yield: 85.5%.

N-butyl[α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.chloro-phenylacetamide. (R = -NH-(CH₂)₃-CH₃; X = 2-Cl)—derivative No. 19.

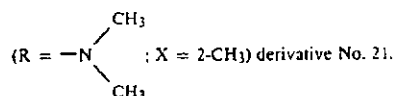
White crystals: M.p. = 101° C. (isopropyl ether).
Yield: 65%.

N,N-dimethyl[α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)phenylacetamide.



White crystals: M.p. = 138° C. (isopropyl ether).
Yield: 39%.

N,N-dimethyl[α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o.methyl-phenylacetamide.



White crystals: M.p. = 119° C. (hexane).
Yield: 15%

The results of pharmacological and toxicological tests reported below demonstrate the properties of the derivatives of the invention both in terms of toxicity and tolerance, and the activity levels of the derivatives, especially their inhibiting action on blood-platelet aggregation and anti-thrombotic activity.

Thus, the invention also relates to a therapeutic composition having in particular an inhibiting action on blood-platelet aggregation and anti-thrombotic action, wherein the active ingredient is a derivative of the formula (I) or an addition salt thereof with a pharmaceutically acceptable mineral or organic acid if Y represents the groups OR or



and with a mineral base if Y is OH.

TOXICOLOGICAL INVESTIGATION

The compounds of the invention exhibit an excellent tolerance and a low toxicity.

In addition, the tests carried out on acute, chronic, sub-chronic and delayed toxicity in various animal species showed no local or general reaction, no disturbances or abnormalities in the biochemical, macroscopic and microscopic examinations performed during this experiment.

PHARMACOLOGICAL INVESTIGATION

(1) Inhibiting action on blood-platelet aggregation

This experiment was performed on rats. The compound to be tested was administered orally in the form of a suspension in gum arabic to the rats over a 3-day period 48 hours, 24 hours and 2 hours before taking a 4 ml blood sample from the animals using Renaud's method from their jugular veins under anaesthetic. The citrated blood was used in the aggregation determinations.

(a) Determination of A.D.P.-induced blood-platelet aggregation.

2 ml of citrated blood were poured quickly into a small beaker placed in a magnetic stirrer, fitted with a magnetic bar. After a few seconds of agitation, 0.4 ml of a solution containing 0.66 ug of adenosine-diphosphate (ADP) per ml was placed in the beaker. After 90 sec-

onds of stirring, two 0.5 ml samples of blood were taken:

the first was mixed with 0.5 ml of an EDTA-formol solution

the second was mixed with 0.5 ml of a solution of EDTA only.

The purpose of adding EDTA formol is to stabilize the blood and thus to fix the aggregation whereas EDTA alone causes disaggregation of all the platelet clusters.

After leaving the mixtures at rest for 10 minutes and centrifuging the two mixtures at low speed for 5 minutes in order to separate the red corpuscles, the supernatant platelet-rich plasma (PRP) was removed, diluted and a platelet count was made.

The intensity of the aggregation was determined by the following ratio:

$$\frac{\text{number of platelets in EDTA-formol}}{\text{number of platelets in EDTA}} \times 100 = \text{percentage of non-aggregated platelets.}$$

The blood-platelet aggregation inhibiting action of the product tested improves as the ratio approaches 100.

The results showing the mean percentage of non-aggregated platelets in groups of 5 rats (treated and reference groups) are shown in Table I.

TABLE I

ADP TEST			
PRODUCT	DOSAGE mg/kg	ROUTE*	RESULT
Reference group	3 × 25	P.O.	16 ± 4
Derivative No. 1	—	—	94 ± 3
Reference group	3 × 5	—	20 ± 11
Derivative No. 1	—	—	82 ± 11
Reference group	3 × 2.5	—	23 ± 15
Derivative No. 1	—	—	56 ± 17
Reference group	—	—	8 ± 0
Derivative No. 10	3 × 25	—	66 ± 2
Derivative No. 10	3 × 12.5	—	49 ± 11
Reference group	3 × 10	—	8 ± 1
Derivative No. 10	—	—	24 ± 5
Reference group	3 × 60	—	11 ± 0
Derivative No. 9	—	—	65 ± 7
Reference group	3 × 100	—	13 ± 3
Derivative No. 4	—	—	89 ± 1
Reference group	3 × 100	—	3 ± 1
Derivative No. 4	—	—	89 ± 4
Reference group	3 × 100	—	4 ± 0
Derivative No. 2	—	—	72 ± 12
Reference group	3 × 100	—	4 ± 0
Derivative No. 12	—	—	27 ± 5
Reference group	—	—	2 ± 0
Derivative No. 17	3 × 100	—	11 ± 3
Derivative No. 20	3 × 100	—	4 ± 1
Reference group	3 × 100	—	18 ± 1
Derivative No. 6	—	—	22 ± 4

*Route of administration

(b) Determination of collagen-induced blood platelet aggregation

0.10 ml of a solution containing 10 ug of collagen per ml was added to 1.5 ml of citrated blood. With the mixture being agitated, the platelets were counted continually.

The reduction of the number of free platelets over time was continuously monitored and a curve was plotted showing the initial aggregation rate.

The results, representing mean values determined in each group of 5 rats (treated and reference groups), are shown in Table II.

TABLE II

COLLAGEN TEST			
PRODUCT	DOSAGE mg/kg	ROUTE*	RESULT
Reference group	3 × 25	P.O.	3.12 ± 0.47
Derivative No. 1	—	—	0.14 ± 0.03
Reference group	3 × 5	—	2.17 ± 0.64
Derivative No. 1	—	—	0.19 ± 0.04
Reference group	3 × 2.5	—	5.00 ± 1.02
Derivative No. 1	—	—	0.60 ± 0.20
Reference group	—	—	3.92 ± 0.63
Derivative No. 10	3 × 25	—	0.16 ± 0.07
Derivative No. 10	3 × 12.5	—	0.54 ± 0.12
Reference group	—	—	2.00 ± 0.35
Derivative No. 7	3 × 100	—	0.66 ± 0.18
Derivative No. 8	3 × 100	—	0.86 ± 0.18
Reference group	3 × 60	—	2.25 ± 0.32
Derivative No. 9	—	—	0.11 ± 0.01
Reference group	3 × 100	—	3.41 ± 0.55
Derivative No. 4	—	—	0.12 ± 0.02
Reference group	3 × 100	—	4.73 ± 0.55
Derivative No. 4	—	—	0.30 ± 0.02
Reference group	3 × 100	—	5.00 ± 1.06
Derivative No. 2	—	—	0.51 ± 0.18
Reference group	3 × 100	—	2.25 ± 0.32
Derivative No. 11	—	—	0.83 ± 0.02
Reference group	3 × 100	—	2.77 ± 0.32
Derivative No. 14	—	—	1.89 ± 0.13
Reference group	3 × 100	—	3.99 ± 0.40
Derivative No. 17	—	—	2.22 ± 0.10
Reference group	3 × 100	—	5.01 ± 0.79
Derivative No. 21	—	—	3.04 ± 0.22
Reference group	3 × 100	—	11.35 ± 1.01
Derivative No. 18	—	—	10.82 ± 0.81

*Route of administration

(c) Determination of bleeding time

The study of the blood-platelet aggregation inhibiting action also concerned the action of the compound of the invention on bleeding time.

The method used is an adaptation of the method of L. STELLA, M. B. DONATI and G. de GAETANO, *Thromb. Res.*, 1975, 7, 709-716.

The experiment was performed on rats. The compound to be tested was administered to the animals per os in suspension in 10 ml/kg of a 5% aqueous solution of gum arabic 65 hours, 41 hours and 17 hours prior to determination. After anesthetizing the animals with pentobarbital, their tails were cut 5 mm from the ends. The blood was carefully sponged at 15-second intervals, taking care not to touch the wound.

Hemostasis was reached when the bleeding stopped for one minute.

The results representing mean bleeding times in seconds, determined in each group of 5 rats (reference and treated groups), are given in Table III. Times longer than 1200 seconds (20 minutes) were not counted.

TABLE III

BLEEDING TIME			
PRODUCT	DOSAGE mg/kg	ROUTE*	RESULT
Reference group	—	P.O.	600
Derivative No. 10	3 × 200	—	1200
Derivative No. 2	3 × 200	—	1200
Reference group	—	—	420
Derivative No. 1	3 × 25	—	1200
Derivative No. 1	3 × 5	—	1080
Reference group	3 × 12.5	—	435
Derivative No. 1	—	—	1200
Reference group	3 × 200	—	780
Derivative No. 3	—	—	1200
Reference group	3 × 200	—	600
Derivative No. 18	—	—	1200
Reference group	3 × 200	—	600

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TABLE III-continued

PRODUCT	BLEEDING TIME		RESULT
	DOSAGE mg/kg	ROUTE*	
Derivative No. 12	—	—	1200

*Route of administration

(2) Anti-thrombotic activity

This activity was studied by the silk thread experimental thrombosis method.

The principle of this study is an adaptation of the experimental thrombosis method using cardiopulmonary by-pass described by TERUHIKO UMETSU and KAZUKO SANAI (THROMB. HAEMOST., 39, 1, 1978).

The animals' left jugular veins and right external carotid arteries were uncovered (the rats were anesthetized by intraperitoneal injection of pentobarbital).

The arteriovenous shunt consists of a central catheter and two lateral catheters; a white raw silk thread is inserted in the central part and circulation is restored for 20 minutes. After stopping circulation by clamping, the thread is gently withdrawn and weighed immediately. As the average weight of a wet silk thread was previously determined, the weight of the thrombus is determined by evaluation the difference.

The treatment is applied 48 hours, 24 hours and 2 hours before the start of blood flow in the shunt by oral administration of the compound to be tested in suspension in 10 ml/kg of 5% gum arabic, while only the 5% gum arabic solution is administered to the reference group.

The results showing the weight of the thrombus in mg are given in Table IV.

TABLE IV

PRODUCT	DOSAGE mg/kg	ROUTE*	ANTI-THROMBOTIC ACTIVITY	
			WEIGHT OF THROMBOSIS IN mg	PERCENTAGE VARIATION
Reference group	3 x 200	P.O.	38.56 ± 2.42	-22
Derivative No. 8	—	—	29.99 ± 3.05	—
Reference group	3 x 200	—	42.65 ± 3.30	-94
Derivative No. 3	—	—	2.60 ± 0.24	—
Reference group	—	—	36.24 ± 2.05	—
Derivative No. 1	3 x 25	—	6.56 ± 0.51	-82
Derivative No. 1	3 x 12.5	—	15.98 ± 1.81	-56
Reference group	3 x 5	—	40.86 ± 2.02	-32
Derivative No. 1	—	—	27.7 ± 2.82	—
Reference group	—	—	40.68 ± 1.74	—
Derivative No. 2	3 x 200	—	8.42 ± 3.28	-79
Derivative No. 10	3 x 200	—	5.89 ± 0.99	-86
Reference group	3 x 200	—	35.76 ± 1.76	-40
Derivative No. 11	—	—	21.38 ± 2.92	—

*Route of administration

The toxicological and pharmacological investigations reported above demonstrate the low toxicity of the compounds of the invention, as well as their excellent tolerance and their inhibiting properties on blood-platelet aggregation, and their anti-thrombotic activity, which make them very useful in medical therapeutic applications.

The drug of the invention may be orally administered in the form of tablets, coated tablets, capsules, drops, granules or syrup. The drug can also be provided in the form of suppositories for rectal administration or in the form of solution for injection for parenteral administration.

Each unit dose contains advantageously from 0.005 g to 0.250 g of a derivative of the invention, the daily dosage regimen varying within a range from 0.005 g to

1.00 g active ingredient according to the age of the patient and the severity of the disease to be treated.

Non-limiting examples of pharmaceutical formulations of the medicine of this invention are given below.

(1) Tablets

Derivative No. 1 . . . 0.050 g;

Excipient: lactose, icing sugar, rice starch, alginic acid, magnesium stearate.

(2) Coated tablets

Derivative No. 10 . . . 0.100 g

Excipient: magnesium stearate, corn starch, gum arabic, shellac, white sugar, glucose, white wax, carnauba wax, paraffin, new cochinealin.

(3) Capsules

Derivative No. 17 . . . 0.100 g

Excipient: magnesium stearate, corn starch, lactose

(4) Solution for injection

Derivative No. 4 . . . 0.075 g

isotonic solvent sufficient to make 3 ml

(5) Suppositories

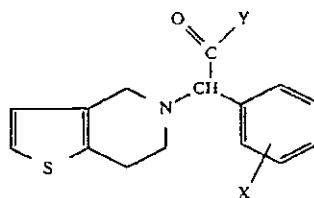
Derivative No. 21 . . . 0.100 g

Semi-synthetic triglycerides sufficient to make 1 suppository.

We claim:

1. A compound of the formula:

(I)



in which Y represents hydroxyl, an OR group wherein R is a straight or branched lower alkyl radical, or



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in which R₁ and R₂ are each independent of each other and represent hydrogen or a straight or branched lower alkyl group; or R₁ and R₂ form together with the nitrogen atom to which they are attached a heterocycle selected from the group consisting of pyrrolidino, piperidino, morpholino, piperazino, N-lower alkyl piperazino and N-benzyl piperazino; and X represents hydrogen, a halogen or a lower alkyl radical; and their addition salts with pharmaceutically acceptable mineral or organic acids if Y represents OR groups or



or with mineral bases if Y represents OH, including both enantiomeric forms or their mixture.

2. Methyl α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o-chlorophenyl-acetate.

3. Methyl α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)phenylacetate.

4. Methyl α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o-fluoro-phenylacetate.

5. Isopropyl α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o-chloro-phenylacetate.

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6. Ethyl α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o-chloro-phenylacetate.

7. N,N-dimethyl-[α -(4,5,6,7-tetrahydro-thieno(3,2-c)-5-pyridyl)-o-chlorophenylacetamide.

8. A therapeutic composition having blood-platelet aggregation inhibiting activities and anti-thrombotic activities containing an effective amount of a compound of claim 1, or an addition salt thereof with a pharmaceutically acceptable mineral or organic acid or with mineral bases, or one of the two enantiomers or their mixture and a pharmaceutically acceptable carrier.

9. The composition as claimed in claim 8, in unit dosage form.

10. The composition as claimed in claim 8 or 9 in unit dosage form, each unit containing from 0.10 g to 1.00 g active ingredient.

11. A method of inhibiting platelet aggregation in mammals which comprises administering an effective amount of a composition comprising a compound of claim 1 and a suitable pharmaceutically acceptable carrier.

12. A method of treating or inhibiting thrombosis in mammals which comprises administering to a mammal in need of treatment an effective amount of a composition comprising a compound of claim 1 and a suitable pharmaceutically acceptable carrier.

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United States Patent [19]

Badore et al.

[11] Patent Number: 4,847,265

[45] Date of Patent: Jul. 11, 1989

[54] DEXTRO-ROTATORY ENANTIOMER OF METHYL ALPHA-5 (4,5,6,7-TETRAHYDRO (3,2-C) THIENO PYRIDYL) (2-CHLOROPHENYL)-ACETATE AND THE PHARMACEUTICAL COMPOSITIONS CONTAINING IT

[75] Inventors: Alain Badore, Roquettes; Daniel Fréhel, Toulouse, both of France

[73] Assignee: Sanofi, France

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[52] U.S. Cl. 514/301; 546/114

[58] Field of Search 546/114; 514/301

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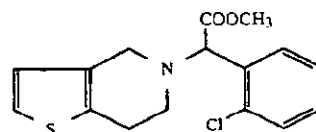
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[57] ABSTRACT

The present invention relates to the dextro-rotatory enantiomer of Formula



and its pharmaceutically acceptable salts with platelet aggregation inhibiting activity.

The invention also relates to a process for the preparation of this compound starting from the racemate and the pharmaceutical compositions containing it.

7 Claims, No Drawings

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(54) Title: POLYMORPHIC CLOPIDOGREL HYDROGENESULPHATE FORM (54) Titre: FORME POLYMORPHE DE L'HYDROGENOSULFATE DE CLOPIDOGREL (57) Abstract The invention relates to a novel polymorphic orthorhombic hydrogenosulphate or (+)-(S)-α-(2-chlorophenyl)-4,5,6,7-tetrahydrothieno [3,2-c] pyridinyl-5-methyl acetate hydrogenosulphate form and to a method for the production thereof. (57) Abrégé Nouveau polymorphe orthorhombique de l'hydrogénosulfate de clopidogrel ou hydrogénosulfate de (+)-(S)-α-(2-chlorophényl)-4,5,6,7-tétrahydrothiéno [3,2-c] pyridinyl-5-acétate de méthyle et un procédé pour sa préparation.		

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ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: POLYMORPHS OF CLOPIDOGREL HYDROGENSULFATE

(57) Abstract: Provided are new crystalline Forms III, IV, V and VI of clopidogrel hydrogensulfate and the amorphous form of clopidogrel hydrogensulfate, as well as their pharmaceutical compositions, and method of treatments with such compositions. Also provided are novel processes for preparation of clopidogrel hydrogensulfate Form I, Form II, Form III, Form IV, Form V, Form VI and amorphous form.

WO 03/051362 A2



United States Patent [19]
Rolski et al.

[11] **Patent Number:** **4,558,053**
[45] **Date of Patent:** **Dec. 10, 1985**

[54] **NAPHTHALENE-1,5-DISULFONATE SALTS
OF DIMERIC INDOLE-DIHYDROINDOLE
ALKALOIDS**

[75] **Inventors:** **Stanislaw Rolski; Ralph R. Pfeiffer,**
both of Indianapolis, Ind.

[73] **Assignee:** **Eli Lilly and Company, Indianapolis,**
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[51] **Int. Cl.⁴** **A61K 31/475; C07D 519/04**

[52] **U.S. Cl.** **514/283; 260/244.4**

[58] **Field of Search** **260/244.4; 424/262;**
514/283

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[57] **ABSTRACT**

Stable, biologically available, orally active 1,5-naphthalenedisulfonate salt of vinzolidine.

8 Claims, No Drawings

EXAMPLE 2

Alternate Preparation of Vinzolidine 1,5-Naphthalene Disulfonate in Three Steps

a. Conversion of vinzolidine free base to vinzolidine sulfate

To a 1% solution of free base in anhydrous ethanol, a stoichiometric amount of 1.0N sulfuric acid is added in dropwise fashion with stirring [1.0 g. of free base (~93% purity) requires ~2 ml. of 1.0N sulfuric acid]. Stirring is continued until some opalescence appears. The solution is allowed to stand at room temperature for two additional hours followed by 2-3 hours at about 0° C. The precipitate is then filtered on a sintered glass funnel, washed with a small amount of anhydrous ethanol and dried in vacuo at 45°; yield = ~85%. Additional salt (ca. 10%) can be obtained from the filtrate.

This sulfate salt contains ca. 9% of water (possibly in the form of a pentahydrate). A sharp X-ray pattern was obtained.

b. Preparation of amorphous vinzolidine 1,5-naphthalenedisulfonate

A 10% aqueous solution of 1,5-naphthalenedisulfonic acid disodium salt is added dropwise with stirring to a 2% aqueous solution of vinzolidine sulfate. A 10% excess of 1,5-naphthalenedisulfonic acid disodium salt is used; i.e., 1.1 mole of acid per mole of base. After holding the mixture at about 0° C. overnight, the precipitate is filtered on a sintered glass funnel, washed with a small amount of water, and dried in vacuo overnight at room temperature. An amorphous material is obtained that contains ~7% of H₂O (yield = >80 percent).

c. Crystallization of amorphous material

The amorphous material is added gradually to methanol with stirring (1 g. per 100 ml. of methanol). After the addition is complete, the suspension is stored in the refrigerator overnight. Subsequent handling is the same as in Example 1. The salt crystallizes as a methanolate (Form I) by both methods and contains three molecules of methanol per molecule of salt.

Vacuum drying removes the methanol but equilibration of the desired salt in air gives a hydrate (Form II); Karl Fisher ~8% water. Form II dissolves to the extent of about 150 mcg./ml. in water at 25° C.

A third crystalline form, Form III, is produced by recrystallizing the salt in boiling methanol and then drying the methanolate in air, which procedure yields a different hydrate containing about 6% water. The X-ray diffraction patterns of these three forms follow. The patterns were obtained using an X-ray tube with a copper target and a nickel filter.

FORM I		
d	I/I ₁	
12.15	.80	
10.88	.16	
9.80	1.00	
8.14	.16	
7.58	.28	
7.24	.20	
6.52	.64	
6.18	.16	
5.90	.04	
5.55	.16	
4.89	.44	
4.57	.36	

-continued

4.42	.44
4.17	.40
3.97	.32
3.76	.64
3.56	.40
3.34	.44
3.26	.24
3.08	.04
2.95	.04
2.89	.04
2.77	.04
2.67	.04
2.54	.04
2.46	.04
2.40	.04
2.28	.04
2.22	.04
2.15	.04
2.08	.06
FORM II	
12.65	.14
10.54	.48
9.47	1.00
7.98	.07
7.32	.17
7.15	.59
6.69	.21
6.11	.17
5.44	.41
5.14	.31
4.71	.52
4.50	.10
4.36	.28
4.16	.14
4.07	.14
3.92	.07
3.78	.14
3.57	.07
3.47	.28
3.34	.17
3.23	.07
3.13	.07
3.03	.03
2.91	.03
2.79	.03
2.68	.03
2.60	.02
2.49	.03
2.38	.02
2.32	.02
2.25	.03
2.22	.02
2.17	.02
2.12	.02
FORM III	
10.85	.55
10.16	.05
9.26	1.00
7.41	.05
6.76	.55
6.28	.80
5.68	.20
5.40	.20
5.07	.10
4.75	.40
4.33	.30
4.20	.15
4.00	.20
3.75	.05
3.42	.15
3.30	.05
3.15	.08
3.02	.05
2.92	.08
2.82	.05
2.69	.05

Naphthalene-1,5-disulfonate salts were also prepared from vinblastine, vincristine and vindesine. The salts were stable, white solids sparingly soluble in water.

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NAPHTHALENE-1,5-DISULFONATE SALTS OF DIMERIC INDOLE-DIHYDROINDOLE ALKALOIDS

BACKGROUND OF THE INVENTION

A number of indole-dihydroindole alkaloids are currently either being marketed or are or have been on clinical trial as anticancer agents. These alkaloids include vincalucoblastine (vinblastine, VLB)—marketed as VELBAN, U.S. Pat. No. 3,097,137; vincristine (VCR, leurocristine)—marketed as ONCOVIN, U.S. Pat. No. 3,205,220; vindesine (VDS)—marketed as ELDISINE, U.S. Pat. No. 4,191,688; vinzolidine, U.S. Pat. No. Re. 30,560; and vinepidine, U.S. Pat. No. 4,143,041. A large number of acid addition salts of these drugs are disclosed, including the naphthalene-1-sulfonate and the naphthalene-2-sulfonate.

In general, the sulfate salts of the above dimeric indole-dihydroindole alkaloids are favored during isolation, synthesis, formulation, etc. The drugs are customarily administered parenterally by the iv route and any pharmaceutically-acceptable soluble salt, such as the sulfate, which can provide the required concentration of drug, can be used. Sulfate salts of VLB and vincristine are prepared by neutralization of the base with dilute sulfuric acid (ethanol for example or other inert polar solvent may also be present to solubilize the base) followed by lyophilization. With these alkaloids, the lyophilized salt had adequate stability, but with vindesine, the lyophilized salt was not stable. Rolski, U.S. Pat. No. 4,259,242 addresses this problem and provides a method of preparing a stable monosulfate salt of vindesine by precipitation during neutralization of vindesine base with sulfuric acid in acetonitrile solution.

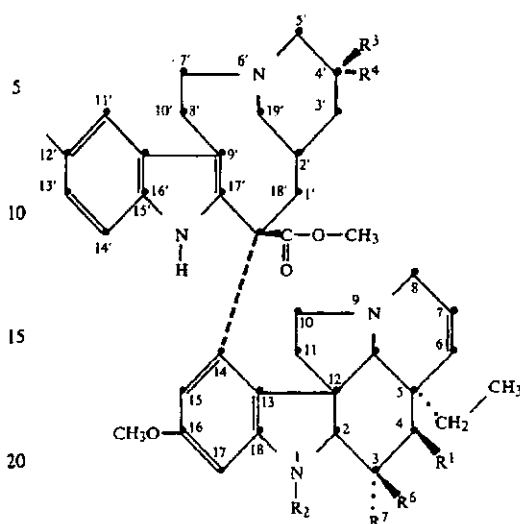
Vinzolidine is another indole-dihydroindole alkaloid whose sulfate salt has been found to be unstable. In contrast to the other alkaloids, vinzolidine is an orally active drug and must be capable of being provided in solid formulations such as capsules and tablets. While an injectable product like vindesine or vincristine can be lyophilized and the lyophilized product sealed in a dry, inert atmosphere, capsules and tablets are constantly exposed to air and water vapor.

Vinzolidine sulfate was prepared in eight different crystalline forms by employing different recrystallization solvents. While the stability of these forms varied considerably, none was found to be sufficiently stable to meet the stability requirements of an oral dosage form.

It is an object of this invention to prepare a vinzolidine salt which is sufficiently stable for use in oral dosage forms and which furnishes adequate vinzolidine blood levels on ingestion.

SUMMARY OF THE INVENTION

In fulfillment of the above and other objects, this invention provides 1,5-naphthalene disulfonate salts of indole-dihydroindole alkaloids of the formula



wherein is OH or O—CO—CH₃, R² is CH₃ or CHO; one of R³ and R⁴ is ethyl and the other is OH; when taken singly, R⁶ is OH and R⁷ is CO—OCH₃ or CO—R⁹, wherein R⁹ is NH₂, NH—C₁₋₃ alkyl-X, wherein X is hydrogen, chloro or bromo, or C₁₋₃ lower-alkanoyloxy; when R⁶ and R⁷ are taken together with the carbon atoms to which they are attached, they form a spiro-oxazolidinedione ring permissibly substituted at N₃'' with C₁₋₃ alkyl or chloro-substituted C₁₋₃ alkyl.

In the above formula, where R¹ is acetoxy, R² is methyl, R⁶ is OH, R⁷ is CO—OH₃, R³ is hydroxyl and R⁴ is ethyl, VLB (vincalucoblastine, vinblastine) is represented; where R¹ is acetoxy, R² is formyl, R⁶ is OH, R⁷ is CO—OCH₃, R³ is hydroxyl and R⁴ is ethyl, vincristine (leurocristine, VCR) is represented; where R¹ is acetoxy, R² is methyl, R⁶ is OH, R⁷ is CO—OCH₃, R³ is ethyl and R⁴ is hydroxyl, leurosidine (vinrosidine) is represented; where R⁶ and R⁷, together with the carbon to which they are attached, form an oxazolidindione ring with a chloroethyl group at N₃'', R² is CH₃, R¹ is acetoxy, R³ is OH and R⁴ is ethyl, vinzolidine is represented; and when R² is CH₃, R³, R⁶ and R¹ are OH, R⁴ is ethyl, R⁷ is COR⁹ and R⁹ is NH₂, vindesine is represented.

The preparation of vinzolidine 1,5-naphthalene disulfonate is illustrated below.

EXAMPLE I

Preparation of vinzolidine 1,5-naphthalenedisulfonate

To 1% (w/v) solution of vinzolidine free base (95+ % purity) in methanol is added dropwise with stirring a stoichiometric amount (1 mole per mole) of a 2% methanolic solution of 1,5-naphthalenedisulfonic acid. The resulting solution was allowed to stand at ambient temperature for about two hours, during which time period crystallization of the vinzolidine 1,5-naphthalenedisulfonate salt begins. The crystallizing solution is kept at about 0° C. overnight and is then filtered. The filter cake is washed with a small amount of methanol and then dried in vacuo overnight at ambient temperature. The salt is then allowed to stand in air for about two hours. The yield of vinzolidine 1,5-naphthalenedisulfonate is 95-97%.

One of the above salts, the orally active vinzolidine 1,5-naphthalenedisulfonate, (vinzolidine napadisilate) is of particular interest since it is approximately bioequivalent to vinzolidine sulfate.

Dosage forms suitable for oral administration have more stringent requirements than do parenteral dosage forms; i.e., the drug must be stable when mixed with pharmaceutically-acceptable excipients customarily used in preparing solid dosage forms such as capsules or tablets. Any such modification of drug form must not substantially increase toxicity nor decrease oral efficacy.

The ability of vinzolidine 1,5-naphthalenedisulfonate to meet these requirements as an oral drug are set forth below.

In the first place, as regards stability, bulk vinzolidine 1,5-naphthalene disulfonate remained virtually unchanged at one year at the following storage temperatures—5° C.; 25° C.; 40° C. In addition, the bulk material was stable for one year at 40° C. and 75% relative humidity. Vinzolidine 1,5-naphthalene disulfonate-containing capsules had a potency of 94.3% of the initial potency after storage for one year at 40° C. and 75% relative humidity. Under the same storage conditions, both bulk and encapsulated vinzolidine sulfate were clearly less stable.

Secondly, as regards toxicity, the LD₅₀ in mice of vinzolidine 1,5-naphthalenedisulfonate is 68–7 mg./kg. compared to 37.7 mg./kg. for vinzolidine sulfate.

Thirdly, as regards oncolytic activity, the 1,5-disulfonate salt shows good antitumor activity against transplanted tumors in mice. For example, using the 1,5-naphthalenedisulfonate salt, against the 6C3HED lymphosarcoma, a single oral dose of 11 mg./kg. (in either acacia or emulphor suspension) gives about a 50% inhibition of tumor growth and single doses of 15–22.5 mg./kg. give 95–100% inhibition.

Finally, vinzolidine 1,5-naphthalenedisulfonate is well-absorbed when administered by the oral route. For example, when each of six monkeys was given a single capsule containing a 1.5 mg./kg. dose of vinzolidine base equivalent or about 2 mg./kg. of vinzolidine 1,5-naphthalenedisulfonate, excellent blood levels of vinzolidine were obtained. Table I which follows gives pooled mean serum concentrations of the drug in mg./ml for the six monkeys at given time intervals with the standard deviation.

TABLE I

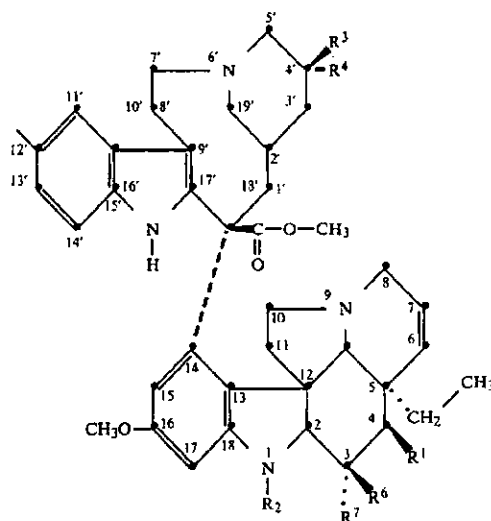
Time in Hours	Mean Serum Concentration Mg./Ml. ± SD
1/12	0
1	0.46 ± 0.28
1	29.38 ± 7.80
4	19.53 ± 3.25
8	5.32 ± 0.93
24	0.66 ± 0.28
48	0.05 ± 0.04
72	0
96	0

The above serum concentrations are comparable to those obtained with vinzolidine sulfate orally.

Oral formulations of vinzolidine 1,5-naphthalenedisulfonate include telescoping gelatin capsules, tablets, suspensions and the like such that each capsule, tablet or unit of suspension (teaspoon) contains a single dosage unit of the drug. Such dosage unit will contain about 30 mgs. of vinzolidine base as the 1,5-naphthalenedisulfonate salt. 1,5-Naphthalenedisulfonate salts of other indole-dihydroindole alkaloids represented by I above can be formulated similarly.

We claim:

1. A 1,5-naphthalenedisulfonate salt of an indole-dihydroindole alkaloid of the formula



wherein R¹ is OH or O—CO—CH₃, R² is CH₃ or CHO; one of R³ and R⁴ is ethyl and the other is OH; when taken singly, R⁶ is OH and R⁷ is CO—OCH₃ or CO—R⁹, wherein R⁹ is NH₂, NH—C₁₋₃ alkyl-X, wherein X is hydrogen, chloro or bromo, or C₁₋₃ lower-alkanoxyloxy; when R⁶ and R⁷ are taken together with the carbon atoms to which they are attached, they form a spiro-oxazolidinedione ring permissibly substituted at N₃'' with C₁₋₃ alkyl or chloro-substituted C₁₋₃ alkyl.

2. A compound according to claim 1, said compound being the 1,5-naphthalenedisulfonate salt of vinzolidine.

3. A compound according to claim 1, said compound being vincristine 1,5-naphthalenedisulfonate.

4. A compound according to claim 1, said compound being vinblastine 1,5-naphthalenedisulfonate.

5. A compound according to claim 1, said compound being vindesine 1,5-naphthalenedisulfonate.

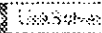
6. A pharmaceutical formulation for oral administration in dosage unit form containing per dosage unit an oncolytic amount of vinzolidine 1,5-naphthalenedisulfonate plus an inert pharmaceutical excipient.

7. A polymorphic form of compound according to claim 2, said polymorphic form being vinzolidine 1,5-naphthalenedisulfonate Form II.

8. A polymorphic form of a compound according to claim 2, said polymorphic form being vinzolidine 1,5-naphthalenedisulfonate Form III.

* * * * *

Lista de resultados combinados | PubMed (6) | EBM Reviews, Cochrane Database (0) | Evidence Based Medicine Review - EBMR (6) | Medline - OVID (6) | OVID - Journals@Ovid (1) | Google Scholar (14) | FindArticles (Free Articles Only) (0) | MEDLINE with Full Text (6)

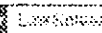
☐ 1Registro Completo  

Título: Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis.

Autores: MH Denninger; J Necciari; E Serre-Lacroix.

Fuente: Semin Thromb Hemost, 1999

En: Google Scholar

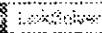
☐ 2Registro Completo  

Título: Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis.

Autores: Denninger MH; Necciari J; Serre-Lacroix E; Sissmann J

Fuente: Seminars in Thrombosis & Hemostasis. 25 Suppl 2:41-5, 1999.

En: Medline - OVID

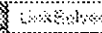
☐ 3Registro Completo  

Título: ... Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on ...

Autores: G Helft; JI Osende; SG Worthley; AG Zaman; OJ

Fuente: Arteriosclerosis, Thrombosis, and Vascular Biology, 2000

En: Google Scholar

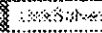
☐ 4Registro Completo  

Título: Complementary, additive benefit of clopidogrel and lipid-lowering therapy in patients with atherosclerosis Journal of the American College of Cardiology. 35(Suppl A):326A, 2000

Autores: Bhatt DL, Feody JM, Hirsch AT, Ringleb P, Hacke W, Topol EJ

Fuente: Journal of the American College of Cardiology. 35(Suppl A):326A, 2000. AN: CN-00371090 Complete Reference

En: Evidence Based Medicine Review - EBMR

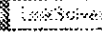
☐ 5Registro Completo  

Título: Acute Antithrombotic Effect of a Front-Loaded Regimen of Clopidogrel in Patients With Atherosclerosis on Aspirin

Autores: Helft, Gerard; Osende, Julio I; Worthley, Stephen G; Zaman, Azfar G; Rodriguez, Oswaldo J; Lev, Eli I; Farkouh, Michael E; Fuster, Valentin; Badimon, Juan J; Chesebro, James H

Fuente: Arteriosclerosis, Thrombosis & Vascular Biology. 20(10):2316-2321, October 2000. AbstractComplete ReferenceTable of Contents

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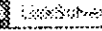
☐ 6Registro Completo  

Título: Acute antithrombotic effect of a front-loaded regimen of clopidogrel in patients with atherosclerosis on aspirin.

Autores: Helft G, Osende JI, Worthley SG, Zaman AG, Rodriguez OJ, Lev EI, Farkouh ME, Fuster V, Badimon JJ, Chesebro JH

Fuente: Arteriosclerosis, thrombosis, and vascular biology. 20(10):2316-21, 2000 Oct. AN: CN-00326948 AbstractComplete Reference

En: Evidence Based Medicine Review - EBMR

☐ 7Registro Completo  

Título: Acute antithrombotic effect of a front-loaded regimen of clopidogrel in patients with atherosclerosis on aspirin.

Autores: Helft G; Osende JI; Worthley SG; Zaman AG; Rodriguez OJ; Lev EI; Farkouh ME; Fuster V; Badimon JJ; Chesebro JH

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Fuente: Arteriosclerosis, Thrombosis & Vascular Biology. 20(10):2316-21, 2000 Oct.

En: Medline - OVID

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Registro Completo  

Título: Clopidogrel: hemorheological effects in subjects with subclinical atherosclerosis.

Autores: Ciuffetti G, Lombardini R, Pirro M, Lupattelli G, Mannarino E

Fuente: Clinical hemorheology and microcirculation. 25(1):31-9, 2001. AN: CN-00377053 AbstractComplete Reference

En: Evidence Based Medicine Review - EBMR

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Registro Completo  

Título: Clopidogrel: Hemorheological effects in subjects with subclinical atherosclerosis

Autores: G Ciuffetti

Fuente: Clinical Hemorheology and Microcirculation, 2001

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Título: Clopidogrel: hemorheological effects in subjects with subclinical atherosclerosis.

Autores: Ciuffetti G; Lombardini R; Pirro M; Lupattelli G; Mannarino E

Fuente: Clinical Hemorheology & Microcirculation. 25(1):31-9, 2001.

En: Medline - OVID

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Registro Completo  

Título: [Effect of therapy with clopidogrel on walking tolerance and parameters of hemostasis in patients with atherosclerosis of lower extremities and intermittent claudication]. [Russian]

Autores: Komarov AL; Kropacheva ES; Tarasova TN; Dobrovolskii AB; Titaeva EV; Mironova IY; Naumov VG; Panchenko EP

Fuente: Kardiologiya. 42(3):45-50, 2002.

En: Medline - OVID

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Título: ... with clopidogrel on walking tolerance and parameters of hemostasis in patients with atherosclerosis ...

Autores: AL Komarov; ES Kropacheva; TN Tarasova; AB

Fuente: Kardiologiya, 2002

En: Google Scholar

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Registro Completo  

Título: Management of atherosclerosis with clopidogrel in high-risk patients with recent transient ischaemic attack or ischemic stroke (MATCH): study results Stroke. 35(6):e181, 2004

Autores: Diener H-C

Fuente: Stroke. 35(6):e181, 2004. AN: CN-00487968 Complete Reference

En: Evidence Based Medicine Review - EBMR

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Registro Completo  

Título: ... effects of aspirin, clopidogrel, and their combination on the progression of atherosclerosis in ...

Autores: Q Gu; JL Chen; YM Ruan

Fuente: Zhongguo Yi Xue Ke Xue Yuan Xue Bao, 2005

En: Google Scholar

☐ 15

Registro Completo  

Título: [Inhibitory effects of aspirin, clopidogrel, and their combination on the progression of

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45**atherosclerosis in rabbits]. [Chinese]**

Autores: Gu Q; Chen JL; Ruan YM

Fuente: Chung-Kuo i Hsueh Ko Hsueh Yuan Hsueh Pao Acta Academiae Medicinae Sinicae. 27(1):87-91, 2005 Feb.

En: Medline - OVID

☐ 16Registro Completo  

Título: Ask the doctors. My mother in her early 90s has been diagnosed with atherosclerosis, had a successful angioplasty a year ago for severe chest pain, takes clopidogrel and aspirin, and in general eats a low-fat diet, although she really likes melted ice cream. But she doesn't like to walk. What else should she be taking, and what can I say when she says she's too old to exercise?.

Autores: Anonymous

Fuente: Heart Advisor. 8(5):8, 2005 May.

En: Medline - OVID

☐ 17Registro Completo  

Título: Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin. [see comment].

Autores: Husted S; Emanuelsson H; Heptinstall S; Sandset PM; Wickens M; Peters G

Fuente: European Heart Journal. 27(9):1038-47, 2006 May.

En: Medline - OVID

☐ 18Registro Completo  

Título: Pharmacodynamics, pharmacokinetics, and safety of the oral reversible P2Y12 antagonist AZD6140 with aspirin in patients with atherosclerosis: a double-blind comparison to clopidogrel with aspirin.

Autores: Husted S, Emanuelsson H, Heptinstall S, Sandset PM, Wickens M, Peters G

Fuente: European heart journal. 27(9):1038-47, 2006 May. AN: CN-00562594 AbstractComplete Reference

En: Evidence Based Medicine Review - EBMR

☐ 19Registro Completo  

Título: Is there a different effect of clopidogrel plus aspirin versus clopidogrel alone on stroke patients with small vessel disease and large artery atherosclerosis? Analysis from MATCH Stroke. 38(2):522, 2007

Autores: Benavente O, Pearce L, Amarenco P, Streifler J, Kase C

Fuente: Stroke. 38(2):522, 2007. AN: CN-00602957 Complete Reference

En: Evidence Based Medicine Review - EBMR

☐ 20Registro Completo  

Título: Effect of clopidogrel on the inflammatory progression of early atherosclerosis in rabbits model.

Autores: Li M; Zhang Y; Ren H; Zhang Y; Zhu X

Fuente: Atherosclerosis. 194(2):348-56, 2007 Oct.

En: Medline - OVID

☐ 21Registro Completo  

Título: ... , additive benefit of clopidogrel and lipid-lowering therapy in patients with atherosclerosis

Autores: DL Bhatt; JM Foody; AT Hirsch; P Ringleb; W Hacke

En: Google Scholar

☐ 22Registro Completo  

Título: ... agent, clopidogrel, in the young and the elderly with and without symptomatic atherosclerosis

Autores: MC Guillin; G Bonnet; J Sissmann; J Necciani; JP

En: Google Scholar

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13☐ 23Registro Completo  Link Solver**Título:** Effects of clopidogrel on the formation of experimental atherosclerosis**Autores:** Q Gu; JL Chen; YM Ruan; WX Si; QZ Wang**En:** Google Scholar☐ 24Registro Completo  Link Solver**Título:** ... oral reversible ADP receptor antagonist, compared with clopidogrel in patients with atherosclerosis**Autores:** S Husted; H Emanuelsson; S Heptinstall; S Clark**En:** Google Scholar☐ 25Registro Completo  Link Solver**Título:** ... front-loaded regimen of clopidogrel on arterial thrombus formation in patients with atherosclerosis ...**Autores:** G Helft; JI Osende; SG Worthley; O Rodriguez; AG**En:** Google Scholar☐ 26Registro Completo  Link Solver**Título:** ... oral reversible ADP receptor antagonist, compared with clopidogrel in patients with atherosclerosis**Autores:** E Husted; C Heptinstall**En:** Google Scholar☐ 27Registro Completo  Link Solver**Título:** ..., additive benefit of clopidogrel and lipid-lowering therapy in patients with atherosclerosis [...**Autores:** DL Bhatt; JM Foody; AT Hirsch**En:** Google Scholar☐ 28Registro Completo  Link Solver**Título:** ... reduces P-selectin and formation of platelet-leukocyte aggregates in patients with atherosclerosis ...**Autores:** U Klinkhardt; R Bauersachs; J Adams; J Graff**En:** Google Scholar☐ 29Registro Completo  Link Solver**Título:** ... agent, clopidogrel, in the young and the elderly with and without symptomatic atherosclerosis [...**Autores:** MC Guillin; G Bennet; J Sissmann**En:** Google Scholar☐ 30Registro Completo  Link Solver**Título:** Ask the doctors. My mother in her early 90s has been diagnosed with atherosclerosis, had a successful angioplasty a year ago for severe chest pain, takes clopidogrel and aspirin, and in general eats a low-fat diet, although she really likes melted ice cream. But she doesn't like to walk. What else should she be taking, and what can I say when she says she's too old to exercise?**En:** MEDLINE with Full Text☐ 31Registro Completo  Link Solver**Título:** [Inhibitory effects of aspirin, clopidogrel, and their combination on the progression of atherosclerosis in rabbits]**Autores:** (chi**En:** MEDLINE with Full Text

41
345
23☐ 32Registro Completo  

Título: [Effect of therapy with clopidogrel on walking tolerance and parameters of hemostasis in patients with atherosclerosis of lower extremities and intermittent claudication]

Autores: Vliianie terapii klopidogrelem na tolerantnost' k khod'be i pokazateli gemostaza u bol'nykh s ateroskleroticheskim porazheniem arterii nizhnikh konechnostei i peremezhaiushcheisia khromotoi

En: MEDLINE with Full Text

☐ 33Registro Completo  

Título: Clopidogrel: hemorheological effects in subjects with subclinical atherosclerosis.

Autores: (eng)

En: MEDLINE with Full Text

☐ 34Registro Completo  

Título: Acute antithrombotic effect of a front-loaded regimen of clopidogrel in patients with atherosclerosis on aspirin.

Autores: (eng)

En: MEDLINE with Full Text

☐ 35Registro Completo  

Título: Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis.

Autores: (eng)

En: MEDLINE with Full Text