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SUPERINDUSTRIA Y COMERCIO

Radicación: 02082770 00000001

Folios: 52

Fecha (AMB): 2002-09-25 10:33:22

Trámite: 011 PCT-PATENT 379 FASE INCI 329 COMPLETOS

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

SEÑORES

DIVISION DE NUEVAS CREACIONES

SUPERINTENDENCIA DE INDUSTRIA Y COMER

E. S. D.

REF: EXPEDIENTE No. 02082770
SOLICITANTE: ORION CORPORATION
SOLICITUD PCT / EP00302207.7 DE LA SOLICITUD
PCT/FI01/00266
FECHA DE LA SOLICITUD: SEPTIEMBRE 16, 2002
TITULO: TRATAMIENTO DE TRASTORNOS RELACIONADOS
CON EL SISTEMA SEROTONERGICO

ASUNTO: PRESENTACIÓN DE DOCUMENTOS

DILIA RODRIGUEZ D'ALEMAN, en mi calidad de apoderada de la sociedad
solicitante, por el presente escrito me permito presentar los siguiente
documentos:

1. Solicitud PCT
2. Documento informando al solicitante de las oficinas designadas para
la presentación de la solicitud PCT
3. Publicación internacional No. WO 01/068067 A2
4. Publicación internacional No. WO 01/068067 A3
5. Información de servicios, archivo BIOSIS XP 002185299
6. Investigación original XP.000937785
7. Información XP-000937794
8. Información XP-000937807
9. Información XP 000937808
10. Información XP-000937809
11. Información XP-000937786
12. Información XP-000937809

Agradezco continuar con el presente trámite

Señor Jefe,

DILIA RODRIGUEZ D'ALEMAN
RCH/srr
P. 450

PCT REQUEST

Original (for SUBMISSION) - printed on 15.03.2001 11:40:21 AM

0	For receiving Office use only	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.91 (updated 01.01.2001)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	National Board of Patents and Registration (Finland) (RO/FI)
0-7	Applicant's or agent's file reference	DERADOSE
I	Title of invention	TREATMENT OF DISORDERS RELATING TO THE SEROTONERGIC SYSTEM
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	ORION CORPORATION
II-5	Address:	Orionintie 1 FIN-02200 Espoo Finland
II-6	State of nationality	FI
II-7	State of residence	FI
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II-9	Facsimile No.	+358-10-429 3477
III-1	Applicant and/or inventor	
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III-1-6	State of nationality	FI
III-1-7	State of residence	FI

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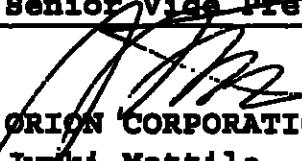
III-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
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III-2-4	Name (LAST, First)	MÄKI-IKOLA, Outi
III-2-5	Address:	Karhunkatu 32 FIN-20750 Turku Finland
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III-2-7	State of residence	FI
IV-1	Agent or common representative; or address for correspondence The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name	ORION CORPORATION
IV-1-2	Address:	ORION PHARMA Industrial Property Rights P.O.Box 65 FIN-02101 Espoo Finland
IV-1-3	Telephone No.	358-10-429 2951
IV-1-4	Facsimile No.	358-10-429 3477
V	Designation of States	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

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V-3	National Patent (States which have become party to the PCT after the issuance of this version of EASY)	CO Colombia ✓	
V-5	Precautionary Designation Statement In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	Exclusion(s) from precautionary designations	NONE	
VI-1	Priority claim of earlier regional application		
VI-1-1	Filing date	17 March 2000 (17.03.2000)	
VI-1-2	Number	00302207.6	
VI-1-3	Regional Office	EP	
VII-1	International Searching Authority Chosen	European Patent Office (EPO) (ISA/EP)	
VIII	Check list	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	9	-
VIII-3	Claims	2	-
VIII-4	Abstract	1	EZABST00.TXT
VIII-5	Drawings	1	-
VIII-7	TOTAL	17	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	Figure of the drawings which should accompany the abstract	<no.>	
VIII-19	Language of filing of the International application	English	
IX-1	Signature of applicant or agent		
IX-1-1	Name	ORION CORPORATION	
IX-1-2	Name of signatory	Timo Lappalainen	
IX-1-3	Capacity	Senior Vice President	
IX-2	Signature of applicant or agent		
IX-2-1	Name	ORION CORPORATION	
IX-2-2	Name of signatory	Jyrki Mattila	
IX-2-3	Capacity	President	

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10-1	Date of actual receipt of the purported international application	
10-2	Drawings:	
10-2-1	Received	
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	
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PCT (ANNEX - FEE CALCULATION SHEET)

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(This sheet is not part of and does not count as a sheet of the international application)

0	For receiving Office use only	
0-1	International Application No.	
0-2	Date stamp of the receiving Office	
0-4	Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet Prepared using	PCT-EASY Version 2.91 (updated 01.01.2001)
0-9	Applicant's or agent's file reference	DERADOSE
2	Applicant	ORION CORPORATION, et al.
12	Calculation of prescribed fees	fee amount/multiplier total amounts (FIM)
12-1	Transmittal fee T	⇒ 800
12-2	Search fee S	⇒ 5 618.71
12-3	International fee	
	Basic fee	
	(first 30 sheets) b1	2 431.8
12-4	Remaining sheets	0
12-5	Additional amount (X)	53.51
12-6	Total additional amount b2	0
12-7	b1 + b2 = B	2 431.8
12-8	Designation fees	
	Number of designations contained in international application	88
12-9	Number of designation fees payable (maximum 8)	6
12-10	Amount of designation fee (X)	523.22
12-11	Total designation fees D	3 139.32
12-12	PCT-EASY fee reduction R	-749.16
12-13	Total international fee (B+D-R) I	⇒ 4 821.96
12-17	TOTAL FEES PAYABLE (T+S++P)	⇒ 11 240.67
12-19	Mode of payment	other: bank account

VALIDATION LOG AND REMARKS

13-2-2	Validation messages Status	Yellow! Additional national designation added: Obtain updated maintenance tables rather than using this field.
13-2-8	Validation messages Contents	Yellow! The power of attorney or a copy of the general power of attorney will need to be furnished unless all applicants sign the request form.

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PCT (ANNEX - FEE CALCULATION SHEET)

DERADOSE

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		Green? Priority 1. The priority document is not enclosed. (The applicant must furnish it within 16 months from the earliest priority date claimed)
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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To: ORION CORPORATION Orion Pharma Industrial Property Rights P.O. Box 65 FIN-02101 Espoo FINLANDE
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Date of mailing (day/month/year) 20 September 2001 (20.09.01)		IMPORTANT NOTICE	
Applicant's or agent's file reference DERADOSE			
International application No. PCT/FI01/00266	International filing date (day/month/year) 16 March 2001 (16.03.01)	Priority date (day/month/year) 17 March 2000 (17.03.00)	
Applicant ORION CORPORATION et al			

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
KP, KR, US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
AE, AG, AL, AM, AP, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EA, EE, EP, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OA, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ.

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 20 September 2001 (20.09.01) under No. WO 01/68067

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 18 months from the priority date.

It is the applicant's sole responsibility to monitor the 18-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 740.14.35	Authorized officer J. Zahra Telephone No. (41-22) 338.83.38
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number
WO 01/68067 A2

- (51) International Patent Classification: A61K 31/00 (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (21) International Application Number: PCT/FI01/00266
- (22) International Filing Date: 16 March 2001 (16.03.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 00302207.6 17 March 2000 (17.03.2000) EP
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicant (for all designated States except US): ORION CORPORATION [FI/PI]; Orionintie 1, FIN-02200 Espoo (FI).
- Published:
— without international search report and to be republished upon receipt of that report
- (72) Inventors; and
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- (74) Agent: ORION CORPORATION; Orion Pharma, Industrial Property Rights, P.O. Box 65, FIN-02101 Espoo (FI).
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/68067 A2

(54) Title: TREATMENT OF DISORDERS RELATING TO THE SEROTONERGIC SYSTEM

(57) Abstract: The present invention relates to deramciclanc, (1R,2S,4R)-(-)-2-[N,N-(dimethylaminoethoxy)]-2-phenyl-1,7,7-trimethyl-bicyclo[2.2.1]heptane, and its use in the treatment of disorders relating to serotonergic system in humans, specifically depression and anxiety.

TREATMENT OF DISORDERS RELATING TO THE SEROTONERGIC SYSTEM

5 FIELD OF THE INVENTION

The present invention is directed to deramciclane, (1R,2S,4R)-(-)-2-[N,N-(dimethylaminoethoxy)]-2-phenyl-1,7,7-trimethyl-bicyclo[2.2.1]heptane, and its use in the treatment of disorders relating to the serotonergic system. Specifically, the
10 present invention is directed to the use of deramciclane in the treatment of depression.

Further, the present invention is directed to the use of deramciclane in the treatment of anxiety, specifically chronic anxiety including GAD, in an oral daily
15 dosage of about 30 mg in humans. The daily dosage may be given as a once-a-day formulation or it may be divided. For example, a once-a-day formulation may be used, and may lead to greater patient compliance than a multiple-dose daily formulation.

20 BACKGROUND

The preparation of deramciclane as a free base and as a fumarate salt has been described in Hungarian Patent No. 212,547. Other pharmaceutically acceptable acid addition salts of deramciclane may be formed with inorganic (e.g., hydrochloric acid,
25 sulfuric acid) or organic acids (e.g., acetic acid, tartaric acid). The fumarate salt is an example of such a pharmaceutically acceptable acid addition salt.

Deramciclane has shown anxiolytic-like effects in some conventional animal models with various routes of administration, and in receptor binding studies *in vitro*
30 deramciclane has shown to bind with high affinity to serotonin 5HT_{2A}- and 5-HT_{2C}-receptor subtypes, being a potent antagonist of these receptors (Gacsalyi, I. et al., Drug Dv Res (1997) 40:333-348). In punished drinking test in rats (Vogel, J.R. et al.,

Psychopharmacologia (1971) 21:1-7) deramciclane was active, after single oral administration at doses of 1 mg/kg and 10 mg/kg. In social interaction test in rats (File, S.E. J. Neurosci Methods (1980) 2:219-238) deramciclane enhanced the social interaction time in some extent, the minimum effective dose after single
5 intraperitoneal administration was 0.7 mg/kg. In two compartment test in mice (Crawley, J. and F.K. Goodwin, Pharmacol Biochem Behav. (1980)13, 167-170. & Crawley, J.N., Pharmacol. Biochem. and Behav. (1981) 15, 695-699) deramciclane was active after single subcutaneous administration at dose of 3 mg/kg. In marble-burying test in mice (Broekkamp, C.L. et al., Eur J. Pharmacol. (1986) 126:223-229)
10 the effective doses were 10 mg/kg and 30 mg/kg orally. Nevertheless, deramciclane was totally ineffective in elevated plus maze test in rats (Handley, S.L. and S. Mithani, 1984, Effects of Alpha-Adrenoceptor Agonists and Antagonists in a Maze-Exploration Model of "Fear"-Motivated Behaviour, Naunyn-Schmiedeberg's Archives of Pharmacology. 327, 1-5) after single intraperitoneal doses at range of 0.1
15 mg/kg – 5 mg/kg. However, deramciclane was able to attenuate the caerulein-induced decrease in exploratory behavior at an intraperitoneal dose of 0.5 mg/kg in the elevated plus maze test.

Also the possible antidepressant activity of deramciclane has been evaluated in
20 various conventional animal models (Gacsalyi, I. et al., Drug Dv Res (1997) 40:333-348). In learned helplessness test in rat (Giral et al. Reversal of helpless behaviour in rats by putative 5-HT1A agonists. Biol. psychiatry 23: 237- 242), deramciclane dose dependently attenuated helpless behaviour induced by inescapable electric foot shocks, when given intraperitoneally 1 or 10 mg/kg, repeatedly 8 times, twice a day,
25 before the test. The effect of deramciclane was found to be negligible, even at relatively high oral doses, 48-160 mg/kg, when evaluated for tetrabenazine-induced ptosis in mice according to the method of Howard et al. (Howard, J.L. et al., (1981) Empirical behavioral models of depression with emphasis on tetrabenazine antagonism. In Enna S.J., Malick J.B., Richelson E. (eds.): Antidepressants:
30 Neurochemical, Behavioral, and Clinical Perspectives. New York: Raven Press, p 107). In the forced swimming test in rats (Porsolt R.D. et al., Eur. J. Pharmacol. (1978) 47:379-391) deramciclane was clearly ineffective at oral doses of 25 and 100 mg/kg.

Thus, deramciclane has been effective in some animal models of anxiety after oral doses at range from 1 mg/kg to 30 mg/kg in mice and rats. Further, deramciclane has showed negligible effects in animal models of depression even after high peroral doses in mice and rats, which is in line with the results reporting that 5-HT_{2C} -
5 receptor agonists are effective in animal models of depression (Moreau J-L. et al. European Neuropsychopharmacology 6:169-175, 1996).

In a whole body autoradiography distribution study with tritium labelled deramciclane in rats (Hazai, I, et al. *J. Pharm. Pharmacol.* 51: 165-174, 1999) at a
10 dose of 3 mg/kg, it was found that after intravenous administration there was high radioactivity (reflecting amount of deramciclane) in several organs including blood and the brain, but after oral administration the amount was substantially lower, especially in the brain.

15 A comparative pharmacokinetic study of orally administered deramciclane in rats, dogs, rabbits and humans (Klebovich et al *Pharm. Pharmacol. Commun.*, 4: 129-136, 1998), it was shown that the plasma concentration curves obtained after the administration of a single 3 mg/kg oral dose of deramciclane to rats (dogs, rabbits) and human show considerable species specific differences. In the peak
20 plasma concentration (C_{max}) values there were significant differences: C_{max} was 5.4 ng/ml in rat and 217.5 ng/ml in human after the same 3 mg/kg oral dose. Thus a 40-times lower oral dose of deramciclane could be used in man to result in the same maximal plasma concentration as in rat. Furthermore, total amount of deramciclane absorbed into blood, calculated as Area Under Curve values (AUC 0 - ∞) from
25 plasma concentrations as a function of time, showed more considerable species difference. The mean AUC 0 - ∞ values after single oral administration of deramciclane were 11.9 ng h/ml and 3737.8 ng h /ml in rat and human, respectively. Thus over 300 times lower oral doses should result in equal exposure in humans than in rats. Basing only the C_{max} difference between rat and man, it can be predicted
30 that considerably lower doses should be centrally active in humans than in rat. The minimum oral effective anti-anxiety dose in rats was 1 mg/kg (1-30 mg/kg the full range; see above), i.e. in a 70 kg-man this would mean 70 mg dose. To reach the same pharmacologically active plasma concentration in human as was shown to be

efficacious in rat, one should divide the rat dose by 40. This would result in 70 mg/40= 1.75 mg (i.e. 0.025 mg/kg) as an effective dose in man.

The binding of deramciclane to serotonin 5-HT_{2A}-receptors in frontal cortex of healthy male volunteers after a single oral dose of 20, 50 and 150 mg of deramciclane is discussed in Kanerva, H., et al., Psychopharmacology (1999) 145:76-81. The determination of the brain 5HT_{2A} -receptor occupancy of deramciclane in humans has shown that 90% and 50% receptor occupancies were reached at deramciclane plasma concentration of about 70 ng/ml and 21 ng/ml, respectively. The pharmacokinetics of single dose of deramciclane and during oral dosing of 10 mg, 30mg and 60mg twice a day for seven days are discussed in Kanerva, H., Pharmacokinetic studies on deramciclane. Kuopio University Publications A. Pharmaceutical Sciences 39.1999. After a single oral administration of 20 mg and 30 mg doses of deramciclane, the C_{max}-values were 24±9.4 ng/ml and 27±6.1 ng/ml, respectively. During repeated administration of deramciclane for one week the C_{min} and C_{max} for 60 mg and 20 mg daily doses were shown to range between 48-91 ng/ml and 16-33 ng/ml, respectively.

As the above experimental animal and human data does not disclose repeated administration of deramciclane rendering steady state plasma concentrations in a treated patient, it was impossible to foresee the exact oral doses of deramciclane effective in treating anxiety in humans. Furthermore, it was totally unexpected that deramciclane was effective in treating depressive symptoms.

Anxiety is a normal emotional feeling related to different situations of threat or fear. External threat is experienced as a fear whereas obscure and unidentified feeling of threat may be experienced as anxiety. When anxiety persists it can develop into a pathological disorder. Anxiety disorders are divided more specifically in diagnostic disorders e.g., panic disorder, phobias, and generalised anxiety disorder (GAD). GAD is a chronic illness associated with excessive anxiety and worry lasting for at least six months. In addition, the anxiety and worry are associated with restlessness, fatigue, difficulties in concentrating or mind going blank, irritability, muscle tension, and sleeping disturbances. The symptoms may be triggered by different events of

life, and the control of anxiety is very difficult to the patient.

Anxiety is currently treated with benzodiazepines, SSRI's and buspirone, which are not optimal treatments regarding the adverse drug reaction and efficacy profiles. Moreover, relapse of the disease, different kinds of withdrawal effects, development of tolerance as well as relapse and recurrence often happen, when traditional anxiolytics are used. For example, to avoid withdrawal effects, the doctors usually gradually taper the dosage of the medicine (i.e. gradually diminish its daily dosage) before the treatment may be stopped. Patients tend to develop tolerance to those traditional compounds as well. Development of tolerance occurs when, for example, a patient requires greater quantities of a compound over time to achieve the same therapeutic effect.

In the treatment of psychiatric disorders with a chronic course, such as anxiety, it is important to prevent the relapse and recurrence of the disease. After the acute treatment phase, the improved condition can be maintained, and relapses can thus be prevented by continuing the treatment in those who have responded to the treatment or who have reached remission during it. After said continuation treatment phase, when recovery has been reached, the disease can be prevented by continuing the treatment further by the so called maintenance treatment, during which the daily dosage may be decreased, for example to a half from the original.

There has thus been a long felt need to obtain an anxiolytic medicament, which is void of withdrawal and discontinuation effects and does not cause development of tolerance in the patients. Furthermore, sufficient efficacy in relapse and recurrence prevention are important qualities of a well functioning anxiolytic drug. It is believed by the inventors that deramciclone satisfies this need in the art.

Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realised and attained by means of the elements and combinations particularly pointed out in the appended claims.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the number of responders (at least 50 % reduction in HAM-A scale).

DETAILED DESCRIPTION OF THE INVENTION

10

An object of the present invention is to provide an oral daily dosage of about 20 to about 30 mg of deramciclane for the treatment of disorders of serotonergic systems in humans. One disorder of the serotonergic system treatable according to the present invention is anxiety, for example chronic anxiety. The present invention also
15 includes the treatment of GAD. Another object of the present invention is directed to the treatment of chronic anxiety where the patient does not experience relapse of the anxiety.

Another object of the present invention is directed to the method of treating
20 depression in a human.

It is still a further object of the invention to provide a treatment regimen of deramciclane that does not require, to avoid withdrawal effects, diminishing the treatment dosage before terminating the treatment. In other words, in this embodiment,
25 the patient may continue receiving the full treatment dosage up to the point of termination of the treatment, and will not suffer withdrawal effects that would have otherwise followed using other conventional treatments. In the regimen of the invention a standard daily dosage of deramciclane is given to the patient for a period long enough, e.g. for from three weeks to ten years, e.g. for from two months to five years, e.g. for
30 from eight months to two years, to cause relief of the symptoms, whereafter the treatment is abruptly terminated. By the term "abruptly terminated" it is meant that the dose is decreased within 24 hours from the standard dosage to less than a fourth of the standard dose, preferably to zero.

A dosage of about 30 mg of deramciclane may be used, for example in a conventional relatively fast release formulation. When the corresponding slow release formulation is used, the dosage may be also lower, for example about 20 mg.

5

In the present invention it has been discovered that when treating disorders relating to anxiety in humans with deramciclane the most effective clinical response was obtained with an oral daily dosage of about 30 mg. When deramciclane was used in the treatment of anxiety, specifically GAD, the oral daily dosage of 30 mg was found to express the best response on Hamilton Anxiety Scale (HAM-A). Further, in the pairwise comparison of the responder criterion (at least 50 % reduction in HAM-A scale) between the deramciclane dosage and placebo, the oral daily dosage of 30 mg was statistically better than placebo.

15 In addition, in the present invention it has been discovered that deramciclane is effective in treating depressive disorders in humans. Specifically, the same oral daily deramciclane dosage of 30 mg, which was effective in the treatment of anxiety was found to be effective on the depressive symptoms in the Montgomery Åsberg Depression Rating Scale (MADRS).

20

The use of the term "about" with respect to the dosage amounts includes the natural industry variation in dosage amounts of drugs administered to patients. For example, a variation of $\pm 5\%$ in a given dosage amount in the methods of the present invention would be included by the term "about" in the present invention. For example, a dosage $30\text{ mg} \pm 5\%$ is included within the phrase "about 30 mg" in an embodiment of the present invention.

EXAMPLE

30 The efficacy of deramciclane in the treatment of anxiety, specifically GAD, was studied in a randomised placebo-controlled double-blind study. In addition, the dosage dependency of the effects of deramciclane was evaluated. Total of 208 patients were included in the study. The subjects were randomly assigned to four parallel groups to receive one tablet twice daily (b.i.d) of a placebo, 5mg

(=10mg/day), 15mg (=30mg/day), or 30mg (=60mg/day) deramciclane. The study started with a one-week placebo run-in period, followed by an eight-week placebo-controlled active treatment and a two-week placebo washout period.

- 5 The efficacy of deramciclane on anxiety symptoms was studied by the efficacy variable Hamilton Anxiety Scale (HAM-A), analyzing the change in the score and using responder criterion (at least 50 % reduction in HAM-A total score).

- 10 The efficacy of deramciclane on depressive symptoms was studied using Montgomery Åsberg Depression Rating Scale (MADRS).

RESULTS

Anxiety

- 15 The HAM-A score decreased 14.5 points from baseline in groups receiving either 15 mg b.i.d or 30 mg b.i.d. dose. However, only the 15 mg b.i.d group differed statistically significantly from placebo ($p=0.006$). No difference was found between the 5 mg b.i.d and placebo. Accordingly, increasing the dose over 15mg b.i.d did not increase the efficacy any more.

20

- Number of responders (at least 50 % reduction in HAM-A scale) is presented in Figure 1. The responder criterion was reached by 54 % ($n=27$), 57% ($n=31$), 76% ($n=39$) and 70% ($n=37$) of patients on placebo, 5 mg, 15 mg and 30 mg b.i.d. dosing, respectively. In comparison between the deramciclane dose levels and placebo, only
25 15 mg b.i.d. was statistically better than placebo ($p=0.020$).

Lack of withdrawal symptoms

- After stopping the above described 8-week treatment period no discontinuation effects were seen (measured by the Physician's Withdrawal Checklist, PWC). This is
30 different and surprising from the experiences with the most other efficacious drugs used for the treatment of anxiety.

Table 1. The PWC values at 8 weeks (when the treatment was stopped) and at 10 weeks (after a two week wash out period)

dosage	after 8 week treatment	after two-week wash-out
placebo	12 (10)	12 (11)
5 mg b.i.d deramciclanc	9 (7)	9 (8)
15 mg b.i.d deramciclanc	7 (8)	8 (7)
30 mg b.i.d deramciclanc	8 (8)	8 (7)

Mean (SD)

5

In conclusion, deramciclanc did not cause any withdrawal symptoms after abrupt discontinuation of the treatment. Therefore, it may be used as a treatment for serotonergic diseases without any withdrawal effects.

10 Depression

MADRS scores decreased similarly and statistically significantly in both 15 mg b.i.d and 30 mg b.i.d groups (7.7 and 8.0 points, $p=0.028$ and 0.016 , respectively). 5 mg b.i.d dose was ineffective. Thus, both the oral daily dosages of 30 mg and 60 mg of deramciclanc were found to be effective on the depressive symptoms.

15

Although the invention has been illustrated by the preceding example, it is not to be construed as being limited to the materials employed therein; rather, the invention is directed to the generic area as herein disclosed. Various modifications and embodiments thereof can be made without departing from the spirit or scope thereof.

20

CLAIMS:

1. Use of deramciclane in the manufacture of a medicament for the treatment of disorders relating to the serotonergic system in humans in an oral daily dosage of about 20 to 30 mg.
5
2. The use according to claim 1, wherein the disorder is anxiety.
3. The use according to claim 2, wherein the anxiety disorder is chronic.
- 10 4. The use according to claim 3, wherein the patient does not experience relapse of the disorder.
5. The use according to claim 4, wherein the disorder is generalised anxiety disorder.
15
6. The use according to claim 5, wherein the daily dosage is about 30 mg.
7. Use of deramciclane in the manufacture of a medicament for the treatment of disorders relating to depression in humans.
20
8. The use according to claim 7, wherein deramciclane is administered orally.
9. The use according to claim 8, wherein an oral daily dosage of about 30 mg is used.
25
10. The use according to any of claims 1 to 9, wherein a once-a-day formulation is used.
11. Manufacture of deramciclane for the treatment of disorders relating to the
30 serotonergic system, to be administered to a human in standard daily dosage for a long enough period to cause relief of the symptoms, followed by an abrupt termination of the administration.

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PCT/FI01/00266

12. Manufacture according to claim 11, wherein the standard daily dosage is given to the patient for at least three weeks.
13. Manufacture according to claim 11 or 12, wherein the standard daily dosage is about 20 to 30 mg.

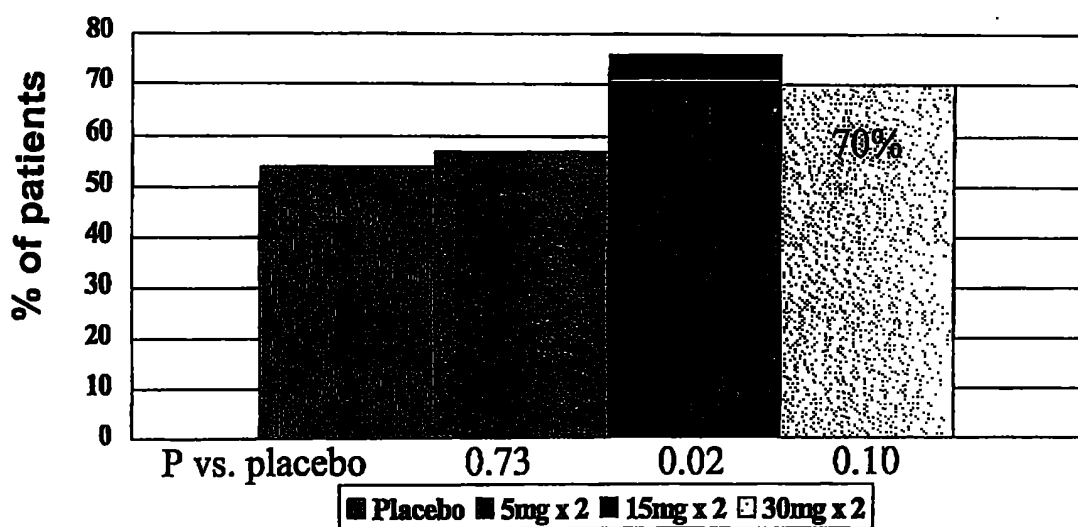


Fig. 1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number
WO 01/068067 A3

(51) International Patent Classification: A61K 31/135, A61P 25/22, 25/24

(21) International Application Number: PCT/FI01/00266

(22) International Filing Date: 16 March 2001 (16.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 00342317.6 17 March 2000 (17.03.2000) EP

(71) Applicant *for all designated States except US*: ORION CORPORATION [FI/FI]; Orionintie 1, FIN-02200 Espoo (FI).

(72) Inventors; and
(75) Inventor/Applicants *for US only*: KANERVA, Harri [FI/FI]; Kalikkivuorinkatu 14, FIN-08100 Lohja (FI). MÄKI-JOKOLA, Outi [FI/FI]; Karhunkatu 32, FIN-20750 Turku (FI).

(74) Agent: ORION CORPORATION; Orion Pharma, Industrial Property Rights, P.O. Box 65, FIN-02101 Espoo (FI).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

(88) Date of publication of the international search report: 11 July 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/068067 A3

(54) Title: USE OF DERAMCICLANE FOR THE TREATMENT OF ANXIETY AND DEPRESSION

(57) Abstract: The present invention relates to deramciclanc, (1R,2S,4R)-(-)-2-[N,N-(dimethylaminoethoxy)]-2-phenyl-1,7,7-trimethyl-bicyclo[2.2.1]heptane, and its use in the treatment of disorders relating to serotonergic system in humans, specifically depression and anxiety.

INTERNATIONAL SEARCH REPORT

International Application No.
PC1/FI 01/00266

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/135 A61P25/22 A61P25/24

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

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

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

BIOSIS, EPO-Internal, WPI Data, PAJ, CHEM ABS Data, MEDLINE, EMBASE, PASCAL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; AN - 1997:459940, GACSALYI, ISTVAN ET AL: "Receptor binding profile and anxiolytic - type activity of deramciclane (EGIS-3886) in animal models." retrieved from STN XP002185299 cited in the application abstract & DRUG DEVELOPMENT RESEARCH, (1997) VOL. 40, NO. 4, PP. 333-348. , -/-	1-13

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "S" document member of the same patent family

Date of the actual completion of the international search

18 December 2001

Date of mailing of the international search report

16/01/2002

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/FI 01/00266

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	KANERVA H ET AL: "BRAIN 5-HT _{2A} RECEPTOR OCCUPANCY OF DERAMCICLANE IN HUMANS AFTER A SINGLE ORAL ADMINISTRATION - A POSITRON EMISSION TOMOGRAPHY STUDY" PSYCHOPHARMACOLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 145, no. 1, July 1999 (1999-07), pages 76-81, XP000937785 ISSN: 0033-3158 cited in the application see in particular the abstract; p. 77, left column, l. 1-4 and 14-20; p. 77 right column, l. 8-13; p. 79, right column, l. 4-6; p. 80, table 1.	1-13
X	KANERVA H ET AL: "PHARMACOKINETICS AND SAFETY OF DERAMCICLANE DURING MULTIPLE ORAL DOSING" INTERNATIONAL JOURNAL OF CLINICAL PHARMACOLOGY AND THERAPEUTICS, DUSTRI-VERLAG, DEISENHOFEN-MUENCHEN, DE, vol. 37, no. 12, December 1999 (1999-12), pages 589-597, XP000937794 ISSN: 0946-1965 see in particular the abstract; p. 595, right column, l. 5-9; p. 596, right column, last two paragraphs.	1-6, 11-13
X	KLEBOVICH I ET AL: "COMPARATIVE PHARMACOKINETICS OF DERAMCICLANE IN RAT, DOG, RABBIT AND MAN AFTER THE ADMINISTRATION OF A SINGLE ORAL DOSE OF 3 MG KG" PHARMACY AND PHARMACOLOGY COMMUNICATIONS, LONDON, GB, vol. 4, no. 2, 1998, pages 129-136, XP000937807 ISSN: 1460-8081 cited in the application see in particular the abstract; p. 129, left column, first paragraph; p. 132, figure 2; p. 133, table 2.	1-6, 11-13
X	KANERVA H ET AL: "THE SINGLE DOSE PHARMACOKINETICS AND SAFETY OF DERAMCICLANE IN HEALTHY MALE VOLUNTEERS" BIOPHARMACEUTICS AND DRUG DISPOSITION, XX, XX, vol. 20, no. 7, October 1999 (1999-10), pages 327-334, XP000937808 see in particular the abstract; p. 333, right column, last paragraph.	1-6, 11-13
	-/-	

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.2

Claims Nos.: 1-13 (in part)

The term "disorders relating to the serotonergic system" in present claims 1-6 and 11-13 are unclear within the meaning of Article 6 PCT. It is not clear which diseases are encompassed by said term, apart from those mentioned in the description, i.e. depression and anxiety (p. 6, l. 11-20). The same applies to the term "disorders relating to depression" in claims 7-10. Consequently, the search has been carried out for those parts of the application which do appear to be clear, namely for the use of deramciclone in connection with the treatment of anxiety and depression for present claims 1-6 and 11-13 and with the treatment of depression for present claims 7-10.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/FI 01/00266

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARMER R E: "INHIBITORS OF MAMMALIAN CENTRAL NERVOUS SYSTEM SELECTIVE AMINO ACIDTRANSPORTERS" CURRENT MEDICINAL CHEMISTRY, BENTHAM SCIENCE PUBLISHERS BV, BE, vol. 7, no. 2, 2000, pages 199-209, XP000937809 ISSN: 0929-8673 see in particular the abstract; p. 200, left column, second paragraph; p. 202, right column, last paragraph - p. 203, left column, first paragraph.	1-6, 11-13
X	PAELVIMAEMI E-P ET AL: "DERAMCICLANE, A PUTATIVE ANXIOLYTIC DRUG, IS A SEROTONIN 5-HT_{2C} RECEPTOR INVERSE AGONIST BUT FAILS TO INDUCE 5-HT_{2C} RECEPTOR DOWN-REGULATION" PSYCHOPHARMACOLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 136, no. 2, March 1998 (1998-03), pages 99-104, XP000937786 ISSN: 0033-3158 see in particular the abstract; p. 102, right column, l. 3-6 and 35-40; p. 103, left column, l. 7-13, right column, last paragraph.	1-6, 11-13
X	BERENIJI ET AL: "EGYT-3886" DRUGS OF THE FUTURE, BARCELONA, ES, vol. 15, no. 12, 1990, pages 1174-1175, XP000937796 ISSN: 0377-8282 see in particular p. 1174, right column, last two paragraphs - p. 1175, left column, first paragraph; p. 1175, left column, last paragraph - right column, first paragraph.	1-6, 11-13

Form PCT/BA/E10 (continuation of second sheet) (July 1992)

XP-002185299

STN Information Services, File BIOSIS

AN - 1997:459940 BIOSIS

DN - PRKV199799759143

TI - Receptor binding profile and
anxiolytic - type activity of deramciclane
(EGIS-3886) in animal models.

IN - Gacsalyi, Istvan; Schmidt, Eva; Gyertyan, Istvan; Vasar, Kero; Lang
Aavo; Haapalinna, Antti; Fekete, Marton; Hietala, Jarmo; Syvalahti,
Erkka; Tuomainen, Paivi; Mannisto, Pekka T. (1)

CS - (1) Univ. Kuopio, Dep. Pharmacol. Toxicol., P.O. Box 1627, FIN-7021
Kuopio Finland

SO - Drug Development Research, (1997) Vol. 40, No. 4, pp. 333-348.
ISSN: 0272-4391.

DT - Article

LA - English

AB - The present series of experiments was done to characterize the
properties of deramciclane, a new antiserotonergic drug, in both
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CC - Pharmacology - General *22002

BC - Suidae 85740
Caviidae 86300
Muridae *86375

IT - Major Concepts
Pharmacology

IT - Chemicals & Biochemicals
DERAMCICLANE; SEROTONIN; DOPAMINE

IT - Miscellaneous Descriptors
ANIMAL MODEL; ANXIOLYTIC-DRUG; DERAMCICLANE; DOPAMINE D-2

RECEPTORS; MALE; PHARMACOLOGY; RECEPTOR BINDING PROFILE;
SEROTONIN 5-HT-2A RECEPTOR; SEROTONIN 5-HT-2C

ORGN- Super Taxa

Caviidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia;

Muridae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia;

Suidae: Artiodactyla, Mammalia, Vertebrata, Chordata, Animalia

ORGN- Organism Name

guinea pig (Caviidae); mice (Muridae); pig (Suidae); Wistar rat
(Muridae)

ORGN- Organism Superterms

animals; artiodactyls; chordates; mammals; nonhuman mammals;

nonhuman vertebrates; rodents; vertebrates

RN - 120444-71-5 (DERAMCICLANE)

50-67-9 (SEROTONIN)

51-61-6 (DOPAMINE)

XP-002185299

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1-2

2

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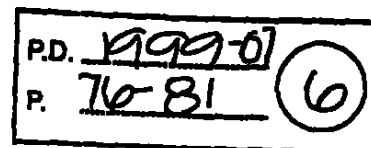
RN - 120444-71-5 (DERAMCICLANE)

50-67-9 (SEROTONIN)

51-61-6 (DOPAMINE)

ORIGINAL INVESTIGATION

Harri Kanerva · Harry Viikman · Kjell Nägren
Olavi Kilkku · Mikko Kuoppamäki · Erkki Syvälahti
Jarmo Hietala



Brain 5-HT_{2A} receptor occupancy of deramciclane in humans after a single oral administration - a positron emission tomography study

Received: 7 September 1998/Final version: 11 January 1999

Abstract Rationale: Deramciclane fumarate is a new 5-HT_{2A} and 5-HT_{2C} receptor antagonist with putative anxiolytic effects. In the present study the binding of deramciclane to serotonin 5-HT_{2A} receptors in frontal cortex of healthy male volunteers was studied using [¹¹C]-N-methyl spiperone ([¹¹C]-NMSP) and positron emission tomography. **Methods:** The receptor occupancy percentage was assessed by the means of inhibition of [¹¹C]-NMSP from the 5-HT_{2A} receptors in the frontal cortex. Single oral doses of 20, 50 and 150 mg deramciclane were given to three subjects at each dose level (total *n* = 9). The receptor occupancy was measured before deramciclane and at 3 and 6 h post-dosing except at the 20 mg dose level where only the 3-h measurement was done. The occupancy percentage was calculated with the ratio method using cerebellum as a reference area. **Results:** Deramciclane inhibited [¹¹C]-NMSP binding dose and concentration dependently. However, deramciclane inhibited maximally only 52% of the [¹¹C]-NMSP binding in the frontal cortex, indicating a non-5-HT_{2A} receptor binding component of this radioligand in frontal cortex. On average, specific [¹¹C]-NMSP binding cerebellum

ratios below 0.355 were not possible to achieve in population. The 52% inhibition was regarded to represent near 100% 5-HT_{2A} receptor occupancy. The and 90% receptor occupancies were reached at deramciclane plasma concentrations of 21 ng/ml and 70 ng/ml, respectively. **Conclusions:** Deramciclane penetrates the blood-brain barrier in humans. Deramciclane binds to the 5-HT_{2A} receptors in the frontal cortex in a saturable manner in vivo. Consequently, increase in deramciclane concentration in plasma above 70 ng/ml will not result in major increase in the 5-HT_{2A} receptor occupancy in the brain.

Key words Deramciclane fumarate · Positron emission tomography · [¹¹C]-NMSP · Serotonin

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) interacts with multiple brain 5-HT receptor subtypes to influence wide range of behaviours. Several main families 5-HT receptors have been described which have been subdivided into further subtypes. These receptors differ in their binding affinity for selective ligands, their receptor-effector coupling mechanisms, and in the involvement in various behavioural processes (Glennon 1990). Antagonism of the two subtypes of 5-HT₂ receptor family, 5-HT_{2A} and 5-HT_{2C}, produces anxiolytic actions in animal models (Critchley and Handley 1986). In addition, a preferential 5-HT_{2C} receptor (formerly 5-HT_{1C}-receptor) agonist, m-CPP, produces anxiogenic-like effects in rodents and in humans (Hoyer 1988; Cruzon and Kennett 1990). Ritalin, ketanserin and spiperone are 5-HT₂ antagonists (Leyse et al. 1985; Hoyer 1988), of which at least ritalin may have clinical anxiolytic and antidepressant properties (Reyntjens et al. 1986; Bersani et al. 1991).

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Deramciclanc (Fig. 1) is a novel non-benzodiazepine type agent which shows activity in several experimental tests suggested to predict anxiolytic activity in humans. The profile of deramciclanc in these tests is clearly distinct from classical anxiolytic agents (Gacsalyi et al. 1997). In vitro receptor binding studies indicate that deramciclanc has no affinity for the GABA_A-benzodiazepine receptor complex, α_1 -, α_2 -receptors, β -receptors, histamine H₁ receptors, 5-HT_{1A} and 5-HT_{1B} receptors. In addition, it has a relatively low affinity for dopamine D₁ or D₂ receptors (1590 and 113 nM, respectively), NMDA receptors (2600 nM) and a moderate affinity for sigma receptors (52 nM) (Gacsalyi et al. 1997). Instead, deramciclanc binds with a high affinity to serotonin 5-HT_{2A} and 5-HT_{2C} receptor subtypes (11 and 9–27 nM, respectively) (Gacsalyi et al. 1997). These serotonin receptor subtypes have shown anxiolytic and possibly also antidepressive actions in experimental preclinical as well as in some clinical studies (Nutt et al. 1990; Hoyer et al. 1994). Therefore, it has been suggested that the mechanism of action of deramciclanc lies in interaction with those receptors belonging to the 5-HT₂ receptor subfamily (Gacsalyi et al. 1997). Deramciclanc has been shown to be an antagonist with an inverse agonist properties at the 5-HT_{2C} receptor with no direct stimulatory agonistic effects (Pälvimäki et al. 1998).

The above mentioned receptor interactions of deramciclanc have been described in vitro in animal tissues. Positron emission tomography (PET) represents an in vivo quantitative autoradiography technique which makes it possible to study pharmacodynamic aspects directly in man. PET has been used successfully to explore the serotonergic system in the living human brain. Currently, there are no 5-HT_{2C} receptor PET tracers that could be used in humans, but 5-HT_{2A} receptors can readily be labelled in vivo with several tracers, of which [¹¹C]-N-methyl-spiperone (NMSP) has been used extensively in humans (Wong et al. 1984; Nordström et al. 1993; Nyberg et al. 1993) [¹¹C]-NMSP has subnanomolar affinities for D₂ receptors and 5-HT_{2A} receptors (Lyon et al. 1986). As there are very few dopamine D₂ receptors in the neocortex, the specific binding of [¹¹C]-NMSP in these areas is generally considered to reflect 5-HT_{2A} receptor binding (Frost et al. 1987; Nordström et al. 1993; Nyberg et al.

1993). In addition, despite the pharmacological similarities of 5-HT_{2A} and 5-HT_{2C} receptors, the spiperone derivative NMSP does not label 5-HT_{2C} receptors (K_i 1540 nM versus [³H]-mesulergine, unpublished data). During the past few years, [¹¹C]-NMSP has been successfully used in 5-HT_{2A} occupancy studies (Nordström et al. 1993; Nyberg et al. 1993).

Deramciclanc has been found to be safe and well tolerated after single oral dosing in the dose range of 0.2–150 mg (Kanerva et al., submitted). Only a few adverse events have been reported, of which dizziness seems to be related to treatment at the maximal tolerated dose (MTD) of 150 mg. In the present study, binding of deramciclanc to the human 5-HT_{2A} receptors in the frontal cortex was evaluated at three different dose levels, including the MTD in vivo. The receptor occupancy was determined using [¹¹C]-NMSP and positron emission tomography. The study was conducted in order to help dose finding procedures for the further clinical development of the drug by producing a 5-HT_{2A} receptor occupancy percentage versus plasma concentration curve.

Materials and methods

This study was performed according to Good Clinical Practice Guidelines of the European Community. The studies followed the recommendations for biomedical research involving humans (current revision of the Declaration of Helsinki of the World Medical Assembly).

The study procedure was approved by The Joint Ethics Committee of the Turku University Central Hospital and the University of Turku.

Chemicals

Deramciclanc 10 mg and 20 mg tablets were provided by EGIS Pharmaceuticals (Budapest, Hungary). These formulations were used in suitable combinations to administer single oral doses of 20, 30 and 150 mg.

[¹¹C]-N-methylspiperone (NMSP) was prepared from [¹¹C]-methyl triflate as recently reported (Nägren and Halldin 1998). An Efremov 103-cm isochronous cyclotron was used to produce [¹¹C]-carbon dioxide. A one-pot procedure (Långström et al. 1986; Nägren et al. 1991) from [¹¹C]-carbon dioxide was used in the preparation of [¹¹C]-methyl iodide, which was converted online to [¹¹C]-methyl triflate by passage through a silver triflate-graphitized carbon column at 150–200°C (Jewett 1992; Nägren et al. 1995). [¹¹C]-NMSP was prepared by reaction of [¹¹C]-methyl triflate with the N-desmethyl precursor (spiperone, Sigma Chemical Company, St Louis, Mo., USA), and the reaction solution was purified on an HPLC column (u-Bondapak C-18, 7.8 × 300 mm, Waters) using a mixture of 10 mM phosphoric acid/acetonitrile, 65/35 at a flow of 6 ml/min. The volume of the formulated (physiological 0.1 M phosphate buffer) [¹¹C]-NMSP solution was determined by weight before and after sterile filtration. The concentration of [¹¹C]-NMSP in the formulated solution was determined by reverse-phase HPLC using a mixture of 10 mM phosphoric acid / acetonitrile, 65/35 at a flow of 2 ml/min. UV absorbance was measured at 250 nm. Samples of [¹¹C]-NMSP were analyzed in triplicate, and concentrations were determined from calibration curves made by injection of three known concentrations of NMSP (RBI, Natick, Mass., USA) on the

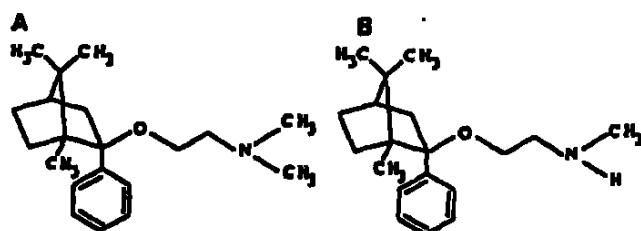


Fig. 1A,B The molecular structures of deramciclanc (A) and its metabolite N-desmethyl deramciclanc (B)

same day as the ^{11}C -synthesis, with standard deviation <2%. The radiochemical purity of [^{11}C]-NMSP was 98–99%, and the specific radioactivity at injection was 555–1486 Ci/mmol.

Subjects

Nine healthy male subjects were recruited. All the subjects gave their written consent prior to the initiation of the study. They were healthy, caucasian male volunteers, whose mean (SD) age, weight, and body mass index were 26.3 (3.4), 75.5 (7.0) and 23.2 (1.4), respectively. They were non-smokers, without clinically significant abnormalities in the physical examination or in the safety laboratory variables. In addition, one female 30-year-old schizophrenic patient (DSM-IV) on stable risperidone treatment (6 mg/day) was recruited.

Study design

An open, uncontrolled study design with three consecutive groups was used. There were three subjects in each group. The first group received 20 mg, the second 50 mg and the third 150 mg single oral dose of deramciclane (Egis Pharmaceuticals, Budapest, Hungary). The study medication was administered in a fasting state. Deramciclane was taken orally in upright position with 200 ml water immediately after the baseline PET measurement (see below). The second and third boluses of [^{11}C]-NMSP were given at 3 h and 6 h after deramciclane intake, respectively. The 6-h measurements were not done at the 20 mg dose level.

Positron emission tomography measurements

Radioactivity in the brain was measured with a whole-body PET scanner (ECAT 931-8/12, Computer Technology and Imaging, Knoxville, Tenn., USA) with 15 slices and spatial and axial resolutions of 6.1 mm and 6.75 mm, respectively.

A vacuum head-holder was prepared for each subject and used in all PET sessions. Identical positioning of the head in both scans was ensured with laser beams and marks on the skin. Before each PET scan, a transmission scan was performed with germanium 68-filled removable ring system. [^{11}C]-NMSP 4–5 mCi (high specific activity) was given as a rapid bolus injection and flushed with saline. Radioactivity in the brain was measured for 51 min using 19 time frames covering almost the entire brain. The regions of interest (ROI) were defined on integrated baseline PET images in the frontal cortex (five slices) and cerebellum (two slices). Subsequently, regional radioactivity was calculated, decay-corrected and plotted against time. The ROI definition and calculation were identical in the second and the third (after deramciclane) PET scans (ROIs from the baseline scan were copied to the other scans).

The frontal cortical ROIs represented preferential binding of [^{11}C]-NMSP to 5-HT $_2\text{A}$ receptors. The cerebellar slices defined the "reference area" or non-specific [^{11}C]-NMSP binding (Nyberg et al. 1993), as the density of 5-HT $_2\text{A}$ receptors is negligible or low in this brain area (Pazos and Palacios 1987). The ratio (R) between frontal cortical to cerebellar uptake of [^{11}C]-NMSP is considered to be an index of 5-HT $_2\text{A}$ receptor density (Lyon et al. 1986; Nordström et al. 1993).

Radioactivity representing specific binding (C_b) to 5-HT $_2\text{A}$ receptors at a given time (t) can be described:

$$C_b(t) = C_F(t) - C_{\text{ref}}(t)$$

where C_F is binding in frontal cortex and C_{ref} is binding in the cerebellum (non-specific binding).

The ratio (R) is calculated from time-radioactivity curves by integrating from 9 to 45 min after tracer injection:

$$R = \int_9^{45} C_b(t)dt / \int_9^{45} C_{\text{ref}}(t)dt$$

The percentage of inhibition of [^{11}C]-NMSP binding was calculated as follows:

$$\text{Inhibition \%} = \frac{R_{\text{baseline}} - R_{\text{after drug}}}{R_{\text{baseline}}} \times 100\%$$

As the study progressed, it became evident that deramciclane was not able completely to inhibit the [^{11}C]-NMSP binding in the frontal cortex. Therefore, a correction was applied to calculate the estimated 5-HT $_2\text{A}$ receptor occupancy as follows:

$$\text{Occupancy \%} = \frac{\text{Inhibition \%}}{\text{Maximum inhibition \%}} \times 100\%$$

Plasma concentrations of deramciclane and *N*-desmethyl deramciclane

Blood samples of 10 ml were taken before the drug intake (baseline) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 8, and 24 h after the 20 mg dose. The blood samples for 50 and 150 mg doses were taken at baseline and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 6.5, 7, and 24 h post-dosing. The plasma samples were analysed using a liquid chromatography-tandem mass spectrometry and a selected reaction monitoring technique. The method has been described in detail elsewhere (Kanerva et al. 1998). The calibration curves for deramciclane and *N*-desmethyl deramciclane were linear over the calibration range of 0.02–100 ng/ml. Samples which had concentrations that exceeded the upper limit were diluted with drug free human plasma and reanalyzed. The assay precision was 7.5 and 12% for deramciclane and *N*-desmethyl deramciclane, respectively, at the lower limit of quantification.

Results

The plasma concentrations of deramciclane and *N*-desmethyl deramciclane increased with the dose. The mean concentration of deramciclane in plasma versus time curves are presented in Figs 2a and b. Deramciclane decreased specific [^{11}C]-NMSP binding in the frontal cortex in a concentration dependent manner. An example of [^{11}C]-decay corrected time-radioactivity curves is presented in Fig. 3. Deramciclane was, however, able to inhibit only 52% of the maximally bound [^{11}C]-NMSP (95% confidence limits 41–63%). In contrast, the reference compound, risperidone, inhibited almost completely the binding of [^{11}C]-NMSP (Fig. 4). The calculated 5-HT $_2\text{A}$ receptor occupancy was 98%.

Figure 5 shows a plot of specific [^{11}C]-NMSP binding in frontal cortex and deramciclane concentration (pooled from all nine subjects). The data fits well with a one-phase exponential function (two-phase exponential did not fit significantly better) concordant with classical receptor binding to a single population of receptors. The data indicates that, on average, B/F (specific binding) ratios below 0.355 could not be achieved in this population with deramciclane. According to calculations presented above, receptor inhibition of about 52% is maximal and corresponds

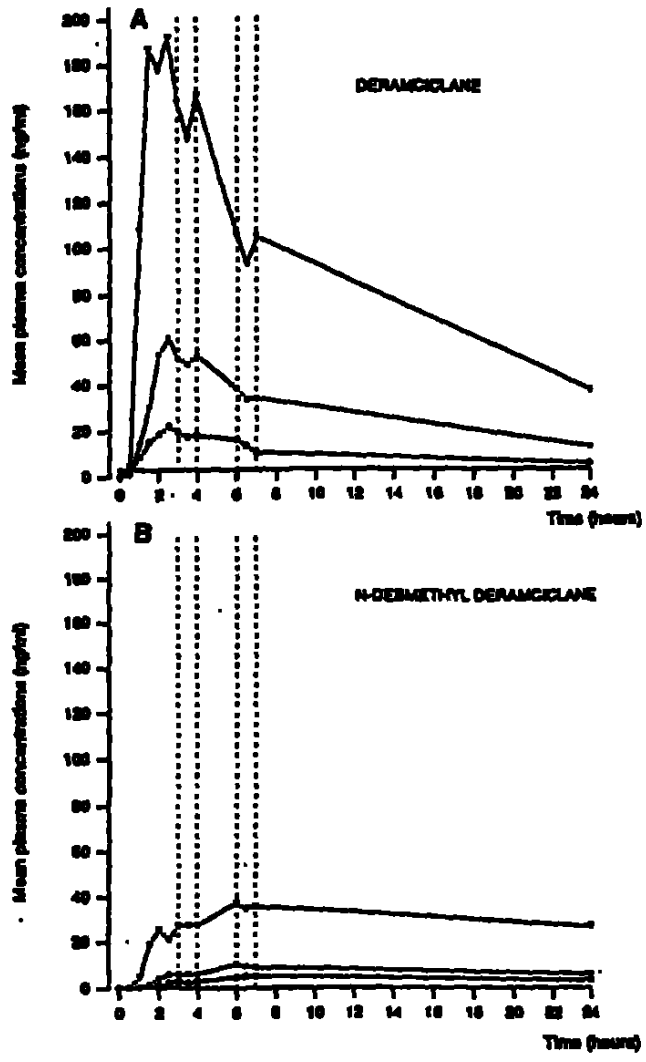


Fig. 2A,B The mean plasma concentration versus time curves of deramciclane (A) and *N*-desmethyl deramciclane (B) up to 24 h post-dosing after single oral doses of 20 (O), 50 (●) and 150 mg (▽) deramciclane. The vertical dotted lines indicate the times of PET measurements. *N* = 3 at each dose level

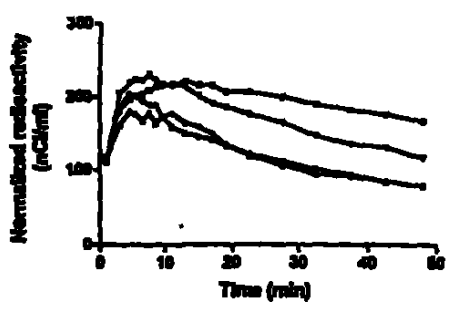


Fig. 3 Regional decay-corrected time-radioactivity curves of [¹¹C]-NMSP in the frontal cortex and the cerebellum in one subject before (baseline measurement) and after the 150 mg dose of deramciclane. Radioactivity is normalized to 4 mCi. ■ Frontal cortex baseline, ▽ frontal cortex 3 h, ▲ cerebellum baseline, ◆ cerebellum 3 h

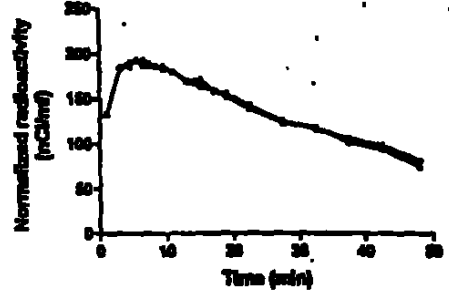


Fig. 4 Regional decay corrected radioactivity of [¹¹C]-NMSP in the frontal cortex (●) and the cerebellum (▲) in a female schizophrenic patient on a stable 6 mg/day risperidone medication

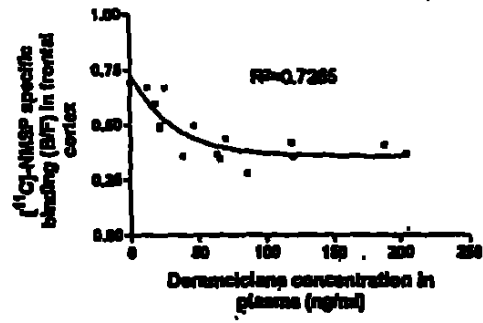


Fig. 5 Relationship between [¹¹C]-NMSP binding (*B/F*) to 5-HT_{2A} receptors and plasma deramciclane concentration in nine healthy male volunteers. Receptor occupancies of 50% and 90% were reached at deramciclane concentrations of 21.1 and 70.0 ng/ml, respectively

to a near complete occupancy of 5-HT_{2A} receptors. Therefore, the estimated 5-HT_{2A} receptor occupancies were calculated using 52% as maximal inhibition. Consequently, the 50% and 90% receptor occupancies were estimated to be reached at plasma concentrations of 21 and 70 ng/ml, respectively.

One subject experienced dizziness after 150 mg intake of deramciclane. This subject had deramciclane 5-HT_{2A} receptor occupancies 38–58%.

Table 1 presents the [¹¹C]-NMSP inhibition data and the calculated 5-HT_{2A} receptor occupancies of deramciclane.

Discussion

In this open exploratory study, we verified the interaction of deramciclane with cortical serotonin 5-HT_{2A} receptors in nine healthy male volunteers using positron emission tomography (PET) and [¹¹C]-NMSP. Deramciclane caused a dose- and concentration-dependent displacement of [¹¹C]-NMSP binding in frontal cortex. Already, the lowest dose caused an average inhibition of [¹¹C]-NMSP which exceeded the test-retest variability of the method in our laboratory (test1/test2 ratios range 0.99–1.08, unpublished data). According to the

Table 1 5-HT_{2A} receptor occupancy and deramciclanc/*N*-desmethyl deramciclanc concentrations after three doses of deramciclanc in healthy male volunteers

Subject no.	¹¹ C]-NMSP inhibition %		5-HT _{2A} occupancy %		Average deramciclanc concentration during PET measurement ^a (ng/ml)		Average <i>N</i> -des methyl-deramciclanc concentration during PET measurement ^a (ng/ml)	
	3 h	6 h	3 h	6 h	3 h	6 h	3 h	6 h
20 mg								
01	17	-	33	-	25.1	-	4.5	-
02	7	-	14	-	12.1	-	1.5	-
03	13 ^b	-	26	-	18.5	-	2.6	-
50 mg								
10	44	36	85	70	70.0	46.5	10.1	13.3
11	24	26	47	49	63.6	38.4	6.3	11.8
12	17	19	33	37	21.6	21.5	2.6	4.1
150 mg								
20	54	43	100	82	86.0	66.0	16.0	22.3
21	30	20	58	38	205	119	34.1	45.1
22	49	56	94	100	189	120	32.1	39.8

^aBaseline study failed; inhibition value was calculated by using a population B/F average 0.692 as baseline value (*n* = 8)

^bMaximum inhibition of [¹¹C]-NMSP binding (52%) corresponds to 100% 5-HT_{2A} occupancy

^cThe average plasma concentration was calculated from the three samples taken during the PET measurement

primary way of analysis, the [¹¹C]-NMSP inhibition percentage varied from 7 to 54% (lowest and highest doses, respectively). It was evident that deramciclanc was maximally able to inhibit on average 52% of [¹¹C]-NMSP binding in frontal cortex (95% confidence limits 41–63%). Considering the selectivity of deramciclanc for the 5-HT₂-like receptors, this could indicate a non-5-HT_{2A} receptor component in [¹¹C]-NMSP binding in the human frontal cortex. [¹¹C]-NMSP has previously been suggested to label 5-HT_{2A} receptors in the frontal cortex (Wong et al. 1984; Lyon et al. 1986; Nyberg et al. 1993). Antipsychotic drugs, like clozapine and risperidone have a high affinity for the 5-HT_{2A} receptor inhibiting up to 94% of [¹¹C]-NMSP binding in these areas (Nordström et al. 1993, 1995; Farde et al. 1994). This is in a good agreement with our own results indicating almost complete (about 98%) inhibition of frontal cortical [¹¹C]-NMSP binding in a risperidone-treated schizophrenic patient. However, clozapine and risperidone are not only selective for 5-HT₂-like receptors, and have high affinity for many neurotransmitter receptor systems.

Despite pharmacological similarities between the 5-HT_{2A} and 5-HT_{2C} receptor subtypes, NMSP has a low affinity for the 5-HT_{2C} receptor. The *K_i* value for rat cloned 5-HT_{2C} receptor was determined to be 1540 nM, indicating over 1000-fold selectivity for the 5-HT_{2A} subtype (Pälvimäki and Hietala, unpublished). In addition, the 5-HT_{2A} receptors clearly outnumber the 5-HT_{2C} receptors in human frontal cortex, making a 5-HT_{2C} receptor binding unlikely (Hoyer et al. 1986). [¹¹C]-NMSP is known to have high affinity for the

dopamine D₂-like receptor subtypes, but the density of these in frontal cortex are also outnumbered many-fold by 5-HT_{2A} receptors. However, NMSP, clozapine and risperidone share a common feature of having a relatively high affinity for α₁-adrenergic receptors. Deramciclanc, in turn, has practically no affinity for the α₁ receptor, which makes an α-adrenergic component of NMSP binding in cortex possible, although this needs to be proven. Nevertheless, this property of [¹¹C]-NMSP does not invalidate the use of this tracer for 5-HT_{2A} receptor occupancy studies for selective drugs, as indicated below. The 52% maximal inhibition can be regarded to represent near 100% occupancy in 5-HT_{2A} receptors. This appears also to be the case for a selective 5-HT_{2A} receptor antagonist, MDL 100 907, which similarly inhibits only 60–70% of frontal cortical [¹¹C]-NMSP binding in healthy volunteers (Grunder et al. 1997). It is, however, also clear that the relatively small number of subjects in the present study decreases the power of the statistical analysis.

Correlation between plasma levels and 5-HT_{2A} receptor availability followed a classical receptor saturation isotherm to a single population of receptors. It should be noted that the reference area, cerebellum, is not totally devoid of 5-HT_{2A} receptors (Pazos and Palacios 1987) but contains up to 10-fold lower 5-HT_{2A} density compared to frontal cortex. This phenomenon may introduce a small (5%) underestimation in the calculated estimates for receptor occupancy (see Nyberg et al. 1993). However, the possible underestimation does not dramatically affect the receptor occupancy values. It may also be that the ratio method used have

underestimated the percentage inhibition relative to the kinetic method (Grunder et al. 1997).

The results suggest that 5-HT_{2A} receptor occupancy (antagonism) is likely to contribute to the clinical effects of deramciclane at least in the upper end of tested doses. Likewise, it may be assumed that 5-HT_{2C} receptors are occupied to the same extent as the affinity of deramciclane for these two receptor subtypes is about the same (Gacsalyi et al. 1997). The data provide relevant concentration ranges to test the efficacy of combined 5-HT_{2A} and 5-HT_{2C} receptor blockade, e.g. in anxiety disorders. The relationship between 5-HT₂-like receptor occupancy (antagonism) and clinical effects are currently not known. In this study, we did not observe any marked subjective effects. Dizziness was the only adverse drug reaction that could have been related to deramciclane and it was observed only in one subject. High 5-HT_{2A} occupancy was not specifically related to this effect. In fact, if anything, this subject had relatively low 5-HT_{2A} receptor occupancies in frontal cortex at 3 h as well as 6 h after deramciclane administration but also a relatively low baseline specific [¹¹C]-NMSP binding. It may be that individual sensitivity in terms of "baseline" 5-HT_{2A} receptor binding sites is important in determining the responses to 5-HT₂ receptor antagonism, but due to the small number of subjects any firm conclusions about this cannot be made.

It is concluded that deramciclane penetrates the blood-brain barrier in humans. Moreover, the maximal displacement of the total [¹¹C]-NMSP from the frontal cortex by deramciclane was about 52%. Deramciclane binds to 5-HT_{2A} receptors in human brain in a saturable manner. The calculated 5-HT_{2A} receptor occupancy of 50% and 90% were reached at plasma deramciclane concentrations of about 21 and 70 ng/ml, respectively.

Acknowledgements The authors wish to thank the staff in the Turku Positron Emission Tomography Laboratory. In addition, Ms. Liisa Huovinen, Ms. Helmi Ruokonen and Dr. Tiina Varis are acknowledged for their skilful assistance in conducting the study.

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XP-000937794

P. 589-597 (9)

International Journal of Clinical Pharmacology and Therapeutics, Vol. 27, No. 124000 (589-597)

Pharmacokinetics and safety of deramciclane during multiple oral dosing

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Key words
deramciclane fumarate -
pharmacokinetics -
tolerability - multiple
administration

Abstract. Deramciclane is a new putative non-benzodiazepine-type anxiolytic compound. It is a selective serotonin 5-HT_{2A} and 5-HT_{2C} receptor antagonist and has also inverse agonist properties. The aim of this study was to reveal the pharmacokinetics and tolerability of deramciclane during repeated oral dosing in healthy male volunteers. **Subjects, material and methods:** A randomized double-blind, placebo-controlled design was used. The study had three consecutive groups that received first a single oral dose of 10, 30 and 60 mg of deramciclane followed by twice a day administration for seven days. The total number of subjects was 28. The pharmacokinetic parameters were calculated for a single dose and after repeated administration. Tolerability was assessed by monitoring safety laboratory variables, electrocardiogram, heart rate, blood pressure and adverse events. **Results:** The steady-state was reached during the seven-day administration. The pharmacokinetics of deramciclane was dose-proportional at steady-state at each dose level. Deramciclane accumulated about three-fold during repeated administration. The relative bioavailability of deramciclane increased about 1.4-fold compared to that of a single dose at each dose level. The mean elimination half-life of deramciclane for 10, 30 and 60 mg doses prolonged from 24.3, 20.9 and 22.9 h after a single dose to 30.5, 25.6 and 28.7 h at steady-state, respectively. Only few adverse events were reported, all mild and transient in nature. The most frequently reported adverse drug reactions were tiredness and headache. There were no deramciclane-induced changes in the clinical chemistry or hematology variables, blood pressure, heart rate or in electrocardiogram. **Conclusions:** In conclusion, the pharmacokinetics of deramciclane is linear over the dose range of 10 - 60 mg at steady-state. The slight non-linearity within the dose levels during repeated administration of seven days was re-

garded as clinically irrelevant. Deramciclane was safe and well tolerated up to doses of 60 mg b.i.d. for seven days.

Introduction

The involvement of central serotonin (5-HT) receptors in different psychiatric disorders has been widely studied since the early 1980s. Introduction of fluoxetine and other selective serotonin re-uptake inhibitors has helped us to understand the neurobiological correlates of depression and anxiety. Consequently, attempts to find anxiolytic agents to replace benzodiazepines has resulted in the discovery of promising new agents, such as the selective 5-HT_{1A} partial agonists buspirone, ipsapirone and gepirone, and the 5-HT₂ antagonists ritanserin and ketanserin [Kastenholz and Crismon 1984, Laysen et al. 1985, Reynjens et al. 1986, Traber and Glaeser 1987]. However, buspirone is the only serotonergic compound which has been approved for the clinical treatment of anxiety [Feighner 1987].

Deramciclane (Figure 1) is a new putative non-benzodiazepine-type anxiolytic compound. In receptor-binding studies in vitro deramciclane has shown selective affinity to 5-HT_{2A} and 5-HT_{2C} receptors [Gacsalyi et al. 1997, Pälvimäki et al. 1998]. Deramciclane is an antagonist of these receptors, and has also been reported to be an inverse agonist in some assays [Pälvimäki et al. 1998]. The psychopharmacology of deramciclane has been studied in a number of animal models, which have shown anxiolytic activity of deramciclane at

Received
February 8, 1998;
accepted in revised form
August 13, 1998

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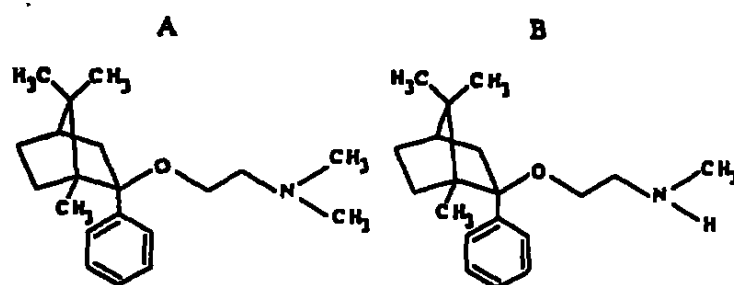


Figure 1. The molecular structures of deramciclane (a) and its metabolite N-desmethyl deramciclane (b).

dose levels 0.5 – 10.0 mg/kg [Gacsalyi et al. 1997].

Deramciclane is rapidly absorbed from the gastro-intestinal tract in rats and dogs [Kanerva et al. 1998a,b, Lengyel et al. 1998]. The absolute bioavailability in rats and dogs is only about 4 and 50%, respectively [Balogh Nemes et al. 1996, Kanerva et al. 1998a,b]. In addition, substantial interspecies variation was observed in the extent of metabolism of deramciclane [Klebovich et al. 1998]. The results of the dog studies suggest that deramciclane has non-linear pharmacokinetics caused by a decrease in total body clearance at doses higher than 1 mg/kg [Kanerva et al. 1998a]. However, in man deramciclane has shown only slight if any non-linearity over the single oral dose range of 3 – 150 mg [Kanerva et al. 1999]. In man the elimination half-life of deramciclane has some dose dependency varying between 20 and 32 h [Kanerva et al. 1999]. In our earlier studies deramciclane was found to be safe and well tolerated after a single oral dose up to 150 mg. There were no changes in hematology or in clinical chemistry. The reported adverse events were mild and transient in nature. The most frequently reported adverse events were headache and dizziness at the highest single dose used (150 mg).

The present study was the first multiple administration of deramciclane in man. The aim of this study was to evaluate the safety of deramciclane in man during repeated administration and to clarify the steady-state pharmacokinetics and possible accumulation of deramciclane and its main metabolite N-desmethyl deramciclane. Moreover, the effect of multiple administration on the linearity of deramciclane pharmacokinetics was evaluated.

Subjects, material and methods

This study was performed according to Good Clinical Practice Guidelines of the European Community. It followed the recommendations for biomedical research involving humans (current revision of the Declaration of Helsinki of the World Medical Assembly).

The study protocol was accepted by the joint Ethics Committee of Turku University Central Hospital and the University of Turku. The Finnish National Agency for Medicines was notified of the study in advance in accordance with national regulations.

Medication

Conventional film-coated deramciclane 10 mg and 20 mg tablets, and matching placebo tablets were used (EGIS Pharmaceuticals, Ltd., Budapest, Hungary). The number of the placebo and deramciclane tablets were equal at each dose level; i.e. 2 × 1 (10 mg) tablet, 2 × 3 (10 mg) tablets and 2 × 3 (20 mg) tablets for the 10, 30 and 60 mg dose groups, respectively, in order to ensure blinding.

Subjects

The subjects were recruited using advertisements placed at the University of Turku Medical School. Altogether 28 healthy Caucasian male volunteers were recruited. All subjects gave their written informed consent prior to the initiation of the studies. They were non-smokers and without clinically significant abnormalities in the physical examination or in the safety laboratory variables. Their demographic characteristics are summarized in Table 1.

Study design

A randomized, placebo-controlled, double-blind study design was applied. The study had three consecutive groups. The first group received 10 mg, the second 30 mg and the third 60 mg of deramciclane twice a day. The study subjects were randomly assigned to re-

Table 1. Demographic data of the healthy male volunteers participating in the study presented as the mean $\pm$ SD (range).

Dose b.i.d.	Age (y)	Weight (kg)	Height (cm)
10 mg	24.2 $\pm$ 1.5 (21.8 – 26.8)	75.4 $\pm$ 8.8 (71.0 – 85.0)	183.0 $\pm$ 8.2 (174 – 188)
30 mg	23.5 $\pm$ 1.0 (22.0 – 24.7)	73.0 $\pm$ 6.8 (66.0 – 85.0)	178.3 $\pm$ 9.8 (170 – 187)
60 mg	22.8 $\pm$ 2.1 (18.7 – 28.1)	76.4 $\pm$ 8.7 (66.0 – 88.0)	183.1 $\pm$ 6.1 (173 – 190)
Placebo	23.0 $\pm$ 2.0 (18.8 – 25.2)	68.1 $\pm$ 3.8 (65.0 – 75.0)	176.0 $\pm$ 4.7 (169 – 183)

ceive either deramciclone or placebo. At the 10 mg and 30 mg dose levels the group size was 8, with 6 subjects on deramciclone and 2 on placebo. In the group receiving 60 mg, the number of subjects was 12; 9 on deramciclone and 3 on placebo. The total number of subjects receiving placebo was 7.

Administration of the drugs

Each subject first received a single oral dose whereafter plasma samples were drawn up to 72 h. Subsequently, on day 4, the twice daily administration was started. The first dose was administered after an overnight fast. The subjects received lunch 4 hours after the first dose. The twice daily administration started on the fourth morning and lasted for 7 days until the morning of the 11th study day when the last dose was given. The tablets were given at 8.00 a.m. and 8.00 p.m. The medication was taken 2 hours before or after meals. Fasting was started at 8.00 p.m. in the evening prior to the last dose and blood samples were then collected as after the first single dose on day one.

Clinical measurements

The subjects were asked to report any symptoms to the study personnel during the study. No leading questions were asked. The severity of all adverse events reported by the subject or observed by the investigator was recorded.

Safety was monitored with laboratory measurements, 12-lead ECG, blood pressure and heart rate measurements and close observation of symptoms. The safety laboratory variables included hematology, serum liver function tests (alanine and aspartate amino-

transferase, γ -glutamyltransferase, total and free bilirubin, alkaline phosphatase), plasma electrolytes (potassium, sodium, calcium), serum glucose and serum creatinine determinations. In addition, level serum lipids were determined at the highest dose.

Blood and urine samples for assessment of safety were taken before the first drug intake, 24 h thereafter and 24 h after the last drug administration. Moreover, samples for hepatic safety assessments were taken in the mornings of the 7th and 10th study day. Supine blood pressure and heart rate were measured and 12-lead ECG was recorded before the drug intake and 3 hours after each drug intake.

Blood sampling

Blood samples for single-dose pharmacokinetic analysis were taken before drug intake (zero sample) and 20 min, 40 min, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 h after the first administration. Single blood samples were taken prior to drug intake in the evening of the 9th day and in the morning and evening of the 10th study day to determine the trough plasma concentrations. To determine the steady-state pharmacokinetics, samples were drawn prior to the last dose and 20 min, 40 min, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h thereafter.

Bioanalytics

The plasma samples were analyzed for deramciclone and N-desmethyl deramciclone concentrations using a liquid chromatography-tandem mass spectrometry and selected ion monitoring technique. The method has been described in detail elsewhere [Kanerva

et al. 1998b]. The calibration curves for deramciclane and N-desmethyl deramciclane were linear over the calibration range of 0.1 – 100 ng/ml and 0.2 – 100 ng/ml, respectively. Samples with concentrations exceeding the upper limit of the calibration range were diluted with drug-free human plasma and reanalyzed. The assay precision was 6.1 and 12% for deramciclane and N-desmethyl deramciclane, respectively, at the lower limit of quantitation.

Pharmacokinetic calculations

The pharmacokinetic parameters were calculated using Siphar/Win for Mac 1.2b program (Simed Biostatistics and Data Processing, Creteil, France). Plasma deramciclane concentration data after the first dose were fitted to a two-compartmental model. The superiority of the two-compartmental model was assessed by using the Akaike criterion. The pharmacokinetic parameters for deramciclane at steady state and for N-desmethyl-deramciclane were calculated using a model-independent method.

The peak (C_{max}) and trough (C_{min}) plasma concentration of deramciclane and the time to reach the peak (t_{max}) were read directly from the observed individual plasma concentration-time data for a single dose and at steady-state. The average concentration at steady state (C_{ss}^{av}) was calculated by

$$C_{ss}^{av} = \frac{AUC_{\tau}}{\tau}$$

The areas under the concentration-time curves (AUC) were calculated using the trapezoidal rule after both single and multiple dosing. The area beyond the last measured time point was estimated by dividing the last concentration with the elimination phase rate constant (λ_z).

The elimination half-life was calculated by

$$t_{1/2} = \frac{\ln 2}{\lambda_z}$$

The percentage of peak trough fluctuation by

$$\%PTF = \frac{C_{max}^{ss} - C_{min}^{ss}}{C_{ss}^{av}}$$

estimated accumulation index by

$$R_{ss} = \frac{1}{1 - e^{-\lambda_z \tau}}$$

where λ_z is the elimination rate for the single dose. The observed accumulation index was calculated by

$$R_{ss}^{obs} = \frac{AUC_1^{ss}}{AUC_1^{sd}}$$

Statistical analysis

The pharmacokinetic parameters are presented as means ($\pm$ SD). Statistical analysis of the pharmacokinetic parameters was performed applying Student's t-test for paired data. A risk level less than 5% ($p < 0.05$) was considered as statistically significant.

Results

Pharmacokinetics of deramciclane

The pharmacokinetic parameters of deramciclane and N-desmethyl deramciclane after the single dose and during repeated administration are presented in Tables 2 and 3. The mean plasma concentration vs. time curves are presented in Figures 2 and 3, respectively.

The mean peak deramciclane plasma concentrations after the first dose (C_{max}) and at steady-state (C_{max}^{ss}) ranged from 12.7 to 73.6 ng/ml and from 33 to 160.8 ng/ml, respectively. Both parameters increased with dose. At each dose level the last 5 consecutive trough values (C_{min}^{ss}) remained comparable (Figure 2). Fluctuation in the peak and trough plasma concentrations at steady-state (PTF%) was about 70% (the mean values ranged from 66 to 76%). It was not affected by the dose.

Time to peak concentration after the first dose (t_{max}) ranged from 1.9 to 3.3 h and was not affected by the dose ($t_{max}^{ss} = 2.7 - 3.3$ h). The dose-normalized $AUC_{0-\infty}^{ss}$ values after a single dose decreased slightly but significantly as the dose was increased assessed using linear regression analysis ($p = 0.0487$), but not significant when assessed using ANOVA. A non-significant trend was observed for the dose normalized steady-state

Table 2. The mean $\pm$ SD pharmacokinetic parameters of deramciclane after a single dose and during multiple dosing of twice a day administration for 7 days in healthy male volunteers.

	10 mg (n = 6)		30 mg (n = 6)		60 mg (n = 9)	
	single dose	last dose	single dose	last dose	single dose	last dose
C_{max} (ng/ml)	12.7 $\pm$ 4.0	33.0 $\pm$ 8.9	38.2 $\pm$ 6.9	91.1 $\pm$ 16.3	73.6 $\pm$ 22.3	160.8 $\pm$ 31.4
t_{max} (h)	2.8 $\pm$ 0.8	3.0 $\pm$ 1.2	3.3 $\pm$ 1.0	3.3 $\pm$ 0.8	1.8 $\pm$ 0.6	2.7 $\pm$ 1.2
AUC_{0-12h} (ng $\cdot$ h/ml)	199.9 $\pm$ 65.8	972.1 $\pm$ 395.6	608.7 $\pm$ 94	2443 $\pm$ 585.8	945.5 $\pm$ 203.7	4489 $\pm$ 1785
AUC_{∞} (ng $\cdot$ h/ml)	93.9 $\pm$ 24.2	289.3 $\pm$ 69.4	261.1 $\pm$ 24.5	799.8 $\pm$ 142.6	453.5 $\pm$ 87.2	1376 $\pm$ 277.7
C_{min} (ng/ml)	—	16.1 $\pm$ 3.9	—	47.7 $\pm$ 10.4	—	75.2 $\pm$ 18.8
C_{tr} (ng/ml)	—	24.1 $\pm$ 5.8	—	69.7 $\pm$ 11.9	—	114.8 $\pm$ 23.1
$t_{1/2\alpha}$ (h)	1.2 $\pm$ 0.52	—	1.6 $\pm$ 0.35	—	0.66 $\pm$ 0.36	—
$t_{1/2\beta}$ (h)	2.2 $\pm$ 1.0	—	1.7 $\pm$ 0.28	—	3.0 $\pm$ 1.2	—
$t_{1/2z}$ (h)	24.3 $\pm$ 5.5	30.5 $\pm$ 7.7	20.9 $\pm$ 1.8	25.6 $\pm$ 4.0	22.6 $\pm$ 2.0	26.7 $\pm$ 6.4
MRT (h)	24.4 $\pm$ 6.5	36.8 $\pm$ 10.6	22.7 $\pm$ 2.6	34.0 $\pm$ 5.5	23.2 $\pm$ 3.2	37.7 $\pm$ 7.9
% $_{ex}$	—	70 $\pm$ 6	—	66 $\pm$ 16	—	76 $\pm$ 17
R_{ac}	3.45 $\pm$ 0.65	—	3.05 $\pm$ 0.21	—	3.3 $\pm$ 0.24	—
R_{ac}^{theor}	—	3.27 $\pm$ 0.85	—	3.11 $\pm$ 0.78	—	3.06 $\pm$ 0.57

* τ equals 12 hTable 3. The mean $\pm$ SD pharmacokinetic parameters of N-desmethyl deramciclane after single and multiple twice a day oral dosing of deramciclane for seven days in healthy male volunteers.

	10 mg (n = 6)		30 mg (n = 6)		60 mg (n = 9)	
	single dose	steady state	single dose	steady state	single dose	steady state
C_{max} (ng/ml)	3.66 $\pm$ 0.66	14.38 $\pm$ 2.82	11.3 $\pm$ 3.8	40.1 $\pm$ 6.5	20.0 $\pm$ 6.0	75.9 $\pm$ 14.6
t_{max} (h)	7.0 $\pm$ 1.1	4.63 $\pm$ 1.83	6.7 $\pm$ 1.0	3.1 $\pm$ 1.4	5.1 $\pm$ 2.6	5.2 $\pm$ 2.21
AUC_{0-12h} (ng $\cdot$ h/ml)	27.8 $\pm$ 6.4	136.7 $\pm$ 31.1	93.9 $\pm$ 26.1	382.2 $\pm$ 68.2	174.3 $\pm$ 39.7	551.4 $\pm$ 195
R_{ac}^{met}	—	5.06 $\pm$ 1.44	—	4.49 $\pm$ 1.83	—	3.39 $\pm$ 1.81

values (AUC_{∞}^{met}). In contrast, within each dose level the AUC_{∞}^{met} values were significantly greater than AUC_{∞}^{par} . The relative bioavailability of deramciclane was about 1.4 times higher at steady-state than after a single dose. Comparison of the AUC_{∞}^{par} and the AUC_{∞}^{met} for 10, 30 and 60 mg b.i.d. dose levels resulted in p-values of 0.0007, 0.0314 and 0.0003, respectively (Student's t-test). Compared with the single dose values, the mean elimination half-life $t_{1/2z}$ was prolonged in repeated administration by 6.3, 4.7 and 5.8 h for 10, 30 and 60 mg dose levels, respectively. The retardation in the elimination was statistically significant for 10 mg ($p = 0.049$) and 60 mg b.i.d. ($p = 0.012$) dose levels and approached statistical significance for the 30 mg b.i.d. ($p = 0.052$) dose level.

Deramciclane accumulation was about three-fold after repeated administration AUC_{∞}^{par} for 7 days compared to the single dose administration (AUC_{∞}^{par}). The theoretical

accumulation index, R_{ac} , did not differ from the observed accumulation R_{ac}^{obs} .

Pharmacokinetics of N-desmethyl deramciclane

The C_{max} of the metabolite was reached at 5.1 – 7.0 h post-dosing, and it increased linearly with the dose. The multiple administration increased the peak concentrations about four-fold. Similar to C_{max} values the AUC_{∞} increased dose-proportionally at each dose level after a single and multiple dosing.

The accumulation of N-desmethyl deramciclane was highest during the deramciclane 10 mg b.i.d. regimen, and it had a tendency to decrease with increasing dose. The metabolite accumulated more than the parent compound at the two lowest dose levels but the accumulation was similar at the highest dose level.

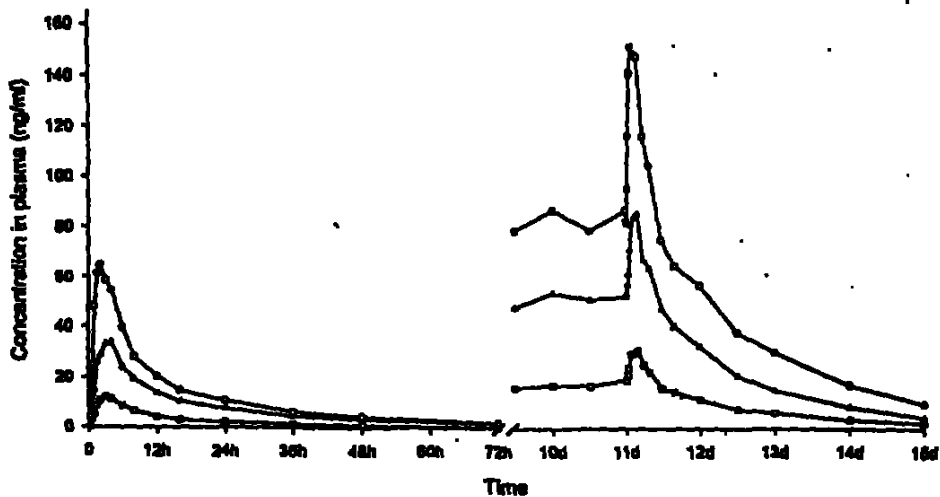


Figure 2. The mean plasma concentration vs. time curves of deramciclane after single oral dosing of 10 (open squares), 30 (filled triangles), and 60 mg (open spheres) and at steady state after 7-day b.i.d. administration. The standard deviations are not shown for clarity.

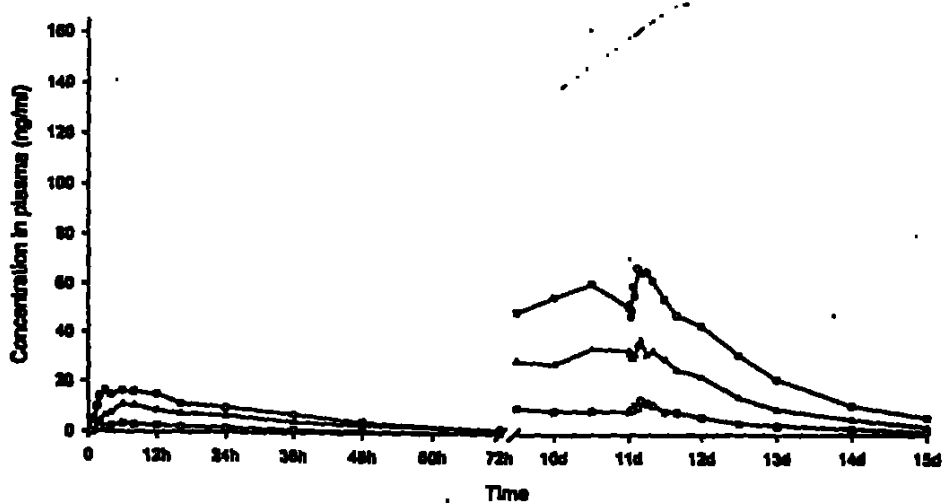


Figure 3. The mean plasma concentration vs. time curves of N-desmethyl deramciclane after single oral dosing of 10 (open squares), 30 (filled triangles), and 60 mg (open spheres) of deramciclane and at steady state after 7-day b.i.d. administration. The standard deviations are not shown for clarity.

Safety and tolerability

Deramciclane was well tolerated over the whole dose range studied. There were no serious adverse events during the study. All of the adverse events were transient and mild or moderate in severity. Tiredness was reported once in the 30 mg and twice in the 60 mg group. In the 60 mg b.i.d. group tiredness occurred during the first study day and was resolved during the second study day; one subject belonging to the 30 mg b.i.d. group

reported tiredness only after the last dose. Feelings of palpitations and chest pain were reported by one subject in the 60 mg group on the 5th and 6th study days, respectively. The ECG was recorded and found to be normal, and the causality with treatment was assessed as unlikely. Both symptoms resolved spontaneously without specific treatment.

There were no treatment-related changes in hematology or clinical chemistry variables. Moreover, there were no dose-dependent changes or trends in the individual safety lab-

oratory variables. There were, however, two subjects in the placebo group, one in the 10 mg group and two in the 60 mg group who had time-dependent elevations in the individual S-GPT values which exceeded the higher reference limit of 60 U/l. It should be noted that the highest elevation from 40 to 245 U/l was seen in the placebo group. In the analysis of mean S-GPT activities, no drug-related differences in changes were seen between the deramciclane and placebo groups.

Fasting serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured only in 3 control subjects and in 9 subjects receiving deramciclane 60 mg b.i.d. No clear drug-related changes were seen in these parameters.

There were no changes or drug-related differences between deramciclane and placebo groups in the mean systolic and diastolic blood pressure, heart rate or ECG in the overall analysis or when the dosage levels were analyzed separately.

Discussion

The objectives of the present study were to examine the pharmacokinetics and tolerability of deramciclane when given as a single dose and repeatedly for 7 days at doses of 10, 30 and 60 mg twice daily to healthy young male volunteers. A placebo group was included into the study for comparison.

The pharmacokinetic parameters of the single doses were similar to those observed in previous single dose studies in man [Kanerva et al. 1999], although the C_{max} and AUC values of deramciclane had a tendency to increase less than proportionately to the dose. In our earlier studies, the pharmacokinetics of deramciclane after single oral administration was linear over the dose range of 3–50 mg. However, a slight non-linearity was suggested over the dose range of 3–150 mg because the $AUC_{0-\infty}$ increased more than expected from the dose increment [Kanerva et al. 1999]. In the present study, linear regression analysis suggests a significant decrease in the dose-normalized AUC values. However, this could be a false-positive finding since in ANOVA model no difference was found. This was not a crossover study, but all 3 groups of subjects received different dose.

Therefore, interindividual variation, rather than increased elimination or decreased absorption, might have resulted in the slight decrease in the dose-normalized AUC values with increasing doses of deramciclane. In conclusion, the pharmacokinetics of deramciclane is probably linear over the dose range of 10–60 mg after a single oral administration.

Since C_{ss} remained constant, the steady-state was reached with twice daily dosing regimen during the 7 days of administration. At each dose level the increase in the $AUC_{0-\infty}$ values was linear and dose-proportional. The accumulation of deramciclane during multiple dosing was about 3-fold over the 7-day dosing period. Substantial accumulation was expected because the dosing interval was about half of the elimination half-life of deramciclane. The $AUC_{0-\infty}$ values were significantly greater than AUC_{0-t} values at each dose level. Multiple dosing caused prolongation in the elimination half-life of deramciclane: the change in $t_{1/2\alpha}$ was significant at 10 and 60 mg b.i.d. but not at 30 mg b.i.d. dose levels, also suggesting non-linearity during multiple administration. Higher $AUC_{0-\infty}$ values than expected could indicate either decreased total body clearance, increased volume of distribution of deramciclane or increased bioavailability. In dogs the non-linearity of deramciclane was caused by decreased total body clearance [Kanerva et al. 1998a]. In opposite, the observed accumulation (R_{ac}^{obs}) did not differ from the theoretical accumulation (R_{ac}) at any dose level, suggesting linear pharmacokinetics. The conflicting results are probably caused by the inaccuracy of the parameters used to calculate the R_{ac} , because deramciclane obeyed the two-compartmental model. The terminal elimination rate constant λ_z does not take into account elimination of deramciclane during the distribution phase. Therefore, it is possible, that R_{ac} is overestimated. In order to obtain more accurate estimation for R_{ac} an effective elimination rate constant should be used. Unfortunately, this is not possible without intravenous administration. Thus, accumulation index may not be a reliable parameter in this study.

These data indicate that deramciclane accumulates more than expected during a 7-day dosing. Therefore, more information on the

accumulation of deramciclane and N-desmethyl deramciclane during repeated administration should be obtained to exclude continuing cumulation during longer treatment periods and to evaluate the relevance of this phenomenon during therapeutic use of deramciclane. Nevertheless, the changes in the relative bioavailability of this magnitude does not have any clinical relevance.

Deramciclane was well tolerated in the dosage range studied. The cardiovascular safety of deramciclane was good. In the previous single dose study a slight decrease in blood pressure (about 7 mmHg) was observed approximately at the t_{max} of N-desmethyl deramciclane (6 h) [Känerva et al. 1999]. However, in this study there were no signs of decreased blood pressure even though t_{max} was reached somewhat earlier during repeated administration of deramciclane than after single dosing.

The subjective adverse effects reported by the volunteers or seen in the laboratory parameters were mild and unspecific. No untoward effects were seen in the hematology parameters which would have been clearly related to deramciclane treatment. The only exception was elevation of liver transaminase activity (about 2-fold over the upper reference limit) in one volunteer receiving 60 mg deramciclane b.i.d. This elevation, however, was not associated with high concentrations of deramciclane in plasma. Moreover, another volunteer receiving placebo had an even more pronounced elevation in liver aminotransferase activity (almost 3-fold the upper reference limit). In both subjects the change was completely reversible after the termination of the study. Among others, some drugs and viral infections may cause elevation of S-GPT activity, which is considered as a fairly specific indicator of damage to liver parenchymal cells. S-GPT activities exceeding 10-fold the upper reference limit can be seen after drug- or virus-induced liver toxicity. Slight to moderate increases of serum alanine aminotransferase activities have been reported in healthy volunteers during hospitalization for clinical studies without any treatment or during placebo treatment [Kanamaru et al. 1989, Kobayashi et al. 1993]. In some studies the elevated enzyme activity has been related to the diet, especially when the carbohydrate content of the diet is

high [Porikos and van Itallie 1983]. Indeed, it was observed that in the present study the subjects who had elevated S-GPT activities ate more than the others and gained weight. Therefore, the elevated S-GPT activity was not regarded to be related to deramciclane treatment.

Deramciclane was found to be safe and well tolerated up to doses of 60 mg b.i.d. for seven days. Since higher doses have not been administered to human at this stage, 60 mg is regarded as the maximal tolerated dose in repeated administration.

The pharmacokinetics of deramciclane was linear between the dose levels for a single dose and at steady-state. In twice daily dosing for 7 days, deramciclane accumulated about 3-fold. After 7-day administration, the relative bioavailability of deramciclane increased about 1.4-fold compared to that of a single dose.

Acknowledgements

The authors wish to thank Ms. Annamari Sauramo, CRA, for her valuable help in collecting the data. Ms. Taina Lehti and Elina Kahra are acknowledged for their expert technical assistance.

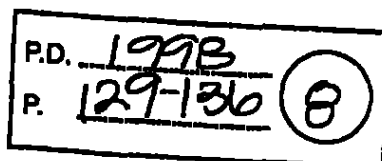
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Pharm. Pharmacol. Commun. 1998, 4: 129-136
Received September 22, 1997
Accepted October 9, 1997



XP-000937807

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Comparative Pharmacokinetics of Deramciclone in Rat, Dog, Rabbit and Man After the Administration of a Single Oral Dose of 3 mg kg^{-1}

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Abstract

A comparative pharmacokinetic study of deramciclone fumarate (EGIS-3886), a new 5-HT_2 antagonist with anxiolytic activity, was performed in rats, dogs, rabbits and healthy volunteers after the administration of a single oral dose of 3 mg kg^{-1} .

Considerable inter-species differences were revealed, particularly with respect to C_{max} , $\text{AUC}_{0-\infty}$ and $t_{1/2}$ values. Deramciclone showed rapid absorption in all cases (t_{max} : 0.3–2.6 h), whereas its elimination differed in different species ($t_{1/2}$: 4.05–12.00 h in animals and 31–61 h in man). After the administration of a single 3 mg kg^{-1} oral dose of deramciclone, the $\text{AUC}_{0-\infty}$ value of the parent compound showed a significant species difference. The $\text{AUC}_{0-\infty}$ in rats, rabbits and dogs were 0.3, 13 and 21%, respectively, of that in man. The percentile ratio of unchanged deramciclone and total radioactivity (R_{DERAM}) appeared to be extremely low and varied between 0.2 and 4.6%. The Vd kg^{-1} values suggested strong tissue binding in all the species studied.

The plasma levels and pharmacokinetic parameters of *N*-desmethyl-deramciclone metabolite (EGIS-7056) were also studied and determined in the above species. The comparative pharmacokinetic study of deramciclone indicated an intensive biotransformation in all species although the rate of biotransformation appeared to be species-dependent.

Deramciclone fumarate (EGIS-3886; (1*R*, 2*S*, 4*R*)-(–)-*N,N*-dimethyl-2-[(1,7,7-trimethyl-2-phenylbicyclo-[2,2,1]-hept-2-yl)oxy]-ethanamine-2-(*E*)-butendioate (1:1)); Figure 1) is a new, non-benzodiazepine type of anxiolytic compound (Budai et al 1980; Gacsályi et al 1988; Mezei et al 1995), synthesized at EGIS Pharmaceuticals Ltd (Budapest, Hungary).

Deramciclone possesses high affinity for 5-HT_{2A} and 5-HT_{2C} receptors, where it exerts antagonistic effects. It has a moderate affinity for sigma receptors and a weak affinity for D_2 receptors. The antiserotonergic effect of the compound is much more pronounced centrally than peripherally (Gacsályi et al 1996a, 1998). Deramciclone has shown anxiolytic effects in animal models over the dose range 0.5–10 mg kg^{-1} .

In animal models deramciclone, unlike benzodiazepines, does not induce muscle relaxation or sedation. Moreover, deramciclone does not potentiate the effects of alcohol even if it is administered in extremely high doses in animals (Kovács et al 1989; Berényi et al 1990; Gacsályi et al 1998).

Radiolabelled deramciclone containing either ^3H or ^{14}C at different positions (Szammer et al 1996, 1997) was not absorbed from the isolated rat, rabbit

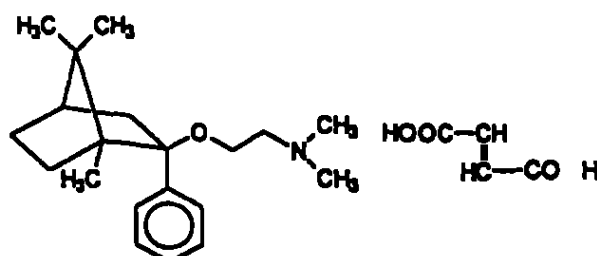


Figure 1. Chemical structure of deramciclone fumarate.

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or dog stomach. However, the absorption of the compound was very fast and complete from various isolated small-intestine sections (duodenum, jejunum, ileum) (Lengyel et al 1998a). In rats treated with radiolabelled deramciclane, the radioactivity distributed very rapidly between various organs and tissues, underwent a relatively rapid elimination and did not appear in the foetus (Hazai et al 1997; Hazai, unpublished results). Within 16 h after treatment with a 3 mg kg^{-1} oral dose, 47 and 16% of the administered radioactivity were excreted with bile in rats and dogs, respectively (Lengyel et al 1998a). A significant enterohepatic recirculation was observed in the rat (Pátfalusi et al 1997).

The administered radioactivity readily penetrated through the blood-brain barrier of the central nervous system and showed a very rapid distribution between various cerebral regions (Magyar, unpublished results).

The absolute bioavailability (F) of deramciclane amounted to 3–4% in rats receiving a 10 mg kg^{-1} dose (Balogh Neimes et al 1996a), to 45–61% in dogs treated with $1\text{--}6 \text{ mg kg}^{-1}$ (Kanerva et al 1997a) and to 43% in rabbits given 3 mg kg^{-1} (Lengyel et al 1998b). Deramciclane was highly (95–98%) bound to plasma proteins (α_1 -AGP, albumin, or both) in all the species studied (mouse, rat, dog, rabbit, man) (Visy et al 1996).

In-vitro metabolism studies using rat, canine and human hepatic microsomal fraction demonstrated that deramciclane was intensively metabolized (Monostory et al 1994; Klebovich et al 1995). The in-vivo metabolism studies in rats and dogs revealed species-specific metabolite patterns (Hazai et al 1995; Klebovich & Ürmös 1996; Ludányi et al 1997).

In the present study, we compared the pharmacokinetic curves and the calculated pharmacokinetic parameters obtained after the administration of a single 3 mg kg^{-1} oral dose of deramciclane fumarate to rats, dogs, rabbits and healthy volunteers (2.76 mg kg^{-1}). In addition, pharmacokinetic properties and the respective species differences of the pharmacologically active metabolite, *N*-desmethyl-deramciclane (EGIS-7056; Gacsályi et al 1996b), were investigated.

Materials and Methods

Standard chemicals

Deramciclane fumarate (Figure 1), *N*-desmethyl-deramciclane fumarate (EGIS-7056) metabolite standard, as well as bencyclane fumarate used as internal standard for the bioanalytical work with rat, dog and rabbit plasma, were synthesized by EGIS Pharmaceuticals Ltd (Budapest, Hungary). ^{14}C -phenyl-deramciclane was obtained from the Central Research Institute for Chemistry of the Hungarian Academy of Sciences (Budapest, Hungary). The specific activity and radiochemical purity of the compound were $1.35 \text{ Gbq mmol}^{-1}$ and >98.5%, respectively. The deuterated deramciclane, used as internal standard in the HPLC-MS-MS bioanalytical method elaborated for the determination of the parent compound and its metabolite in human plasma, was synthesized by Orion Pharma (Espoo, Finland).

Study design for the treatment of various species

Treatment of rats and rabbits. After five days of acclimatization, healthy male Wistar rats and female white New Zealand rabbits kept in a standardized environment received a single oral dose of 3 mg kg^{-1} after fasting. The rats received unlabelled deramciclane and the rabbits received ^{14}C -phenyl-deramciclane. The test compound was applied through a gastric tube as a 0.4–0.5% suspension in methylcellulose in a volume of 1.5 or 5 mL kg^{-1} , respectively (Klebovich et al 1996; Lengyel et al 1998b). The animals had free access to drinking water throughout the study.

The demographic data of the experimental animals are shown in Table 1.

For studying the pharmacokinetics of deramciclane in rats, five animals were treated for each sampling time. Blood samples (5 mL from each animal) were supplemented with 0.5 mL of 3% $\text{Na}_2\text{-EDTA}$ solution applied as anticoagulant. The study in rabbits was of self-control design in which $150 \mu\text{L}$ of 2% $\text{Na}_2\text{-EDTA}$ was added as anticoagulant to 1.5 mL blood.

For both species, the plasma samples separated by centrifugation were kept below -18°C until sample processing.

Table 1. Data relating to the animals used and human subjects participating in the comparative study.

Parameter	Rat	Dog	Rabbit	Healthy volunteers
Dose (mg kg^{-1} , p.o.)	3.0	3.0	3.0	2.76
Sex	Male	Male	Female	Male
Age (years)	0.2	0.8	0.3	23.8
Body weight (kg)	0.19 ± 0.01	12.2 ± 1.0	2.6 ± 0.3	76.1 ± 6.8

Treatment of dogs. After 19 days of acclimatization, male Beagle dogs kept in a standardized environment were treated with 3 mg kg^{-1} deramciclane (Kanerva et al 1997a, b). The animals received the appropriate amount of the active substance calculated on the basis of their body weight in a hard gelatine capsule after 12 h of fasting. Water was freely available. The data of the experimental animals are shown in Table 1. Blood samples were collected with EDTA-treated 10-mL vacutainers (Venject, Leuven, Belgium). The plasma samples sequestered by centrifugation of blood were kept at -70°C .

Human pharmacokinetic study. In a clinical trial, healthy male volunteers ingested deramciclane fumarate in tablets containing 208.3 mg of the active substance (150 mg deramciclane base). By using the mean body weight of the subjects in the present study the relative dose was $2.76 \pm 0.24 \text{ mg kg}^{-1}$ (in deramciclane fumarate). The tablets were produced by EGIS Pharmaceuticals Ltd (Budapest, Hungary).

The dose calculated for a mean body weight of 76.1 kg was almost identical with the 3 mg kg^{-1} oral dose administered in animal experiments. Accordingly, the dose applied in the present series of investigations was independent of the species and did not require extrapolation, whereby it allowed a direct comparison of the single dose pharmacokinetics of deramciclane across the four species.

Healthy volunteers ingested the tablet with 250 mL water after overnight fasting and they were not allowed to eat for 4 h after treatment. The demographic data of the subjects participating in the clinical trial are shown in Table 1. Blood samples were collected through an indwelling catheter. The $\text{Na}_2\text{-EDTA}$ -containing plasma samples separated from blood were kept below -70°C until processing.

Bioanalytical methods

The bioanalytical method applied for the simultaneous determination of deramciclane and *N*-desmethyl-deramciclane metabolite in rat and rabbit plasma was a gas chromatographic method using mass selective detection (GC-MSD) (Hewlett-Packard HP 5890 Series II-HP 5971, Palo Alto, CA) (Lengyel et al 1998b). Before chromatographic analysis, the plasma samples were processed with solid-phase extraction (SPE) (RP-18) optimized with ^{14}C -deramciclane due to the extremely low plasma levels (Balogh Nemes et al 1996b). The ions followed by selective ion monitoring (SIM) were 58, 162 and 86 m/z for the parent compound, the metabolite and the bency-

clane internal standard, respectively. The sensitivity (LLOQ) of the validated GC-MSD method was 100 pg/0.5 mL for deramciclane and 300 pg/0.5 mL for *N*-desmethyl-deramciclane (Lengyel et al 1998b).

In dog plasma, the compounds of interest were determined after automatized SPE (RP-18) sample processing with a gas chromatographic method using nitrogen selective detection (GC-NPD) (Hewlett-Packard HP 5890 Series II). Bencyclane was used as an internal standard (Klebovich et al 1994). The sensitivity (LLOQ) of the validated bioanalytical method was 2.5 ng mL^{-1} for both solutes (Kanerva, unpublished results).

For the determination of deramciclane and *N*-desmethyl-deramciclane in human plasma, deuterated deramciclane was applied as internal standard. Following liquid-liquid extraction with diethyl ether, the solutes were determined with high-performance liquid chromatography-tandem mass spectrometry (HPLC-API-MS-MS) (Sciex API III, Perkin Elmer, Thornhill, Ontario, Canada) using selected reaction monitoring technique (SRMT). The sensitivity (LLOQ) of the method was 20 pg mL^{-1} for deramciclane and 100 pg mL^{-1} for the metabolite (Heikkinen 1995a, b).

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated from the plasma concentration-time data with SIPHAR/WIN ver. 1.2b, or SIPHAR/WIN for Mac 1.2 (SIMED SA.. Biostatistics and Data Processing, Creteil Cedex, France) validated pharmacokinetic software.

In the case of maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}), measured and observed values are presented. The area under the plasma concentration-time curve was determined by a non-compartmental method applying the trapezoidal rule (AUC_{0-t}) and extrapolation to infinity ($\text{AUC}_{0-\infty}$). The AUC_{Res} values denote the percentile ratio, the extrapolated area ($\text{AUC}_{1-\infty}$) represents in the total AUC ($\text{AUC}_{0-\infty}$). The terminal elimination rate constant (β) was calculated by linear regression using the least-squares method, which fitted a straight line to terminal phase of the semi-logarithmic plot of the plasma concentration-time data. Half-life of elimination ($t_{1/2}$) was obtained from the formula of $0.693/\beta$. The values of total body clearance (CL_{Tot}) and those of the apparent volume of distribution (V_d) were calculated as $\text{Dose}/\text{AUC}_{0-\infty}$ and as $\text{Dose}/\beta \text{ AUC}_{0-\infty}$, respectively. The formula of $\text{AUMC}/\text{AUC}_{0-\infty}$ was applied to determine the mean residence time (MRT) values.

The approximate percentage (R_{DERAM}) of unchanged parent compound in the systemic circulation in rabbits was determined according to $AUC_{0-\infty} \text{ Deramciclane} / AUC_{0-\infty} \text{ Total radioactivity}$.

These AUC values were determined from the same plasma samples by different bioanalytical methods (GC-MSD; liquid scintillation counting).

The percentile ratio (R_{METAB}) of *N*-desmethyl-deramciclane, the pharmacologically active metabolite (Monostory et al 1994; Gacsályi et al 1996b) and the parent compound in the systemic circulation was calculated with the following formula:

$$AUC_{0-\infty, \text{ Metabolite}} / AUC_{0-\infty, \text{ Deramciclane}}$$

Statistical analysis

The results of the pharmacokinetic calculations are given as mean $\pm$ s.d. values. Statistical evaluation was made with Statistica for Windows ver. 4.5 software (StatSoft Inc., Tulsa, OK), Excel ver. 5.0 software (Microsoft Corp., USA), and with SAS ver. 6.11. software (SAS Institute Inc., Cary, NC).

Results

The course of the plasma concentration curves obtained after the administration of a single 3 mg kg^{-1} oral dose of deramciclane fumarate to

rats, dogs, rabbits and healthy volunteers showed considerable species-specific differences (Figure 2). The time to reach maximum plasma concentration ($t_{\max}$) was different in the four species studied and varied between the extremely fast absorption (0.3 h) in the rat and the somewhat slower absorption (2.6 h) in man. The maximum plasma concentration ($C_{\max}$) values also showed significant differences. The peak plasma levels detected in rats, dogs and rabbits were 2, 48 and 92%, respectively, of that measured in man (Table 2). The $AUC_{0-\infty}$ values showed a similar tendency; the values calculated in rats, dogs and rabbits were 0.3, 21 and 13%, respectively, of that observed in man.

The elimination half-life ($t_{1/2}$) appeared to be significantly lower in rats (4.05 h); and rabbits (5.77 h), than in dogs (12.00 h) or man (31.61 h) (Table 2). Accordingly, plasma levels of deramciclane could be followed up to 12 h after dosing in rats, and for more than 96 h after drug ingestion in healthy volunteers (Figure 2).

There was a reversed tendency concerning total body clearance ($CL_{\text{Tot}} \text{ kg}^{-1}$) values (rats: $251.6 \text{ L h}^{-1} \text{ kg}^{-1}$; man: $0.9 \text{ L h}^{-1} \text{ kg}^{-1}$). The values of the apparent volume of distribution ($V_d \text{ kg}^{-1}$) indicated a strong tissue binding in all the species studied, although the extent varied from species to

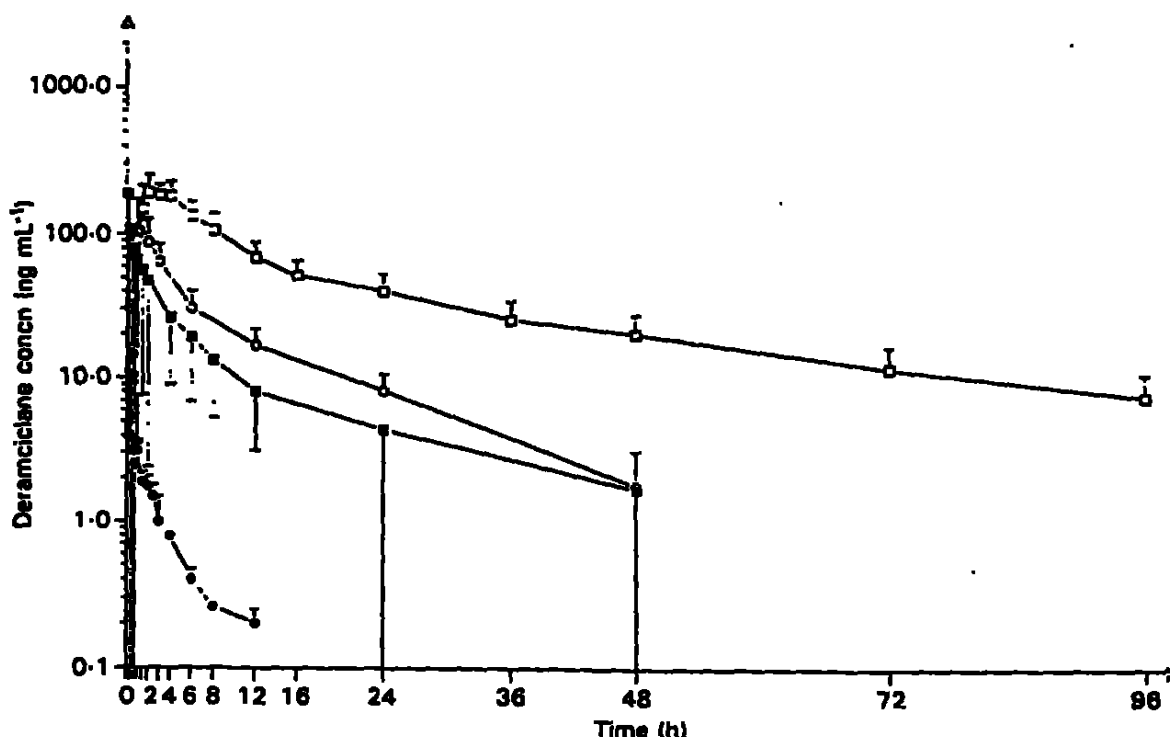


Figure 2. Comparison of the pharmacokinetic curves of deramciclane administered as a single 3 mg kg^{-1} oral dose to ● rat ($n=5$); ○ dog ($n=5$); ■ rabbit ($n=5$); □ man ($n=12$). Data represent means $\pm$ s.d.

PHARMACOKINETICS OF ORAL DERAMCICLANE IN VARIOUS SPECIES

Table 2. Comparison of the pharmacokinetic parameters of deramciclane in various species treated with a single 3 mg kg⁻¹ oral dose.

Pharmacokinetic parameter	Rat (n = 5)	Dog (n = 5)	Rabbit (n = 5)	Healthy volunteers (n = 12)
C _{max} (ng mL ⁻¹)	5.4	105.3 ± 29.2	199.4 ± 185.6	217.5 ± 53.9
t _{max} (h)	0.3	1.8 ± 0.7	1.4 ± 2.6	2.6 ± 0.9
AUC _{0-∞} (ng h mL ⁻¹)	11.9	802.2 ± 216.8	503.8 ± 286.6	3737.8 ± 1017.9
t _{1/2} (h)	4.05	12.00 ± 2.47	5.77 ± 1.99	31.61 ± 8.55
CL _{tot} /kg (L h ⁻¹ kg ⁻¹)	251.6	3.8 ± 1.0	13.7 ± 16.4	0.9 ± 0.4
Vd/kg (L kg ⁻¹)	1469.1	65.2 ± 19.3	111.2 ± 124.4	38.0 ± 12.9
MRT (h)	4.1	15.6 ± 5.7	6.0 ± 2.0	33.5 ± 8.6
AUC _{REM} (%)	15.2	NA	5.4	9.4
R _{DERAM} (%)	0.2*	0.4†‡	4.6	NA

NA = no data; * From Pátfalusi et al (1997); † Dose: 1 mg kg⁻¹; ‡ From Hazai (unpublished results).

species. The MRT values ranged between 4.1 and 33.5 h in the four species investigated (Table 2).

In rabbits the ratio of the AUC_{0-∞} of the unchanged deramciclane and the AUC_{0-∞} of the total radioactivity (parent compound and metabolites) was 4.6% (R_{DERAM}).

The most important pharmacokinetic parameters of deramciclane determined in the four species after the administration of a single 3 mg kg⁻¹ oral dose of the compound are compared in Figure 3.

The peak concentration (C_{max}) of *N*-desmethyl-deramciclane metabolite (Gacsályi et al 1996b) measured in plasma samples of different species, appeared between 0.33 and 5.5 h (t_{max}) and amounted to 1.2 ng mL⁻¹, 56.9 ng mL⁻¹, 21.1 ng mL⁻¹ and 56.1 ng mL⁻¹ in the rat, dog, rabbit and man, respectively. The AUC_{0-∞} value of the

metabolite was three magnitudes higher in man than in the rat (1921.1 vs 3.7 ng h mL⁻¹) (Table 3). The elimination half-life (t_{1/2}) of the *N*-desmethyl-metabolite showed great species differences (rat: 2.68 h; man: 27.06 h) (Table 3). The pharmacokinetic profile of the metabolite is shown in Figure 4. The plasma levels of *N*-desmethyl-deramciclane could be followed for 12 h in rats and for a minimum of 96 h in man.

The *N*-desmethyl-metabolite/deramciclane ratio (R_{METAB}) showed a great variation from species to species (Table 3; Figure 5).

Discussion

The comparative pharmacokinetic study of orally administered deramciclane in rats, dogs, rabbits and

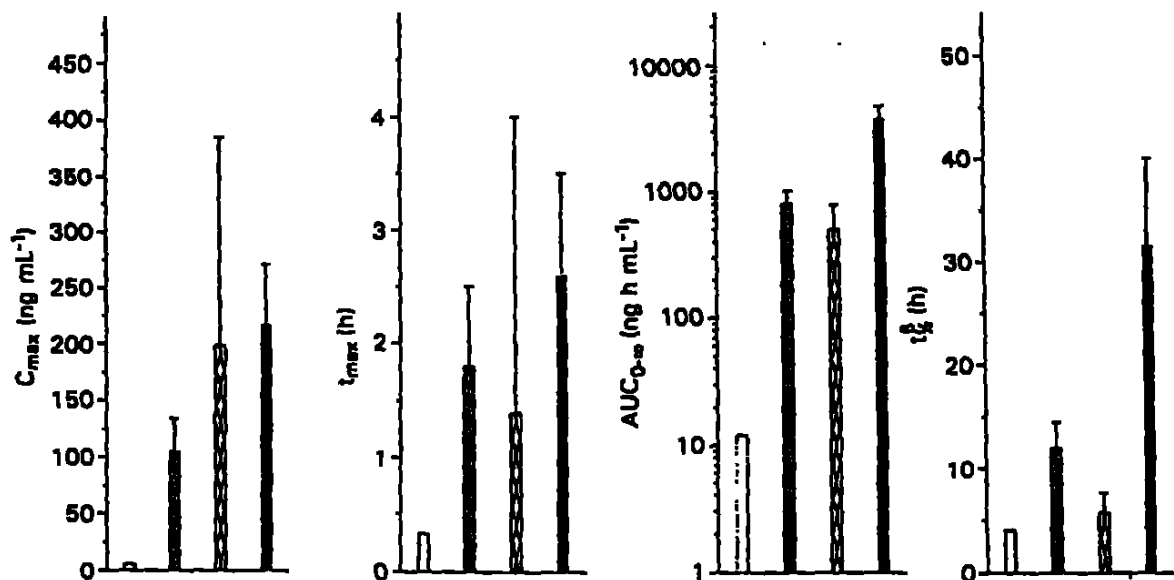


Figure 3. Comparison of the main pharmacokinetic parameters of deramciclane administered as a single 3 mg kg⁻¹ oral dose to □ rat (n = 5); ▨ dog (n = 5); ▩ rabbit (n = 5); ■ man (n = 12). Data represent means ± s.d.

Table 3. Comparison of the pharmacokinetic parameters of *N*-desmethyl-deramciclane metabolite in different species after the administration of a single 3 mg kg⁻¹ oral dose of deramciclane.

Pharmacokinetic parameter	Rat (n = 5)	Dog (n = 5)	Rabbit (n = 5)	Healthy volunteers (n = 12)
C _{max} (ng mL ⁻¹)	1.2	56.9 ± 14.3	21.1 ± 14.8	56.1 ± 18.6
t _{max} (h)	0.33	2.1 ± 0.6	0.2 ± 0.1	5.5 ± 1.5
AUC _{0-∞} (ng h mL ⁻¹)	3.7	888.7 ± 44.7	52.2 ± 15.3	1921.1 ± 225.1
t _{1/2} (h)	2.68	9.94 ± 0.73	4.42 ± 2.79	27.06 ± 4.92
R _{METAB} (%)	31.1	110.8	8.5	51.4

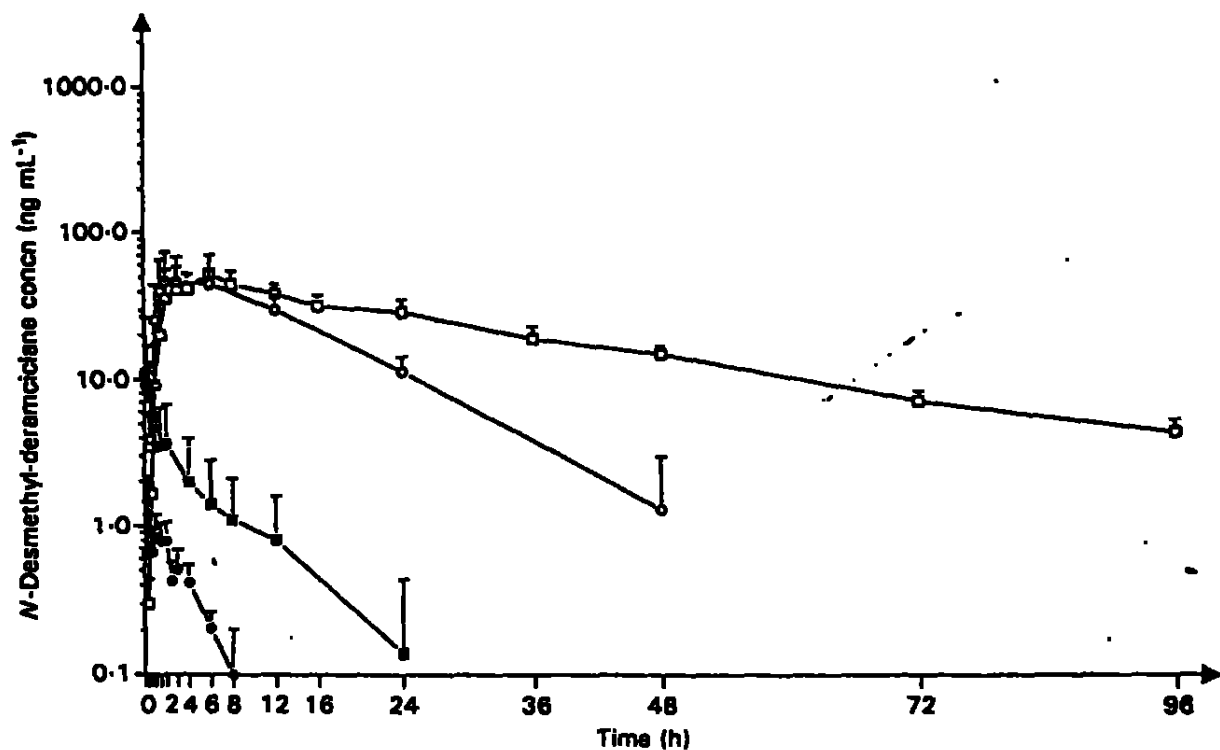


Figure 4. Comparison of plasma concentration curves of *N*-desmethyl-deramciclane after the administration of a single 3 mg kg⁻¹ oral dose of deramciclane to ● rat (n = 5); ○ dog (n = 5); ■ rabbit (n = 5); □ man (n = 12). Data represent means ± s.d.

man treated with a single 3 mg kg⁻¹ dose revealed significant species differences concerning the main pharmacokinetic parameters (Figure 3). Species differences appeared to be the most prominent with respect to the pharmacokinetic parameters of C_{max}, AUC_{0-∞} and t_{1/2}. Deramciclane was rapidly absorbed (t_{max}: 0.3–2.6 h) in all the species studied. The elimination of the compound was highly species dependent (t_{1/2}: 4.05–31.61 h).

Absorption studies with radiolabelled deramciclane (³H and ¹⁴C) unequivocally demonstrated that the compound was completely absorbed from various sections of the small intestine (Lengyel et al 1998a), therefore the different systemic exposure (AUC_{0-∞}) to the unchanged parent drug should be ascribed to species differences in the rate and extent of the

metabolism of deramciclane. The value of the apparent volume of distribution indicated strong tissue binding in all the species studied.

Comparing the AUC_{0-∞} values of unchanged deramciclane to that of the total radioactivity in rabbits with those previously reported in rats (Pátfalusi et al 1997) and dogs (Hazai, unpublished results) it was apparent that due to the intensive metabolism only a negligible portion of the blood level of total radioactivity could be ascribed to unchanged deramciclane (R_{DERAM} = 0.2–4.6%) (Table 2).

The *N*-desmethyl-deramciclane metabolite appeared in the plasma samples of each species. Although the levels of metabolite were different in various species, the plasma level of *N*-desmethyl-

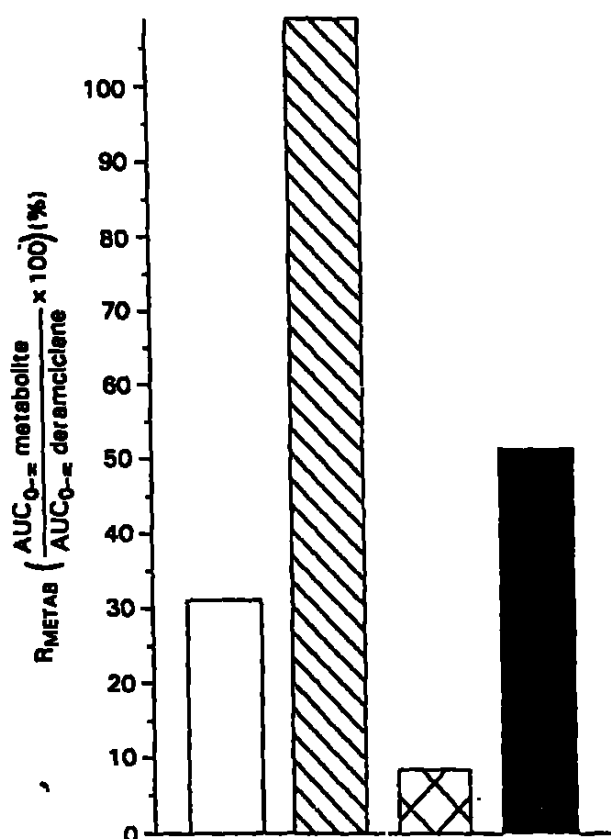


Figure 5. Comparison of *N*-desmethyl-metabolite/deramciclane ratios (R_{METAB}) in □ rat; ▨ dog; ▩ rabbit; ■ man.

deramciclane was always significant as compared with that of the parent compound. The preliminary metabolism studies in rats and dogs indicated that only a small portion (1–3%) of the administered dose was excreted as unchanged deramciclane (Hazai et al 1995; Klebovich & Ürmös 1996). Similarly, only about 5% of the administered deramciclane dose was excreted as *N*-desmethyl metabolite (Klebovich et al 1995; Klebovich & Ürmös 1996). This finding indicated a multistep biotransformation, as more than 90% of the dose was excreted as other metabolites.

Acknowledgements

The authors wish to thank Mr Gyula Grézel and Mr Olavi Kilkku for their statistical support and Mrs Margit Bernáth and Mrs Gabriella Szöke for their skillful and valuable technical assistance.

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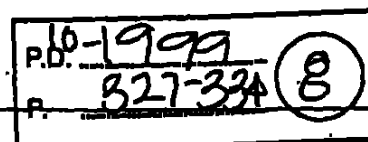
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P. 456 - 0056

BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. 20: 327-334 (1999)



The Single Dose Pharmacokinetics and Safety of Deramciclone in Healthy Male Volunteers

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ABSTRACT: The pharmacokinetics and tolerability of a new putative non-benzodiazepine type anxiolytic compound deramciclone was studied in two consecutive studies. An open dose-escalation design was used to study doses from 0.2 to 50 mg in 18 healthy male volunteers. In the second study doses from 50 to 150 mg were investigated in 14 healthy males in a double-blind, placebo-controlled, dose escalation study. Deramciclone was rapidly absorbed from the GI-tract and T_{max} was 2–4 h. The elimination half-life increased from about 20 h to about 32 h with the increasing dose. Nevertheless, the $AUC_{0-\infty}$ values increased linearly within the studies over the dose ranges of 3–50 and 50–150 mg. However, the increase was more than the ratio of the dose over the total dose range of 3–150 mg. Therefore, non-linear pharmacokinetics of deramciclone at high doses cannot be excluded. *N*-desmethyl deramciclone, which is the active metabolite of deramciclone, was determined in plasma. C_{max} was reached at about 6 h. The AUC_{0-6h} for the *N*-desmethyl metabolite was about one third of the $AUC_{0-\infty}$ of the parent compound and the ratio remained constant at each dose level. Deramciclone was safe, and was well tolerated at each dose level. Copyright © 1999 John Wiley & Sons, Ltd.

Key words: deramciclone fumarate; pharmacokinetics; tolerability; first administration

Introduction

The prevalence of anxiety disorders is approximately 20% in the adult population [1,2]. The pathophysiological basis of anxiety disorders is still largely unknown, but major theories centre around brain neurotransmitter dysfunction [3–5]. Benzodiazepines are still the primary drugs for anxiety disorders despite their disadvantages, such as sedation, tolerance, addiction and withdrawal symptoms [6]. Therefore, new strategies for the treatment of anxiety disorders have been sought.

Over the past decade there has been growing interest in the brain serotonin system and its role in psychiatric disorders. Several 5-HT receptors and their subtypes have been described [7]. These receptors differ in their binding affinities, their receptor-effector coupling mechanisms, and the behavioural processes they mediate [8]. Drugs acting at several different 5-HT receptor subtypes and selective serotonin re-uptake inhibitors (SSRIs) produce anxiolytic effects [9–12]. Antagonism of the two

receptor subtypes of the 5-HT₂ family, 5-HT_{2A} and 5-HT_{2C}, produces anxiolytic-like effects in animal models and in clinical practice [13–18].

Deramciclone fumarate, (1*R*,2*S*,4*R*)-(–)-*N,N*-dimethyl-2-[(1,7,7-trimethyl-2-phenylbicyclo[2.2.1]hept-2-yl)oxy]ethanamine 2-(*E*)-butenedioate (1:1), was synthesized and developed by Egis Pharmaceuticals Ltd., Budapest, Hungary (Figure 1). In receptor binding studies *in vitro* deramciclone has shown high affinity to 5-HT_{2A} and 5-HT_{2C} receptors [19,20]. Deramciclone is an antagonist of these receptors, and has also been reported to be an inverse agonist in some assays [20]. The psychopharmacology of deramciclone has been studied in a number of

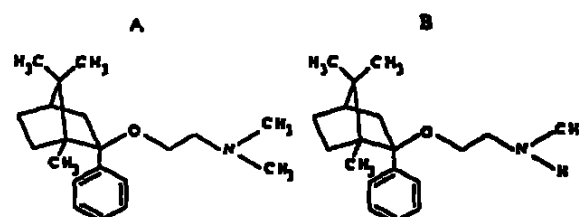


Figure 1. The molecular structures of deramciclone (A) and its metabolite *N*-desmethyl deramciclone (B)

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Blood samples of 10 mL were taken before drug intake (zero sample) and 20 min, 40 min, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h thereafter.

Clinical Measurements

The subjects were asked to spontaneously report any symptoms to the study personnel during the study. No leading questions were asked. The severity of all adverse events—reported by the subject or observed by the investigator—was recorded.

The safety of the subjects was monitored with safety laboratory variables, 12-lead ECG, blood pressure and heart rate measurements and close observation of symptoms. Blood and urine samples were taken in order to determine the safety variables before the drug intake and 24 h after it. The safety laboratory variables included haematology (blood counts, erythrocyte sedimentation rate, prothrombin time), serum liver function tests (alanine and aspartate aminotransferase, gamma glutamyltransferase, total and free bilirubin, alkaline phosphatase), plasma electrolytes (potassium, sodium, calcium), serum glucose and serum creatinine. The supine blood pressure, heart rate and 12-lead ECG were recorded before the drug intake and 1, 2, 3, 4, 6, 8, 12, and 24 h thereafter to assess possible cardiovascular changes during the study.

Chemical Analyses

The plasma samples were analysed using a liquid chromatography-tandem mass spectrometry and a selected reaction monitoring technique, ($^3\text{H}_3$)-deramciclane was used as an internal standard for both analyses. The liquid chromatographic system consisted of a HP Model 1090 Series II HPLC pump and it was equipped with Sciex API III triple quadrupole mass spectrometer with a standard atmospheric pressure ionization source. The method has been described in detail elsewhere [23]. The calibration curves for deramciclane and *N*-desmethyl deramciclane were linear over the calibration range of 0.1–100 ng/mL. Samples, with concentrations exceeding the upper limit of the calibration range were diluted with drug free human plasma and reanalysed. The assay precision for deramciclane and *N*-desmethyl deramciclane varied between 2.5–7.2 and 3.6–12%, respectively, over the whole calibration range. The lower limit of quantitation was 0.1 ng/mL.

Pharmacokinetic Calculations

The pharmacokinetic parameters were calculated using the Siphar/Win program (Simed Biostatistics and Data Processing, Creteil, France). Plasma deramciclane concentration data were fitted for a

two-compartmental model using the peeling algorithm. Powell's minimization algorithm was used to determine either all of the fitting parameters or at least the slope of the elimination phase (λ_2). The plasma concentration data for *N*-desmethyl-deramciclane were calculated model-independently.

The area under the concentration-time curve (AUC) was calculated using the linear trapezoidal rule. The area beyond the last measured time point ($C_{t_{\text{last}}}$) was extrapolated to infinity by dividing $C_{t_{\text{last}}}$ by the slope of the terminal phase (λ_2). The dose-normalized AUC values were calculated using the equation:

$$\text{AUC}_{\text{norm}} = \text{AUC}(\text{D mg}) \cdot \frac{3 \text{ mg}}{\text{D mg}}$$

where $\text{AUC}(\text{D mg})$ is the $\text{AUC}_{0-\infty}$ for doses (D).

The mean residence time (MRT) was calculated using:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}}$$

where AUMC is the area under the first moment curve [24].

The peak plasma concentration (C_{max}) of deramciclane and the time to reach it (T_{max}) were read directly from the observed individual plasma concentration-time data.

Statistical Analysis

The dose-dependency of the absorption of deramciclane and the formation of *N*-desmethyl deramciclane were estimated on the basis of the $\text{AUC}_{0-\infty}$ for both compounds. Relations (linear and/or quadratic effect) of these parameters to the dose were assessed using non-linear regression analysis (power function) for the original values. Statistical comparisons between the dose levels were performed by analysis of variance (ANOVA) followed by Tukey's comparison of means if necessary. To allow direct comparison between doses, individual AUC-values were dose-normalized. Kruskal-Wallis and Wilcoxon's rank sum tests were used for T_{max} . A risk level less than 5% ($p < 0.05$) was considered as statistically significant.

Results

Seventeen subjects completed the trial according to the protocol in the first study. However, one subject was withdrawn after taking the 40 mg dose because the AUC value increased four times more than expected from the dose increment. In the second study all subjects completed the study according to the protocol.

animal models, which have shown anxiolytic activity of deramciclane at dose levels of 0.5–10.0 mg/kg [19].

Deramciclane is readily and completely absorbed from the gastrointestinal tract in rat and dog [21]. The absolute bioavailability of deramciclane is only about 50% in dogs due to the first-pass metabolism [22]. Furthermore, the $AUC_{0-\infty}$ values of deramciclane increase more than proportionally to the dose in dogs [22]. The non-linearity was observed at doses higher than 1 mg/kg after intravenous and oral administration, and it was thought to be caused by a decrease in the clearance of deramciclane.

In the present study, the safety and tolerability of deramciclane was evaluated by giving escalating single oral doses to healthy male volunteers. Moreover, the linearity of the pharmacokinetics of deramciclane in man was studied and its maximal tolerated dose level determined.

Materials and Methods

These studies were performed according to the Good Clinical Practice Guidelines of the European Community. The studies followed the recommendations for biomedical research involving humans (current revision of the Declaration of Helsinki of the World Medical Assembly).

The study procedures were accepted by the Ethics committee of the Helsinki Deaconess Hospital (study 1) and by The Joint Ethics Committee of the Turku University Central Hospital and the University of Turku (study 2). The Finnish National Agency for Medicine was notified in advance, in accordance with national regulations.

Subjects

The subjects were recruited from the healthy volunteers interested in clinical trials. In addition, advertisements were placed at the University of Helsinki Medical School, School of Pharmacy, School of Chemistry and University of Turku Medical School. Eighteen subjects were recruited for study 1 and 14 for study 2. All subjects gave their signed informed consent prior to the initiation of the studies. They were healthy, Caucasian males, whose mean (range) age was 24.6 (19.6–30.8) years, the mean (range) weight was 76.1 (66.0–88.0) kg and the mean (range) body mass index was 23.3 (21.0–26.0) kg/m². They were non-smokers, without clinically significant abnormalities in the physical examination or in the safety laboratory variables.

Study Designs

Study 1. This study was conducted in the Helsinki Deaconess Hospital. Open within subject rising

dose design with overlapping groups was used. Four groups received an escalating single oral dose of deramciclane with at least one-week washout periods between dose levels. In each group the first subject, a leader, received the increased dose first. After the safety parameters of the leader were known the next subject was administered the same dose. The first group ($n = 3$) received 0.2, 0.4, 0.7, 1.4, 3 and 6 mg doses of deramciclane. The second group ($n = 3$) received doses of 3, 6, 9, 12, and 15 mg. In the third group ($n = 6$) the deramciclane doses were 9, 12, 15, 17, and 20 mg, and in the fourth group ($n = 6$) 20, 30, 40 and 50 mg. Deramciclane was administered in conventional hard gelatine capsules at the strengths of 0.2, 0.4, 1.4, 3, 6 and 14 mg and were prepared by Egis Pharmaceuticals. Combinations of these formulations were taken with 200 mL water. Dosing was preceded by an over-night fast. The subjects were supine until four hours post dosing at which time lunch was served. Thereafter, they were allowed to walk but not to leave the ward. Caffeine-containing beverages were not allowed during the first 24 h post dosing. The subjects were kept in the hospital until 48 h post dosing.

Blood samples of 10 mL were taken before drug intake (zero sample) and 20 min, 40 min, 1, 1.57 2, 3, 4, 6, 8, 12, 16, 24, 36 and 48 h thereafter. In addition, one blood sample was taken one week after the 50 mg dose.

Study 2. The study was conducted in the laboratory of Clinical Research Services Turku (University of Turku, Turku, Finland). A placebo-controlled double-blind dose escalation design with two groups was used. The placebo was randomly added within each subject's treatment sequence. In the first group each subject received single oral doses of 50, 100, 150 mg and one dose of placebo with wash-out periods of three weeks between each dose. At each dose escalation level, there were six subjects on active medication and two on placebo making a total of eight subjects.

The maximal dose was set at 150 mg on the basis of the available toxicological data. After reaching this dose level, a new group of six subjects was recruited to increase the sample size. In this group four subjects were allocated on active treatment and two on placebo.

Ten and 20 mg film-coated conventional tablets (Egis Pharmaceuticals) were used in combinations to make up 50, 100 and 150 mg doses. The placebo tablet (Egis Pharmaceuticals) was identical in appearance to the deramciclane tablets and the number of placebo tablets administered corresponded to that of the active medication at each dose level. The tablets were given after an overnight fasting with 200 mL water. Fasting was maintained until 4 h post dosing. Caffeine-containing beverages were not allowed during the 48 h that the subjects stayed at the study site.

Pharmacokinetics of Deramciclane

Deramciclane was rapidly absorbed and was detected in plasma 20 min after dosing. The plasma profile showed a biphasic decline and thus an open two-compartmental model was used in the pharmacokinetic calculations (Figure 2). The means ($\pm$ S.D.) of pharmacokinetic parameters for the parent compound and the desmethylated metabolite are presented in Tables 1 and 2, respectively. The pharmacokinetic parameters were not affected by the formulation at the 50 mg dose level. Thus, the parameters for capsules and tablets are not separated in the tables.

The peak plasma concentration of deramciclane was reached at about 2.5–3 h. T_{max} was not affected by the dose. The peak plasma concentration of deramciclane increased slightly but significantly more than in proportion to the dose ($p < 0.0023$) as assessed by power function. The distribution phase was followed by relatively slow elimination of deramciclane from plasma. The mean terminal $t_{1/2}$ values increased with dose from 21.5 h at the dose of 3 mg to 32.8 h at the 100 mg dose ($p = 0.0007$, ANOVA). However, no significant differences were found in the doses in pairwise comparisons using Tukey's post-hoc analysis. The MRT tended to increase with the dose, but this was not statistically significant.

The $AUC_{0-\infty}$ increased more than in proportion to the dose ($p = 0.048$, non-linear regression) (Figure 3) over the whole dose range from 3 to 150 mg. Moreover, the dose normalized AUC values (AUC_{norm}) increased significantly ($p = 0.017$, ANOVA) which suggested disproportionality to the dose. The only significant differences in the Tukey's post-hoc analysis were found between 3 and 150 mg, and 6 and 150 mg dose levels. However, there were no quadratic effects within the two studies, i.e. over the dose ranges from 3 to 50 mg and 50 to 150 mg, because the power function did not differ from unity.

Pharmacokinetics of N-Desmethyl Deramciclane

The maximum plasma concentration of the *N*-desmethyl metabolite was reached at about 6–9 h. C_{max} for the metabolite increased linearly and proportionally with increasing doses of deramciclane. The linear and non-linear regression analysis for the $AUC_{0-t_{last}}$ values against the dose are plotted in Figure 4. $AUC_{0-t_{last}}$ values increased more than in relation to the dose ($p = 0.025$). However, linear regression resulted in a slightly better fit than non-linear regression ($r^2 = 0.94$ and $r^2 = 0.88$, respectively). The AUC of *N*-desmethyl deramciclane in plasma was two times smaller than the AUC of deramciclane and this ratio remained constant at different dose levels suggesting linearity of this metabolic pathway over the dose range studied.

Cardiovascular Effects

Deramciclane did not cause changes in blood pressure or in the heart rate over the dose range of 0.2 and 50 mg. The changes in blood pressure and heart rate in study 2 are presented in Figure 5.

After dosing of 150 mg the diastolic blood pressure (DBP) increased significantly ($p = 0.0486$) from the baseline when compared with the placebo at the time of deramciclane peak plasma concentration (2–3 h). In contrast, at 6 and 12 h blood pressure assessments both DBP and SBP were 5–10 mmHg lower for the 50–150 mg deramciclane doses than for the placebo. At the 6 h time point DBP (–8.2 mmHg) and SBP (–7.6 mmHg) were significantly lower for 100 mg dose than for the placebo ($p = 0.0216$ and 0.0377, respectively).

During the first 4 h after administration, when the subjects were lying supine, heart rates were lower than at the baseline. The changes in the heart rate during the study were similar for all deramciclane

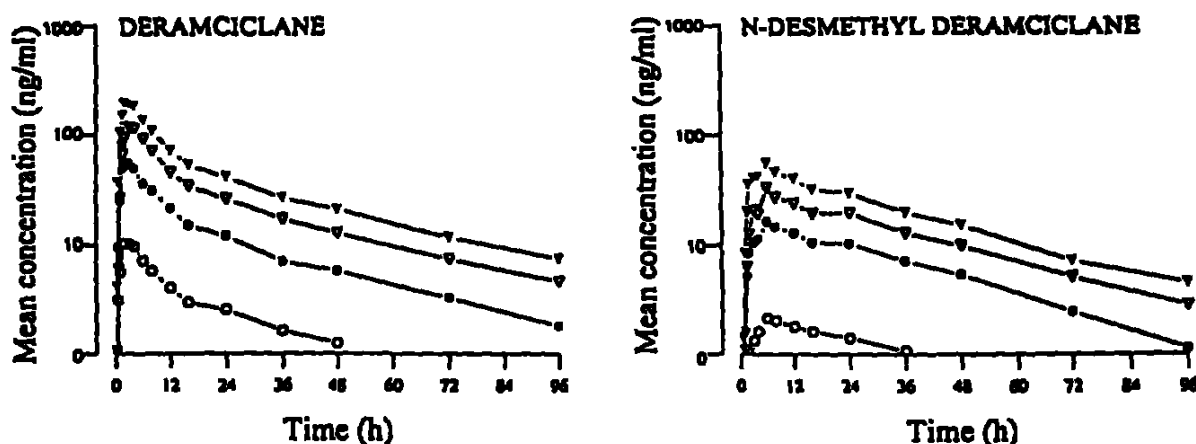


Figure 2. The mean plasma concentration versus time curves of deramciclane and *N*-desmethyl deramciclane for 9 (open spheres), 50 (solid spheres), 100 (open triangles) and 150 mg (solid triangles) single oral doses. The standard deviations are not shown for clarity.

Table 1. Pharmacokinetic parameters of deramciclanc after a single oral administration to healthy male volunteers

Dose (mg)	N	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-∞} (ng·h/mL)	AUC _{0-12h} (ng·h/mL)	t _{1/2β} (h)	MRT (h)
3	6	2.4 ± 0.6	3.2 ± 0.8	40.9 ± 16.5	40.9 ± 16.5	21.5 ± 2.7	26.0 ± 3.5
6	6	4.5 ± 1.1	3.5 ± 0.5	78.9 ± 22.9	39.4 ± 11.5	19.9 ± 3.1	25.2 ± 3.9
9	9	12 ± 10.6	2.7 ± 1.1	201 ± 170	67.1 ± 56.6	22.4 ± 3	27.8 ± 5.5
12	9	13 ± 5.7	2.4 ± 0.8	221 ± 95	55.3 ± 23.6	22.0 ± 4.0	27.1 ± 5.5
15	9	16 ± 5.9	3.3 ± 1.3	282 ± 154	56.4 ± 30.7	22.2 ± 4.4	27.5 ± 6.5
17	6	21 ± 11.2	2.8 ± 0.4	346 ± 230	61.1 ± 40.7	25.4 ± 5.8	30.1 ± 7.0
20	12	24 ± 9.4	3.2 ± 1.4	428 ± 170	64.2 ± 25.6	25.2 ± 7.2	30.8 ± 10.0
30	6	27 ± 6.1	3.8 ± 1.2	584 ± 160	58.4 ± 16.0	26.5 ± 6.9	34.3 ± 9.4
40	6	36 ± 8.0	3.7 ± 0.5	824 ± 405	61.8 ± 30.4	28.3 ± 11.1	36.4 ± 14.8
50*	13	59 ± 16	2.7 ± 0.9	1099 ± 318	63.5 ± 19.1	27.0 ± 6.0	30.8 ± 7.1
100	8	124 ± 44	3.3 ± 0.7	2333 ± 775	70.0 ± 23.3	32.8 ± 10.0	34.0 ± 9.0
150	12	218 ± 54	2.6 ± 0.9	3738 ± 1018	74.8 ± 20.4	31.6 ± 8.5	33.5 ± 8.6

* The mean (± S.D.) parameters from both studies.

Table 2. Pharmacokinetic parameters of N-desmethyl deramciclanc after a single oral administration to healthy male volunteers

Dose (mg)	N	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-∞} (ng·h/mL)	AUC _{0-12h} (ng·h/mL)	T _{1/2β} (h)
3	6	0.6 ± 0.2	7.7 ± 2.3	16.9 ± 5.2	16.9 ± 5.2	25.7 ± 10.3
6	6	1.3 ± 0.3	7.0 ± 1.1	32.8 ± 4.3	16.4 ± 2.1	23.7 ± 7.3
9	9	2.2 ± 0.8	6.9 ± 1.1	62.7 ± 26.8	20.9 ± 8.9	27.9 ± 6.5
12	9	3.0 ± 1.0	8.4 ± 2.2	78.9 ± 25.8	19.7 ± 6.5	27.1 ± 8.9
15	9	3.7 ± 1.4	7.6 ± 3.4	98.1 ± 33.3	19.6 ± 6.7	19.8 ± 3.4
17	6	4.8 ± 1.7	7.0 ± 2.8	128 ± 48.0	22.6 ± 8.5	25.6 ± 8.9
20	12	5.2 ± 1.5	9.8 ± 3.2	137 ± 39.6	20.6 ± 5.9	25.1 ± 7.3
30	6	6.5 ± 2.4	9.0 ± 2.4	190 ± 73.4	19.0 ± 7.3	25.2 ± 6.7
40	6	9.6 ± 4.5	8.0 ± 3.3	282 ± 133	21.2 ± 9.9	28.3 ± 9.6
50*	13	16 ± 4.0	6.9 ± 2.3	452 ± 166	27.1 ± 10.0	25.1 ± 7.4
100	8	34 ± 8.3	5.9 ± 1.4	835 ± 126	25.0 ± 3.8	29.7 ± 14.3
150	12	56 ± 19	5.5 ± 1.4	1339 ± 225	26.8 ± 4.5	27.1 ± 4.9

* The mean (± S.D.) parameters from both studies.

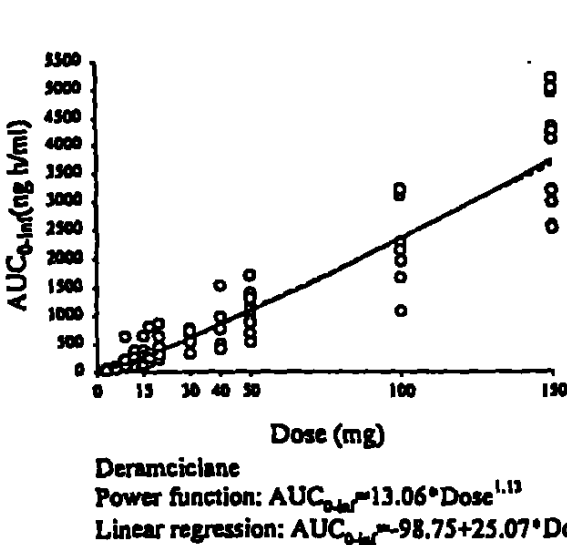


Figure 3. The power function (dashed) and linear regression (solid) lines for AUC_{0-12h} of deramciclanc against the dose over the dose range 3–150 mg in healthy male volunteers. Statistical analysis resulted in $p = 0.048$ for power effect and $r^2 = 0.88$ for both lines

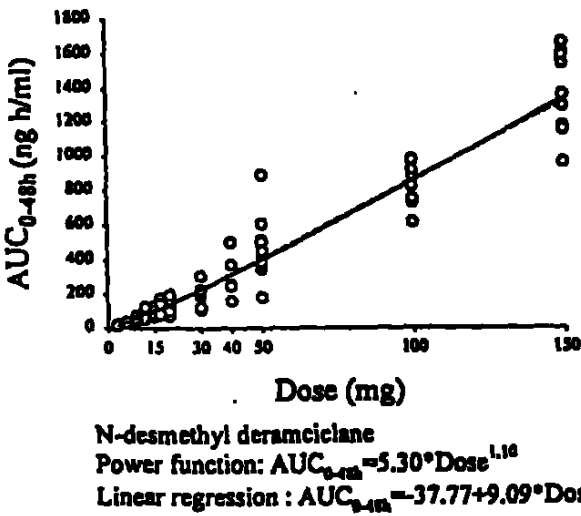


Figure 4. The power function (dashed) and linear regression (solid) lines for AUC_{0-48h} of N-desmethyl deramciclanc against the dose over the dose range 3–150 mg in healthy male volunteers. Statistical analysis resulted in $p = 0.025$ for power effect. $r^2 = 0.88$ and $r^2 = 0.94$ for the power function and linear regression, respectively

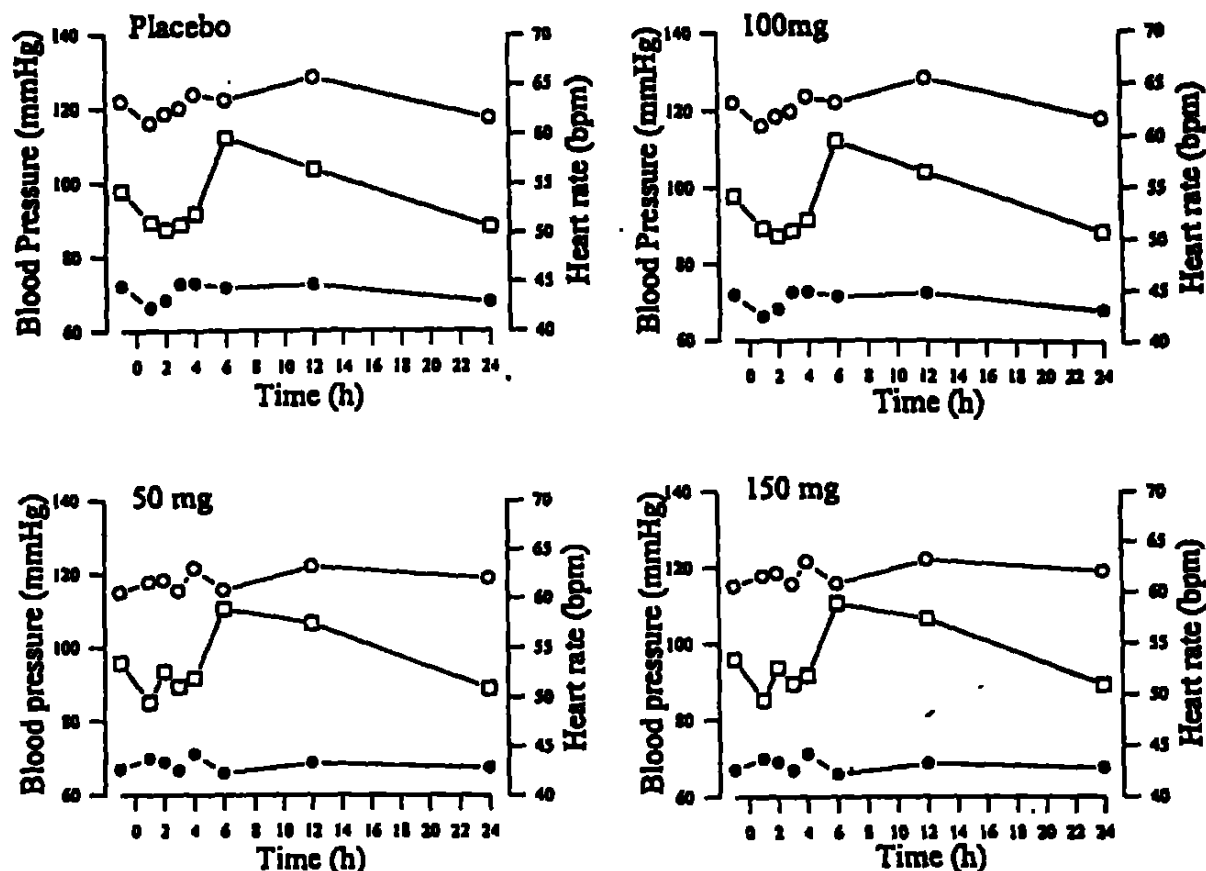


Figure 3. Changes in the systolic (open spheres) and diastolic (solid spheres) blood pressure, and heart rate (rectangles) over the first 24 h after a single oral dose of placebo, 50, 100, and 150 mg of deramciclane in eight healthy male volunteers

doses and they did not differ from placebo. There were no dose-dependent or clinically relevant changes in the conduction times of the ECG.

Safety and Tolerability

Deramciclane was well tolerated over the whole dose range studied. There were no serious adverse events during the study. Moreover, all of the adverse events were transient, and mild or moderate in severity. The adverse events reported within five elimination half lives from the time of the drug intake are presented below without causality assessments. None of the adverse events were reported more than once by the same subject. However, a dose dependency in the frequency of the reported adverse events can be seen for the 150 mg deramciclane dose.

Common cold and related symptoms, such as pharyngitis, rhinitis and fever were reported to the same extent for placebo and active treatment groups. Moreover, some isolated adverse events such as diarrhoea, dyspepsia, flushing and urticaria was reported but these symptoms did not recur when a higher dose was given to the same subjects. At the highest dose of 150 mg the most frequently reported adverse event for deramciclane was headache which was not reported at all for placebo. However, two of

three reported headaches appeared on the day following the drug intake. Dizziness was reported once for placebo and twice at 150 mg dose level 3–6 h after dosing.

There were no statistically significant changes in any of the routine safety laboratory variables. Moreover, there were no dose-dependent changes or trends in the individual safety laboratory variables.

Discussion

The pharmacokinetics and safety of deramciclane, a new putative anxiolytic compound was studied after single oral administration over a dose range of 0.2–150 mg. The absorption of deramciclane was rapid and the kinetic profile was fitted with open two-compartmental model. The pharmacokinetic parameters for 50 mg capsules and tablets did not differ from each other. Thus, the change of the formulation from a conventional hard gelatine capsule to a conventional film coated tablet did not affect the dissolution and/or the absorption of the drug *in vivo*.

In our earlier studies in dogs the reduction in the elimination rate of deramciclane with increasing dosage resulted in non-linear pharmacokinetics [22].

This is possibly due to saturation of metabolism in the dose range of 1–6 mg/kg. Similarly, in man a tendency for prolongation of the elimination half-life from 21 to 33 h with an increasing dose was seen. Unfortunately, it is impossible to determine the mechanism causing the prolongation in man without administering deramciclane intravenously. However, these results could indicate saturation of some metabolic pathway(s) at higher deramciclane doses. Consequently, the dose normalized AUC-values increased slightly, but yet significantly with dose. It should be noted, however, that the increase in the $AUC_{0-\infty}$ values was linear within the two studies over the dose ranges from 3 to 50 mg and from 50 to 150 mg. In addition, in man there were no differences in the kinetic parameters within the groups of subjects receiving the same doses. This could be due to the relatively narrow dose ranges within the groups. On the other hand, the number of subjects was small and the inter-individual variabilities of $AUC_{0-\infty}$ and C_{max} values were relatively high. By taking into consideration the inter-individual variation and small number of subjects definite conclusions on the linearity or non-linearity cannot be drawn. Nevertheless, these results suggest slight non-linearity for deramciclane over the dose range from 3 to 150 mg.

Similar non-linearity was observed also for *N*-desmethyl deramciclane. However, in the case of the metabolite the linear regression model gave better fit assessed using the mean square of regression (r^2) as a criterion. From Figure 2 it seems that the concentration of the metabolite in plasma declines with a similar rate as the parent compound. Therefore, it is suggested, that the elimination of metabolite might be formation rate limited.

Substantial inter-individual variations in the pharmacokinetic parameters are common for many psychiatric drugs [25]. In the case of deramciclane the coefficients of variation were in the range of 30 and 60% for $AUC_{0-\infty}$. Variation in absorption, distribution and elimination (both excretion and metabolism) depend on the physico-chemical properties of the compound itself [24]. In addition, the variations in kinetic parameters of many psychiatric drugs are due to genetic polymorphism of cytochrome P450 isoenzymes [25,26]. In the case of fluoxetine and tricyclic antidepressants, polymorphism of CYP2D6 results in substantial variation in kinetic parameters [27]. However, CYP2D6 does not seem to be responsible for the variation in the kinetic parameters of deramciclane because all the subjects in study 1 were extensive debrisoquine hydroxylators (Orion Pharma, data on file). However, the involvement of other CYP450 isoenzymes, and the role of their genetic polymorphisms in the metabolism of deramciclane remains unknown.

There were no observable changes in the conduction times in the ECG measurements. A slight

(5–10 mmHg) reduction in the systolic and DBP values were observed for the active treatment groups at the dose levels of 50, 100 and 150 mg when compared with placebo. The hypotensive effect could be caused by a decrease in resistance in the peripheral blood vessels because the heart rate did not change during the study. Interestingly, the significant reductions in blood pressure were observed at the peak plasma concentration (6 h) of *N*-desmethyl deramciclane. The metabolite has high affinity to 5-HT_{2C} receptors. Stimulation of 5-HT₂ receptors in smooth muscle cells produces vasoconstriction of most vessels [28]. Moreover, it has been shown, that a 5-HT₂ antagonist, ketanserin, blocks the receptor responsible for 5-HT-induced contraction of most vascular smooth muscle. It should be noted that ketanserin also possesses α_1 -receptor affinity, which makes the interpretation of that result more difficult. Actually, both serotonin and α_1 -receptor actions may be necessary to produce the antihypertensive effect of ketanserin [29]. Therefore, it is possible that the decrease in blood pressure was related to the metabolite or it was caused by a synergistic action with the parent compound. The effect did not, however, reach clinical relevance and a dose response to this effect was not observed.

No deramciclane related changes in the haematology or clinical chemistry variables were observed. Deramciclane was well tolerated up to a 150 mg single oral dose. All the subjective adverse events were either mild or moderate and transient. The most frequently reported adverse effects of deramciclane were headache and dizziness. The other reported adverse effects occurred randomly and their frequency was not dose-dependent. 5-HT₂ antagonists, such as ketanserin and ritanserin, have similar adverse effect profiles and the most frequently reported adverse effects of ketanserin are dizziness, orthostatic hypotension, fatigue and QTc-prolongation [30–32]. Therefore, it is possible that dizziness is a pharmacological effect of deramciclane and is mediated via the 5-HT₂ receptors in the cerebral vascular bed or in the brain.

In conclusion, deramciclane, a new putative anxiolytic compound was safe and well tolerated up to a single oral dose of 150 mg. This dose level was considered to be the maximal tolerated dose, since higher doses have not been studied. The pharmacokinetics of deramciclane was slightly non-linear. However, the clinical relevance of this finding is probably negligible.

Acknowledgements

The authors wish to thank Ms Helinä Ruokonen, CRA and Ms Annamari Sauramo, CRA for their valuable help in collecting the data.

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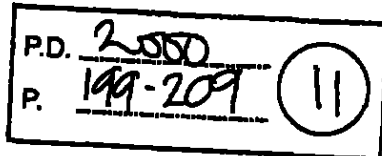
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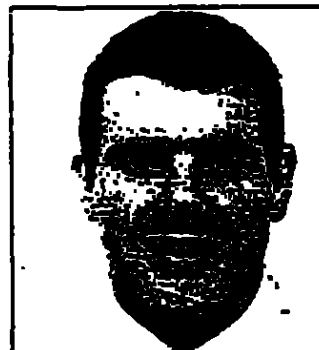
Inhibitors of Mammalian Central Nervous System Selective Amino Acid Transporters

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XP-000937809

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Abstract: Central nervous system (CNS) selective amino acid transporters provide an important function in maintaining tonic extracellular levels of amino acids that act as neurotransmitters, synaptic modulators or neurotransmitter precursors. Small molecule inhibitors of these transporters have been postulated and in some cases demonstrated to be useful in the treatment of a range of CNS driven disorders such as epilepsy, anxiety, psychosis, depression, pain and neurodegenerative disease. Although much of the research to date in this field has focussed on inhibition of the γ -amino butyric acid (GABA) transporters more recent reports have also generated interest in modulation of glycine, glutamate and proline transporters. This article will review the current medicinal chemistry literature and structure activity relationships known for mammalian CNS selective amino acid transporters.

Introduction

The uptake of amino acids into mammalian cells is mediated by energy dependent and passive transporters. These transporters are characterised by their general ability to transport amino acids in a stereoselective fashion with broad substrate specificity. Amino acid transporters in the central nervous system (CNS) maintain tonic extracellular levels of amino acids that act as neurotransmitters (or precursors to neurotransmitters) or synaptic modulators. Their role in maintaining intra and extra cellular CNS amino acid homeostasis is critical in preventing neurotoxicity by removing neurotransmitter-amino acids from synaptic clefts and also by providing a supply of precursors in the CNS.

Recent advances in the molecular biological aspects of plasma membrane amino acid transporters have been extensively reviewed [1, 2]. More information about their structure, function and tissue distribution is discovered on a regular basis. Breakthrough's in the identification of subtypes of classes of CNS amino acid transporters has fuelled further medicinal chemistry research to find selective inhibitors as potential new medicines for a range of CNS driven diseases such as epilepsy, anxiety, psychosis, depression, pain and neurodegenerative disease.

The aim of this review is to provide the reader with an overview of key medicinal chemistry advances in the development of CNS amino acid transport inhibitors. Each class of transporter will be reviewed in turn covering those that are expressed in CNS tissue - γ -amino butyric acid (GABA) transporters, glutamate transporters, glycine transporters, proline transporter, taurine transporter and the brain specific cationic amino acid transporter.

γ -Amino butyric acid (GABA) Transporters

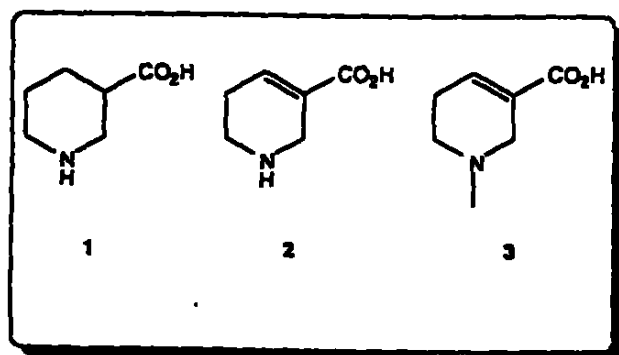
The GABA transporters form part of the superfamily of sodium and chloride dependent neurotransmitter transporters. The rat and human brain GABA transporter (GAT-1) was one of the first transporters of this family to be cloned in 1990 and was the starting point for the isolation of related cDNA constituting this superfamily [1]. The GABA transporters, like all transporters of this superfamily, are proteins with a 12-membrane spanning domain topology. Four mammalian GABA transporters have been cloned to date from mouse, rat and human brain [2]. They possess a high level of sequence homology but vary in their tissue distribution and pharmacological properties. The murine clones are referred to as mGAT-1, mGAT-2, mGAT-3 and mGAT-4, however, they correspond to the human (and rat) homologues hGAT-1, hGAT-2 and hGAT-3 respectively in nomenclature terms [3]. mGAT-1 (hGAT-1) and mGAT-4 (hGAT-3) are high affinity neuronal GABA transporters and are found

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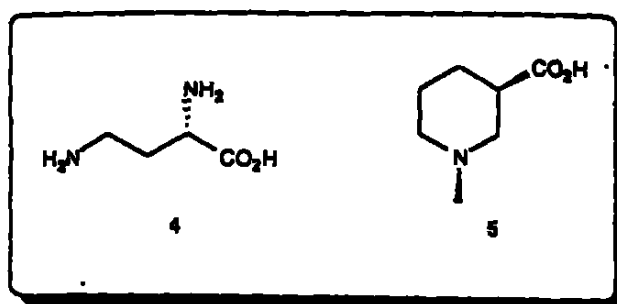
specifically in the CNS. mGAT-3 (hGAT-2) is a moderate affinity GABA transporter with primarily a glial distribution in the brain but is also expressed in liver and kidney. mGAT-2 (hBGT-1) is a low affinity GABA transporter that also transports the osmolyte betaine. mGAT-2 (hBGT-1) has primarily a glial distribution in the brain but is also expressed in liver and kidney [1].

Inhibition of GABA transporters has been a successful strategy in the treatment of epilepsy as demonstrated with the launch of Tiagabine (14) (Novo Nordisk) in 1996. GABA transporter inhibitors have also been implicated for use as analgesics, anxiolytics, and hypnotics and in the treatment of muscle movement disorders, depression and psychosis [4, 5, 6]. Recent studies have also indicated their potential as anti-tussive agents [7].

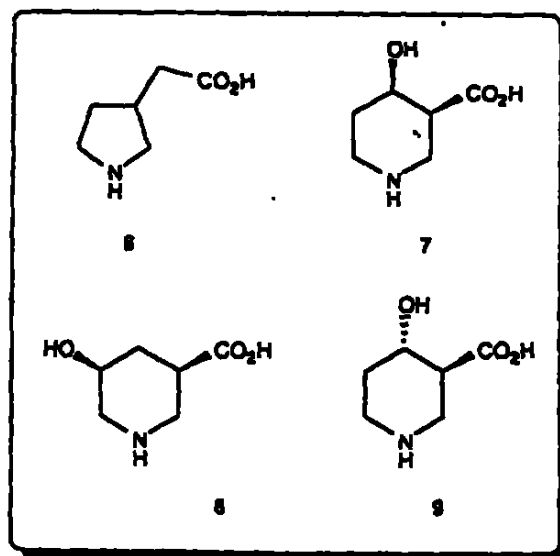
Medicinal chemistry research into GABA transporters is the most established of all the amino acid transporter classes and has periodically been covered in general GABA medicinal chemistry review articles [8, 9, 10]. The first major breakthrough in GABA transport research came when it was found that the heterocyclic amino acids, nipecotic acid (1) and guvacine (2) were potent inhibitors of GABA uptake (IC_{50} = 9 and 8 μ M respectively) whilst the simple N-methyl analogue of guvacine, arecaldine (3), was considerably less potent (IC_{50} = 122 μ M) [11]. Nipecotic acid and guvacine have subsequently been shown to be inhibitors of both neuronal and glial GABA uptake with some selectivity for the glial system [8] and no affinity for GABA receptors. Whilst (1) and (2) are inhibitors of GABA transport they are also substrates which can complicate interpretation of pharmacological data [10].



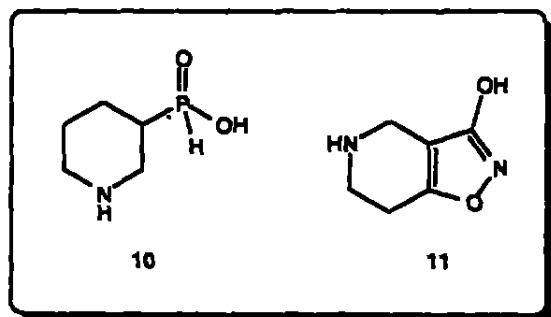
SAR studies with simple acyclic analogues of GABA lead to inhibitors of GABA uptake in rat brain slices, however, they showed little selectivity for transport inhibition over inhibition of GABA binding in rat brain [12]. The GABA analogues DABA (4) and ACHC (5) are selective inhibitors of neuronal GABA uptake and there is some evidence that they also act as substrates [8, 13].



SAR studies have also been carried out based on variation of the heterocyclic ring structure found in (1) and (2). Attempts were made to produce irreversible transport inhibitors to further probe biochemical function by introducing thiol and epoxide functionality to the heterocyclic ring, however, this led to only very weak inhibitors [14]. Attempts to improve inhibitor potency by decreasing or increasing heterocyclic ring size were unsuccessful, as was the introduction of amino, methyl or phenyl groups onto (1) [9, 15, 16, 17, 18]. Transposing the carboxylic acid moiety from C-3 to C-4 of the heterocyclic ring also proved to be an unsuccessful strategy [9, 15, 16, 17], however, appending an acetic acid group to pyrrolidine to give homo- β -proline (6) yielded a potent inhibitor of GABA uptake (IC_{50} = 2.5 μ M) which also had significant affinity for GABA_A receptors [9, 19]. One compound worthy of mention from this series is *cis*-4-hydroxypiperidine-3-carboxylic acid (7). This compound retains potent inhibitory properties (IC_{50} = 11 μ M) [17] of GABA uptake and has been shown to be selective for the glial system [9]. Interestingly, the related *cis*-5-hydroxy analogue (8) and the *trans*-4-hydroxy analogue (9) were inactive. X-ray crystallography and NMR studies subsequently showed that (7) possesses an essential axial hydroxyl group whilst (8) and (9) have equatorially aligned hydroxyl groups [9, 19].



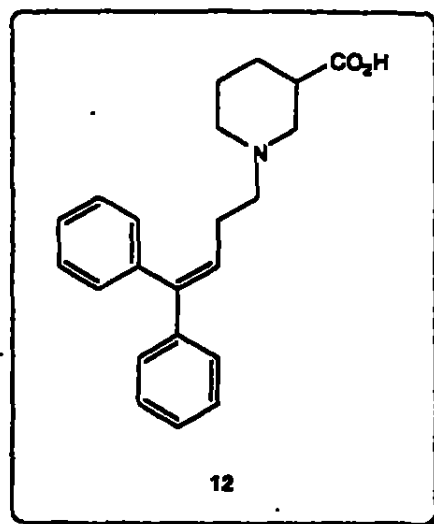
Bioisosteric replacements for the carboxylic acid functionality in (1) have recently been reported [20] with the phosphinic acid analogue (10) retaining uptake inhibitory activity ($IC_{50} = 24 \mu M$). Bioisosteric carboxylic acid replacements have also been used to address the pharmacokinetic problems associated with (1) and (2). Nipecotic acid (1) and guvacine (2) both suffer from poor blood brain barrier penetration upon systemic administration due to the fact that at physiological pH they exist in fully zwitterionic forms [8, 10]. The bioisosteric analogue THPO (11) overcomes this problem to some extent as it penetrates the brain upon systemic administration [8]. THPO (11) is highly selective glial uptake inhibitor ($IC_{50} = 160 \mu M$) that has been shown not to act as a substrate at the transporter [8, 13, 21]. The corresponding isothiazole to (11) is less active as are regioisomeric analogues of (11) [21]. Classical approaches to improve blood brain barrier penetration using lipophilic ester prodrugs of (1) met with limited success. The ethyl and pivaloyloxymethyl esters of (1) demonstrated *in vivo* activity in seizure models upon systemic administration, however, the weak cholinergic side-effects seen with the ethyl ester of (1) seem to have precluded their further development [6, 8].



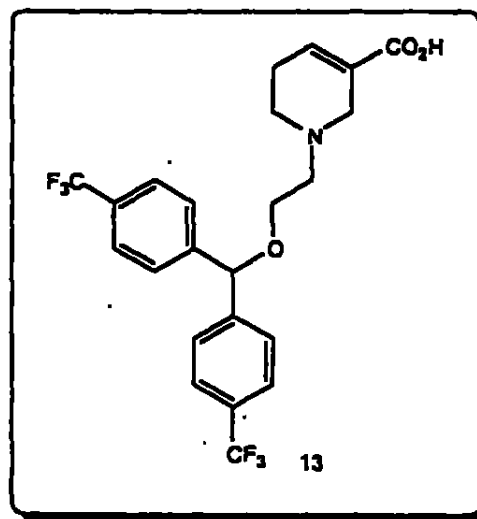
Exciting advances in medicinal chemistry GABA transport research were made in the early 1980's when workers at Smith Kline and French discovered that appending bulky lipophilic side chains to the nitrogen atoms of known GABA uptake inhibitors such as (1) and (2) led to marked increases in inhibitory potency *in vitro* [22]. SKF 89976-A (12) has an inhibitory IC_{50} of $0.2 \mu M$ in a brain synaptosome assay and does not act as a substrate at the transporter or have affinity for GABA receptors [10, 22]. It has improved pharmacokinetic properties by virtue of increased lipophilicity ($\log D$ 1.14 compared to -2.66 for (1)) and appears to readily enter the CNS as it protects rodents from bicuculline induced seizures when dosed orally [22]. SAR studies on (12) indicated that varying the side chain length or removing the double bond led to losses in inhibitory activity [22].

Subsequently, several groups have appended lipophilic side chains to known GABA uptake inhibitors to try and improve potency and pharmacokinetic

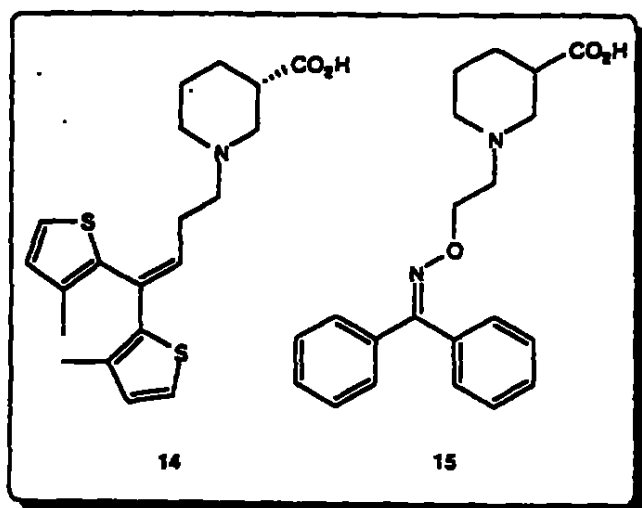
parameters. The 4,4-diphenyl-3-butenyl (DPB) substituted THPO derivatives showed improved potency over THPO ($IC_{50} = 7 \mu M$ compared to $160 \mu M$ for THPO) [13] and whilst DPB substituted GABA ($IC_{50} = 16.5 \mu M$) is weaker as an inhibitor than GABA [22] this potency can be regained by subsequent N-methylation of DPB GABA ($IC_{50} = 4 \mu M$) [23].



Workers at Parke-Davis reported on a series of N-(benzhydryl ethyl ether) derivatives of (1) and (2) [24]. Extensive SAR studies were carried out to probe chain length, spacer groups and aromatic substitution patterns. This work led ultimately to the discovery of the potent lipophilic inhibitor CI-966 (13) ($IC_{50} = 0.34 \mu M$) which like (12) also inhibited seizures *in vivo* upon oral dosing [24, 25]. The compound was progressed to the clinic where unfortunately it demonstrated severe adverse CNS symptoms such as memory deficits, myoclonus and tremors, unresponsiveness and subsequent severe psychological disturbances [22, 26]. For these reasons clinical trials with CI-966 (13) were terminated.

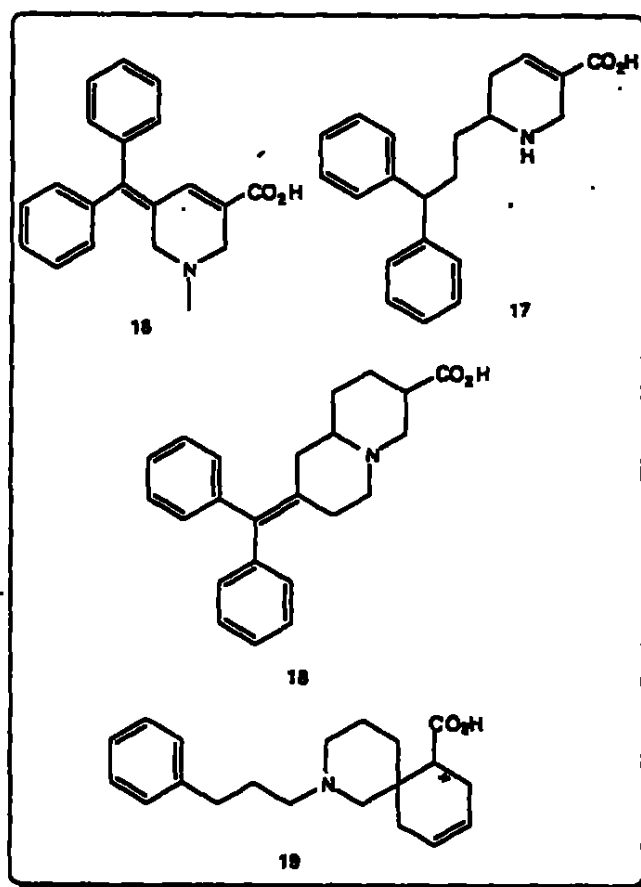


The most successful compound to come out of GABA transport research to date arose from work carried out at Novo Nordisk. Once again lipophilic N-substituents were added to nipepic acid (1) to give a range of 4,4-diaryl or 4,4-diheteroarylbutenyl derivatives [27]. One of the most potent analogues, Tiagabine (NO-328) (14) ($IC_{50} = 0.067 \mu M$), demonstrated anticonvulsant activity in mice following i.p. administration at low doses. One key observation from the SAR study carried out at Novo Nordisk was the requirement for an ortho or pseudo ortho substituent in the aryl or heteroaryl rings to give potent levels of inhibition [27]. Tiagabine (14) has subsequently been progressed through the clinic and was launched in 1996 for the treatment of specific types of epilepsy. Tiagabine has been shown to be 90% orally bioavailable in man with a half-life of 3.8-9 hours requiring 2-4 times daily administration [28]. Novo Nordisk have also recently patented a range of lipophilic N-substituted ethers [29] and oxime ethers [30] of (1) and (2) and N-substituted lipophilic carboline derivatives of 4-phenyl piperidines [31] as GABA uptake inhibitors, some of which are believed to be in preclinical development. The oxime ether NNC-711 (15) is one of the most potent known inhibitors to date ($IC_{50} = 0.047 \mu M$) and demonstrates protective efficacy against seizures *in vivo* [32].

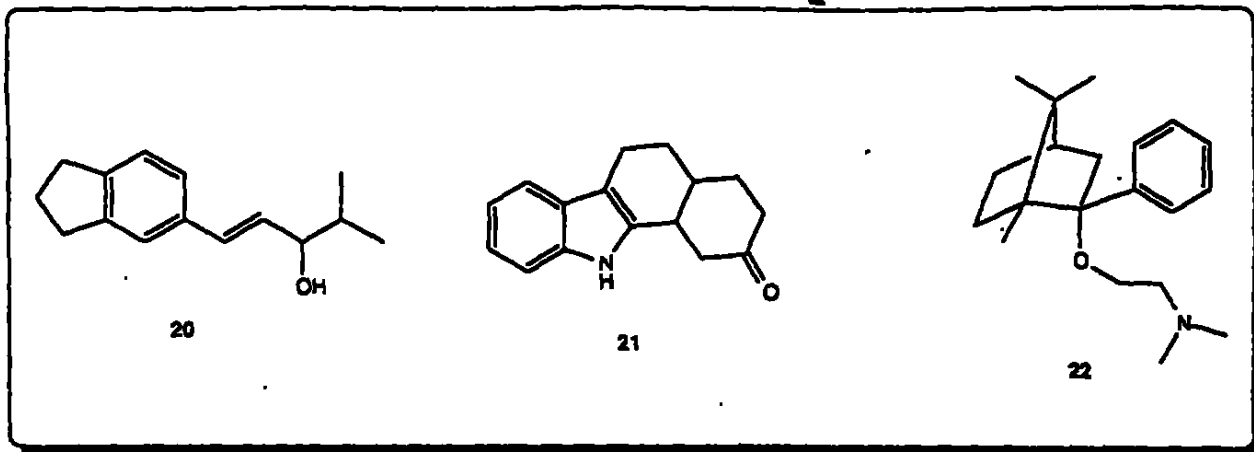


Inspired by the advances made in affinity for lipophilic N-substituted derivatives of (1) and (2), several groups investigated the effects of appending lipophilic functionality to other positions on the heterocyclic amino acid core. A range of 5-substituted derivatives of (3) have been reported [6, 33] with the 5-benzhydryl derivative analogue (16) retaining reasonable inhibitory activity ($K_i = 13 \mu M$). Interestingly the N-demethylated version of (16) was considerably less active ($K_i = 104 \mu M$) [6]. The 6-(1,1-diphenylpropyl) substituted guvacine analogue (17) was synthesised after a pharmacophore model based on all known GABA inhibitors at that time had predicted it would be a

potent inhibitor of GABA transport [34]. The model proved successful as (17) is a potent inhibitor ($IC_{50} = 0.1 \mu M$), however, it lacks any *in vivo* activity in seizure models. This lack of *in vivo* activity has been attributed to the inability of (17) to cross the blood brain barrier due to its increased hydrogen bonding capability compared to tertiary amino analogues [34]. Attempts to improve potency by appending lipophilic ethers at the 6 position of (2) were unsuccessful [35]. Workers at Synaptic reported on a series of conformationally restricted analogues of (1), which were designed using the previously described pharmacophore model [34, 36]. The quinolizidine compounds such as (18) were shown to only be weak inhibitors indicating that the lipophilic side chains present in (12-14) may bind to the transporter in their fully extended conformation [36]. Spirocyclic analogues of (1) retain moderate affinity as GABA transport inhibitors with (19) having an $IC_{50} = 42 \mu M$ [37].



Several non-heterocyclic amino acid derivatives have been reported to be inhibitors of GABA transport. The anticonvulsant, stiripentol (20) is a moderate potency inhibitor ($IC_{50} = 50 \mu M$) [38] as are the quinolizidine derivatives exemplified by (21) ($IC_{50} = 8.5 \mu M$) [39]. The bicycloheptane derivative EGYT 3886 (22) acts as an anticonvulsant *in vivo* and displays moderate non-competitive inhibition of GABA transport



(IC_{50} = 19-44 μ M) [40]. EGYT 3886 is apparently in phase III trials.

Recent advances in GABA transport inhibition have focussed on the search for selective inhibitors of the cloned mammalian transporters in an attempt to further unravel their potential as therapeutic targets. Workers at Synaptic have described the potency of many known inhibitors at cloned mammalian GABA transporters [41, 42] (Table 1). The lipophilic derivatives, (12, 13, 14), are all highly selective for hGAT-1 suggesting that their anticonvulsant activity is mediated *via* inhibition of this transporter. The Synaptic team went on to discover (S)-SNAP-5114 (23), a selective inhibitor of hGAT-3 (Table 1), from a set of compounds designed around (22), and SNAP-5294 (24), a selective inhibitor of rGAT-2 (Table 1) [41,42]. Workers at Novo Nordisk have reported a selective mGAT-2 (hBGT-1) inhibitor, NNC 05-2090 (25) [3], and have patented a series of mGAT-

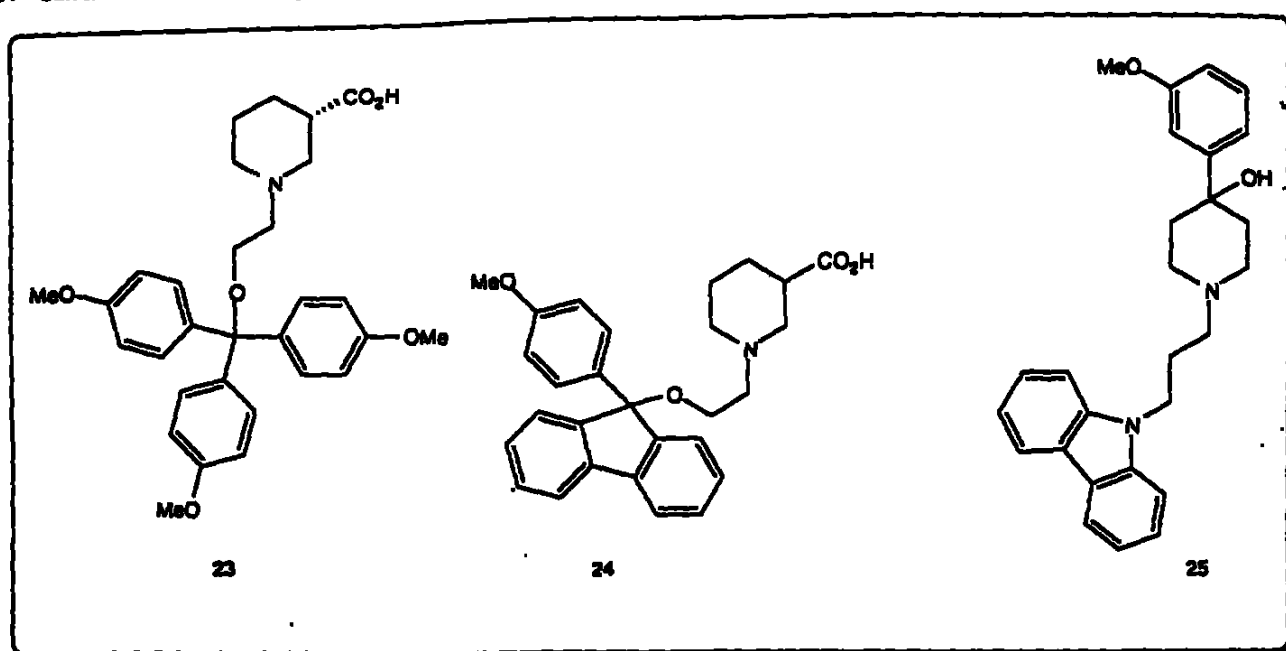
4 (hGAT-3) inhibitors based on similar structures to (25) [31]. NNC 05-2090 (25) has a K_i = 1.4 μ M at mGAT-2 and is 10-30 fold selective over the other GABA transporters [3]. Future *in vivo* studies utilising subtype selective GAT inhibitors will allow their pharmacological and physiological role to be probed further.

Glycine Transporters

The glycine transporters also form part of the superfamily of sodium and chloride dependent neurotransmitter transporters. Two glycine transporter genes have been identified to date which give rise to two transporters with approximately 50% amino acid sequence homology. GLYT1 has three variants (1a, 1b, 1c) arising from alternative splicing and human GLYT2 exists only in one form [1]. GLYT1 and GLYT2 transporters are distinct from the classical system A,

Table 1. Potency of Compounds at Cloned GABA Transporters (IC_{50} μ M) [41, 42]

Compound	hGAT-1 (mGAT-1)	rGAT-2 (mGAT-3)	hGAT-3 (mGAT-4)	hBGT-1 (mGAT-2)
GABA	5	5	7	38
(R, S)-(1)	8	39	105	2370
(R)-(1)	8	19	51	2310
(S)-(1)	116	763	2320	6410
(2)	14	58	119	1870
(12)	0.13	550	1990	7210
(13)	0.26	1280	333	300
(14)	0.07	1410	917	1670
(22)	25	30	48	39
(23)	368	21	5	140
(24)	133	51	142	27% at 100 μ M

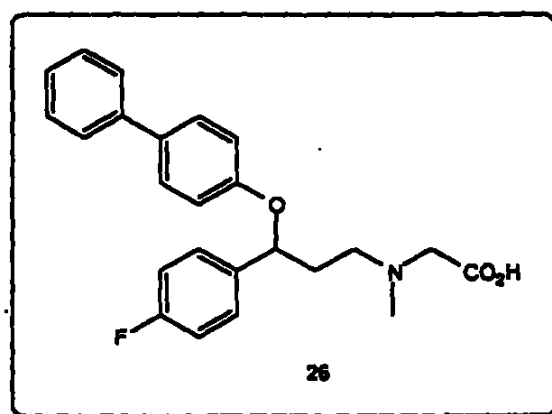


ASC and 'imino' transporters which have broad substrate affinity and selectivity whereas GLYT transporters are more specific and are expressed in different regions of the body [2]. GLYT1 is expressed centrally and peripherally whilst GLYT2 has a CNS specific distribution [1]. The expression of GLYT2 is consistent with the distribution of the inhibitory glycine receptor and neurones of high glycine content (spinal cord, auditory system). GLYT2 is located axonally at presynaptic terminals whereas GLYT1 has predominantly a glial distribution [1]. GLYT1 is not only expressed in areas corresponding to strychnine-sensitive and strychnine-insensitive glycine receptors but also outwith these areas. The role of GLYT1 in these areas has been postulated to involve modulation of NMDA receptor function or non-strychnine-sensitive glycine receptors [1]. Inhibition of the glycine transporter has been hypothesised to be useful in the treatment of epilepsy and pain directly and of schizophrenia and dementia via modulation of NMDA receptor function [43, 44].

There are only a few reports of glycine transport inhibitors in the literature. Simple substrate specificity and potency has been reported by several groups and has been summarised [1]. Glycine acts as a moderate affinity substrate at both GLYT1 and GLYT2 ($K_m = -20-50 \mu M$). GLYT1 is inhibited by sarcosine (*N*-methyl glycine) ($K_i < 100 \mu M$) but alanine, GABA, serine, glycine esters, glutamate and proline have no affinity [1, 45]. In contrast GLYT2 is not inhibited by sarcosine, alanine, β -alanine, serine, cysteine, glycine esters or *N*-methylaminoisobutyric acid [1, 46].

Trophix pharmaceuticals have recently patented two series of glycine transport inhibitors based on lipophilic

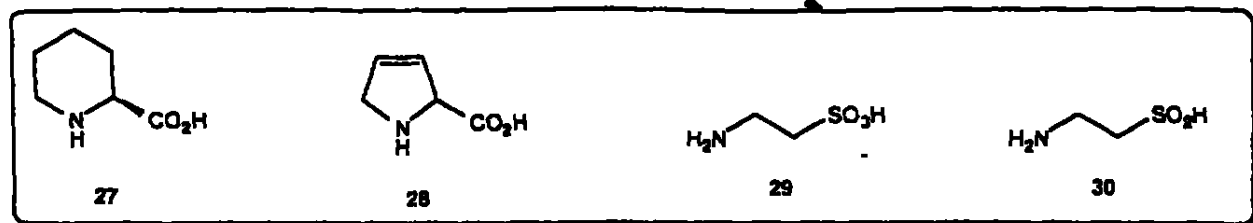
analogues of sarcosine and lipophilic piperidine *N*-acetic acid derivatives [43, 44]. The lipophilic sarcosine derivative (26) has reported to be a potent ($K_i = 0.005 \mu M$) and selective GLYT1 inhibitor [47].



Proline Transporter

The proline transporter (PROT) is another member of the superfamily of sodium and chloride dependent neurotransmitter transporters. It has a CNS specific tissue distribution being found in the olfactory bulb, cerebral cortex and hippocampus [1]. The PROT transporter is distinct from the classical system 'imino' transporters which have broad substrate affinity and selectivity. It has approximately 45% sequence homology to the related glycine transporters [1].

Only simple inhibitors and substrates for PROT have been reported to date. L-Proline is a high affinity substrate ($K_m = \sim 10 \mu M$) whilst L-pipecolic acid (27) ($K_i = 14 \mu M$), sarcosine ($K_i = 30 \mu M$) and 3,4-



dehydroproline (28) ($K_i = 31 \mu\text{M}$) act as inhibitors [48]. Other moderately potent inhibitors of PROT include norleucine, phenylalanine, histidine and cysteine, but many other amino acids show no affinity [48]. Interestingly, *N*-methyl and *N*-acetyl derivatives of proline are inactive as are the carboxylic ester derivatives [48,49]. More recently enkephalins and their analogues have been shown to be potent inhibitors of PROT with des-Tyr-Leu-enkephalin having an $\text{IC}_{50} = 0.26 \mu\text{M}$ [50]. It is still unclear as to the role of PROT in the CNS, however, circumstantial evidence implicates L-proline as a putative synaptic regulatory molecule [1].

Taurine Transporter

The final member of the superfamily of sodium and chloride dependent neurotransmitter transporters worthy of mention is the Taurine transporter, TAUT. Although the expression of TAUT is widespread throughout the body it is found in the brain along with an abundance of taurine which may suggest a specific function of TAUT in the CNS [1, 2]. TAUT has a high sequence homology (~60-70%) with BGT-1 and has a high affinity for the transport of taurine (29) ($K_m = <10 \mu\text{M}$). TAUT has moderate affinity for the transport of β -alanine ($K_m = -60 \mu\text{M}$) and is inhibited by hypotaurine (30) ($K_i = <10 \mu\text{M}$). GABA, alanine, glycine and proline have all been shown to lack affinity for TAUT [1, 51].

Cationic Amino Acid Transporters

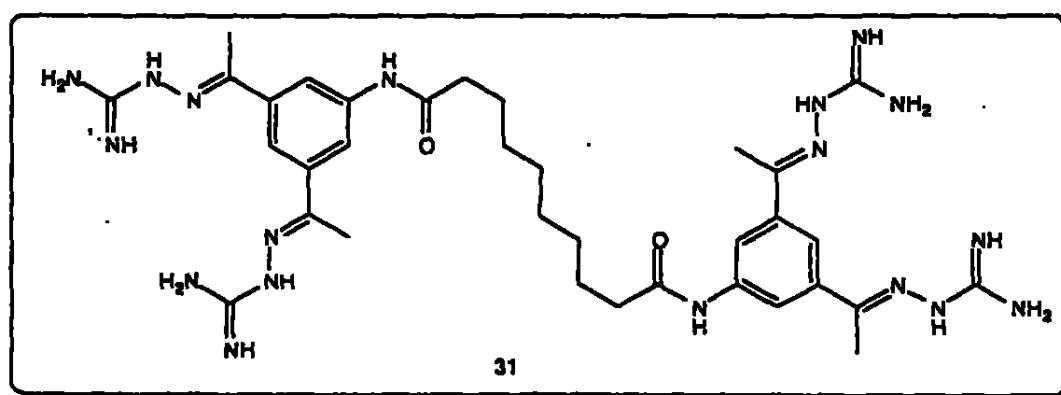
Four mammalian genes defining a family of cationic amino acid transporters (CAT-1, -2, -3, -4) have been

identified. CAT-1, CAT-2a, CAT-2b and CAT-4 have been reported to have a widely expressed tissue distribution, however, the remaining transporter CAT-3 has been shown to have a brain specific expression in rat and mouse [1]. CAT-3 exhibits a 53-58% amino acid sequence homology with CAT-1, CAT-2a and CAT-2b. It is related to the common cationic amino acid transporter group y^+ and is believed to exist structurally in a similar fashion to mCAT-1 that has been postulated to exist with a 12 or 14 trans-membrane topology [1]. CAT-3 transports L-arginine ($K_m = -100 \mu\text{M}$) as a substrate in a sodium and chloride independent manner. It also transports L-lysine ($K_m = -150 \mu\text{M}$) and L-ornithine ($K_m = -150 \mu\text{M}$) [1, 52]. Although the specific role of CAT-3 in the CNS is as yet undefined, it is known that cationic amino acids are required for protein and urea synthesis (arginine) and as precursors for the synthesis of bioactive molecules such as nitric oxide, urea and polyamines (arginine and ornithine respectively) [1]. Hence, CAT-3 may have a CNS specific role in the provision of these key biochemical entities.

No CAT-3 specific inhibitors have been reported to date, however, moderately potent general inhibitors of arginine transport such as the guanilylhydrazone (31) ($\text{IC}_{50} = 10-50 \mu\text{M}$) have been disclosed [53].

Glutamate Transporters

CNS glutamate transporters, their substrates and competitive inhibitors have recently been reviewed in detail [54]. Glutamate transporters form part of the superfamily of sodium dependent transporters for anionic and zwitterionic amino acids which have been

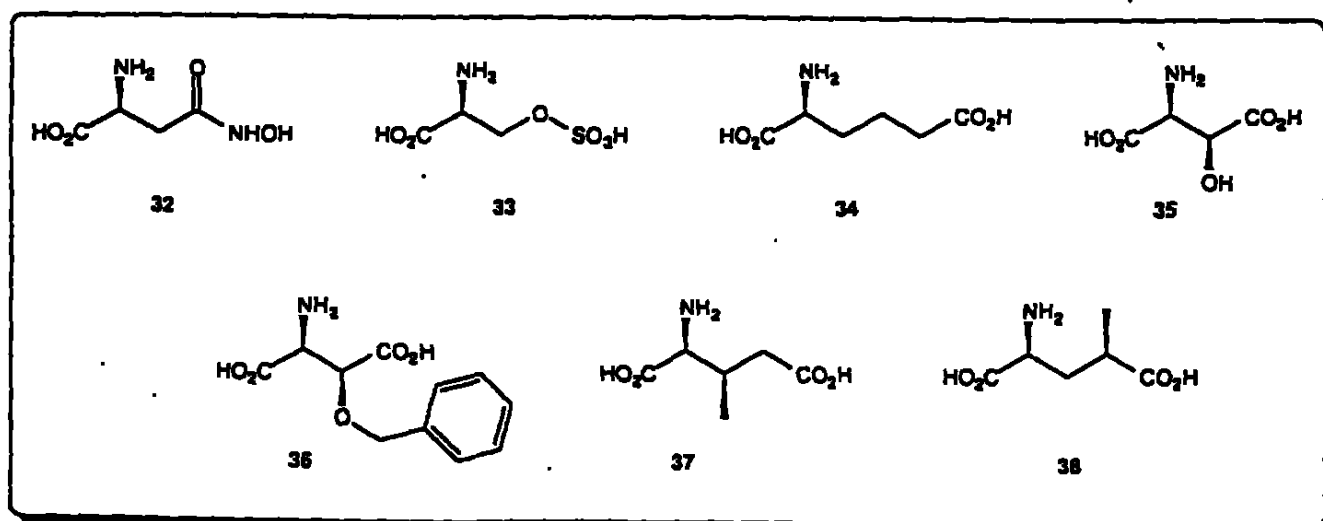


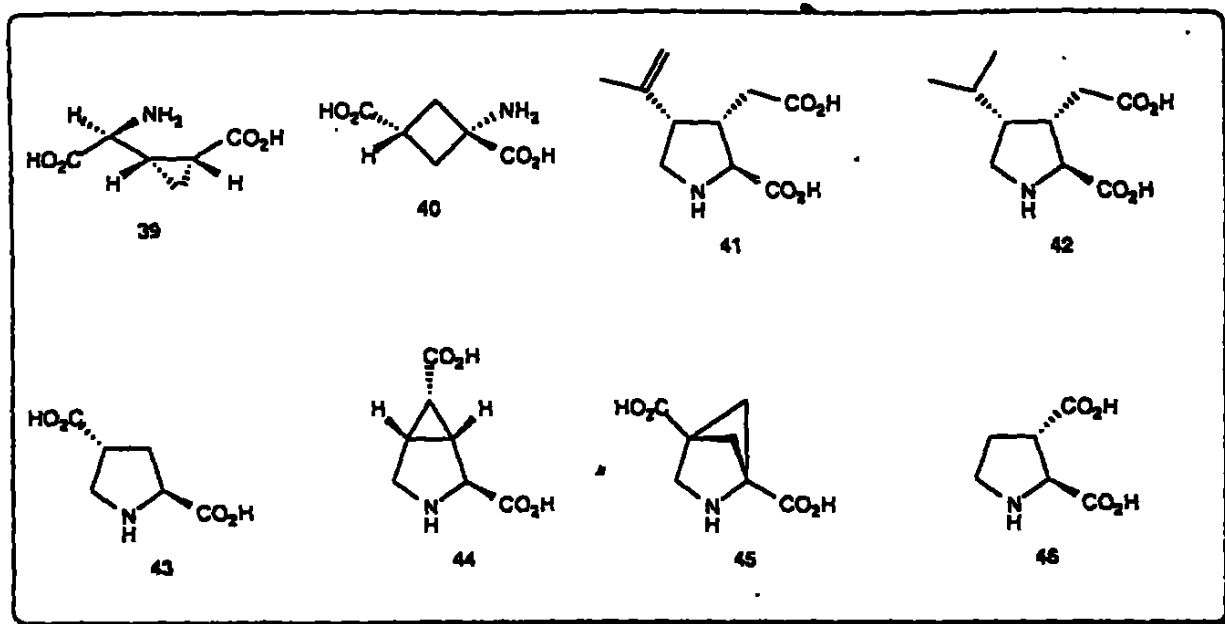
postulated to have a substrate (amino acid and sodium)-gated chloride channel mode of action in addition to their transport role [1]. Five glutamate or anionic amino acid transporters have been identified and labelled excitatory amino acid transporters (EAAT 1-5) [1, 54]. EAATs 1-5 show a sodium and potassium dependent transport and are likely to be neural and nonneural variants of the anionic amino acid transport system X⁻_{AG}. With the possible exception of EAAT-5 the transport mechanism has been shown to be electrogenic with glutamate being co-transported with 2 or 3 sodium ions and an optional proton with counter-transport of a potassium ion and an optional hydroxyl anion [1]. Their topology is not fully defined with models suggesting 6-10 transmembrane domains consisting of α -helices and β -sheets. EAAT 1-5 show 36-65% amino acid homology with each other and have varying tissue distributions. Human EAAT-1, 3 and 4 are expressed in the brain and other tissues, EAAT-2 is brain specific whilst EAAT-5 is expressed in the retina. EAAT-1 and 2 are present in astroglial cells whereas EAAT-3 is present in neurons. [1]. In terms of application to the treatment of CNS disease, inhibition of glutamate transport may prove useful in the treatment of schizophrenia by improving glutamatergic function [55], however the excitotoxic potential in raising glutamate levels is always present [54].

Simple substrates for EAATs 1-5 are the anionic amino acids L-glutamate and L- or D-aspartate. EAATs 1, 2, 3, and 5 have moderate affinity ($K_m = 10$ -64 μ M) and EAAT-4 has a high affinity for these substrates ($K_m = 2$ -3 μ M) [1]. Simple structure activity relationships indicated that α -amino acids with a second acidic group 2-4 carbons away from the α -carboxyl moiety were competitive inhibitors [54]. The nature of the second acidic group has been varied and led to some selective inhibitors being identified. L-aspartate- β -hydroxamate (32) has some selectivity for neuronal EAAT-3 ($K_i = 133$ μ M compared to 369 and 184 μ M for EAAT 1 and 2

respectively). L-serine-O-sulfate (33) has some selectivity for EAAT-1 ($K_i = 107$ μ M compared to 1157 and 150 μ M for EAAT 2 and 3 respectively) and L- α -aminoadipic acid (34) has good selectivity for EAAT-4 as a substrate ($K_m = 168$ μ M at EAAT-4 and >1 mM at EAAT 1-3) [54]. The addition of substituent groups to the β and γ positions of the side chain in aspartate and glutamate has yielded some interesting analogues. L- β -Threo-hydroxyaspartate (35) (and the corresponding D- isomer) are substrates for EAAT-1-4 but act as an inhibitor at EAAT-5 ($K_i \sim 1$ μ M). Hydroxy ester and ether derivatives of (35) have been reported which vary in their pharmacological profile [56, 57]. Alkyl substituted hydroxy esters of (35) act as substrates whereas the benzoate and 1-naphthoate esters are moderately potent inhibitors of EAAT-1. The benzyl ether derivative (36) is a potent inhibitor of EAAT-1 and 2 ($K_i = 9$ and 0.1 μ M respectively) that also has some weak affinity for NMDA and kainate receptors [54, 56, 57]. The β -methyl derivative L-threo-3-methyl glutamate (37) shows no affinity for EAAT-1 but acts as an inhibitor at EAAT-2 ($K_i = 18$ μ M) whereas the corresponding *erythro* isomer has no affinity for either transporter [54, 58]. The γ -methyl derivative (2S, 4R)-4-methylglutamate (38) acts as a substrate at EAAT-1 but as an inhibitor at EAAT with $K_i = 3.4$ μ M, however, it is also a potent kainate receptor agonist [54, 59].

Conformationally constrained cyclic glutamate derivatives have also been investigated as potential inhibitors of glutamate transport. The cyclopropyl analogue (39) is a potent inhibitor of glutamate transport (EAAT-1 $K_i = 7.5$ μ M, EAAT-2 $K_i = 2.5$ μ M, EAAT-3 $K_i = 10$ μ M) whilst related stereoisomers tend to be less potent [54, 59]. The *cis*-cyclobutyl analogue (40) shows moderate inhibitory action (EAAT-1 $K_i = 120$ μ M, EAAT-2 $K_i = 660$ μ M, EAAT-3 $K_i = 244$ μ M) whilst the corresponding *trans* isomer is inactive [54]. Pyrrolidine dicarboxylate derivative medicinal chemistry research has yielded some of the most interesting





compounds to date. Kainic acid (41) and the reduced analogue dihydrokainate (42) act as specific inhibitors ($K_i \sim 10\text{--}80 \mu\text{M}$) of EAAT-2 with no appreciable affinity for other EAATs and more interestingly (42) has reduced affinity for AMPA and kainate receptors compared to (41) [1, 54]. The 2,4 dicarboxylate derivative, L-trans-2,4-PDC (43) acts as a substrate at EAAT-1-4 with similar potency to L-glutamate, however, it acts as a potent inhibitor of EAAT-5 ($K_i = 6.2 \mu\text{M}$) [54, 60]. In an attempt to constrain the somewhat flexible pyrrolidine ring in (43), the bicyclic analogues (44) and (45) were synthesised [54, 61]. Both (44) and (45) show promise as potent modulators of glutamate transport with (44) showing potent inhibition of EAAT-2 ($K_i = 1.6 \mu\text{M}$) whereas (45) acts as a moderately potent substrate for EAAT-2 ($K_m = 45 \mu\text{M}$). The related 2,3 pyrrolidine dicarboxylate analogue (46) is a potent inhibitor of EAAT-2 ($K_i \sim 10 \mu\text{M}$) but has little activity at EAAT-1 or 3; however the use of (46) as a pharmacological tool may be hampered by its excitotoxic side effects mediated through excitatory amino acid ionotropic receptors [54].

Conclusions

The use of amino acid transport inhibitors as effective medicines has now been demonstrated by Tiagabine (14). With the recent advances in molecular biology, medicinal chemists will be able to target transporter subtypes to generate potent and selective chemical entities to probe pharmacological and physiological function of CNS specific amino acid transporters. Improvements in pharmacokinetic parameters will in turn hopefully provide suitable candidates to progress into the clinic with the aim of

treating a wide range of psychiatric, neurodegenerative and CNS driven disorders.

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Inhibitors of Amino Acid Transporters

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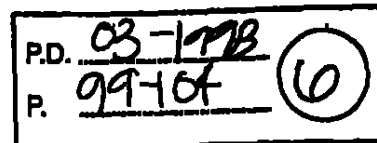
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ORIGINAL INVESTIGATION

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Deramciclane, a putative anxiolytic drug, is a serotonin 5-HT_{2C} receptor inverse agonist but fails to induce 5-HT_{2C} receptor down-regulation

Received: 24 October 1996/Final version: 8 August 1997

Abstract Deramciclane (EGIS-3886) is a novel anxiolytic agent that binds with high affinity to 5-HT_{2A/2C} receptors. The interactions of deramciclane with the serotonin 5-HT_{2C} receptor were characterized further using receptor phosphoinositide hydrolysis assays and receptor autoradiography. Deramciclane antagonized 5-HT_{2C} receptor mediated 5-HT-stimulated phosphoinositide hydrolysis with an IC₅₀ value of 168 nM. Deramciclane also decreased basal phosphoinositide hydrolysis by up to 33% (EC₅₀ = 93 nM) in a physiological system in the choroid plexus, suggesting that deramciclane possesses inverse agonist properties at this receptor. Administration of single doses of 0.5 mg/kg and 10 mg/kg resulted in a maximal 5-HT_{2C} receptor occupancy of up to 45% and 79%, respectively, in the choroid plexus. Chronic (14 days) treatment with 0.5 mg/kg or 10 mg/kg deramciclane did not alter [¹²⁵I]DOI (agonist) or [³H]mesulergine (antagonist) binding to 5-HT_{2C} receptors in the choroid plexus compared to saline-treated controls, as determined by quantitative receptor autoradiography. In comparison, the effects of deramciclane on 5-HT_{2A} binding characteristics and receptor occupancy were also studied. Deramciclane treatment resulted in 5-HT_{2A} receptor occupancy of up to 78%, but no significant effect of chronic treatment on 5-HT_{2A} receptor agonist binding levels was found. In conclusion,

these data indicate that deramciclane is a 5-HT_{2C} receptor inverse agonist and occupies 5-HT_{2C} receptors during treatment, and that chronic treatment with deramciclane does not lead to 5-HT_{2C} receptor down-regulation.

Key words Deramciclane · Anxiolytic drug · 5HT_{2C} · 5HT_{2A} · Serotonin receptor · Receptor regulation · Inverse agonist

Introduction

The 5-HT₂ receptor subfamily has recently attracted interest as a possible target for new anxiolytic drug development. This approach is based on observations that drugs acting at these receptors have anxiogenic or anxiolytic properties. For instance, the anxiogenic properties of *m*-chlorophenylpiperazine (mCPP) in rats (Kennett et al. 1989), pigeons (Gleason et al. 1989) and humans (see Kahn et al. 1988; Handley and McBlane 1993; Kennett 1993) have been attributed to its agonist activity at 5-HT₂-like receptors, mainly the 5-HT_{2C} (Kennett 1992; Gibson et al. 1994). One of the novel putative anxiolytic drugs with antiserotonergic properties is deramciclane (Gacsályi et al. 1988, 1996). It has relatively high affinity for 5-HT_{2A} and 5-HT_{2C} receptors, intermediate affinity for sigma receptors and low or very low affinity for many other neurotransmitter receptors, including other serotonin receptors subtypes as well as dopamine, adrenergic, GABA, CCK, benzodiazepine, histamine and NMDA receptors. Deramciclane demonstrates anxiolytic-like activity in Vogel's test, social interaction, two-compartment box and marble burying tests and does not reduce motor activity at the established anxiolytic doses (Gacsályi et al. 1988, submitted). In the present study, we investigated the interactions of deramciclane with the 5-HT_{2C} receptors to characterize further the

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interactions of deramciclane with the receptor, to find out if deramciclane occupies 5-HT_{2C} receptors after single-dose administration and whether chronic treatment with deramciclane alters the quantity of 5-HT_{2C} agonist or antagonist binding sites in the rat brain. Clozapine, known to induce 5-HT_{2C} down-regulation, was included as a positive control (Hietala et al. 1992).

Materials and methods

Animals

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Ind., USA) weighing 200–250 g were used. Rats were housed under standard laboratory conditions (temperature 21°C; humidity 55 ± 5%; lights on from 6.00 a.m. to 6.00 p.m.). Standard pelleted food and tap water were available ad libitum. Experiments were conducted according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Finnish Statute Book 1360/90).

Drug treatments

Deramciclane (EGIS Pharmaceuticals, Hungary) was diluted in 0.9% saline and the solution was emulsified with a drop of Tween 80. Clozapine (Leponex, 25 mg/ml; Sandoz Pharma AG, Basle, Switzerland) was used as commercially available ampoules. In the chronic treatment experiment, four groups of rats ($n = 6$ in each group) received SC injections of clozapine (25 mg/kg), deramciclane (0.5 or 10 mg/kg) or an equal volume (1 ml/kg) of saline (with Tween 80 added) once a day for 14 days. In the receptor occupancy experiment, rats were given a single dose of deramciclane (0.5 or 10 mg/kg), clozapine (25 mg/kg), or an equal volume (1 ml/kg) of saline. Doses refer to the free base of a given drug.

Brain dissection

Rats were decapitated 68 h (chronic treatment experiment) or 1 h, 2 h, 4 h, 8 h and 24 h (occupancy experiment) after the last drug injection, and the brains were immediately dissected, frozen on dry ice and stored at –80°C for later use. Coronal brain sections were cut on a cryostat (–16°C) at 18 µm thickness and thaw-mounted onto gelatin-coated glass slides. The thawed sections were then dried at room temperature for several hours and subsequently stored at –80°C until used for receptor autoradiography.

5-HT_{2C} receptor mediated phosphoinositide hydrolysis assay

5-HT-induced phosphoinositide hydrolysis was measured in rat choroid plexus as previously described (Kuoppamäki et al. 1993). Choroid plexi were rapidly dissected out after decapitation and a single choroid plexus (about 0.5–1.0 mg wet weight) was placed into 5 ml Krebs-bicarbonate (KRB) buffer (118 mM NaCl, 5.0 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃) containing 10 mM glucose. KRB buffer was changed once before incubation for 1 h at 37°C in a shaking water bath with two intermediate changes of buffer. The samples were subsequently labeled with 1 µCi of [³H]myo-inositol (New England Nuclear, Boston, Mass., USA) for 90 min in the presence of O₂/CO₂ (95:5).

10 µM pargyline and 10 mM lithium were added and the incubation was continued for 15 min. The antagonists, if present, were added at the same time with pargyline and lithium. Thereafter, 5-HT or an equal volume of buffer was added to give a final volume of 300 µl and the samples were incubated for 30 min. The reaction was stopped by addition of 940 µl chloroform/methanol (1:2 v/v). After vortexing and standing for 15 min, 300 µl chloroform and 300 µl deionized H₂O were added and the tubes were vortexed and centrifuged. A portion (750 µl) of the upper aqueous phase containing water soluble inositol phosphates was removed and applied to a column of Dowex-1 anion-exchange resin in the formate form. Free inositol was eluted with 10 ml of 10 mM myo-inositol, followed by 10 ml of 5 mM sodium tetraborate/60 mM ammonium formate to elute glyceroinositol phosphate. Inositol monophosphate (IP) was eluted with 10 ml of 200 mM ammonium formate/0.1 M formic acid. The first 5 ml of inositol monophosphate was eluted directly into scintillation vials, while the additional 5 ml was discarded. OptiPhase "Hisafe" 3 (LKB Wallac, Loughborough, UK) was used as a scintillation fluid and the IC₅₀ values were calculated with the software GraphPad InPlot 4.1 (GraphPad Software, San Diego, Calif., USA).

Receptor autoradiography

For determination of 5-HT_{2C} antagonist binding (chronic treatment experiments), slides were first preincubated in 170 mM TRIS-HCl buffer at room temperature for 15 min, then incubated in 170 mM TRIS-HCl plus 5 nM [³H]mesulergine (Amersham, Little Chalfont, UK) and 100 nM spiperone at room temperature for 120 min. Non-specific binding was determined in the presence of 5 µM methysergide. Slides were subsequently washed in 0°C 170 mM TRIS-HCl (2 × 10 min) and dipped in ice-cold deionized water. For determination of 5-HT_{2C} receptor agonist binding, dried, room-temperature slides were first preincubated in a 50 mM TRIS-HCl buffer containing 10 mM MgSO₄ and 0.1% (w/v) bovine serum albumin (BSA) at room temperature for 10 min to remove endogenous serotonin. Slides were then incubated in a similar buffer plus 200 pM (±)-1-(2,5-dimethoxy-4-[[¹²⁵I]iodophenyl]-2-aminopropane ([¹²⁵I]DOI, New England Nuclear, Boston, Mass., USA) and 100 nM spiperone (to block 5-HT_{2A} receptors) at room temperature for 60 min. Non-specific binding was defined in the presence of 1 µM methysergide. After incubation, the slides were rinsed in a similar ice-cold buffer (2 × 10 min) and dipped in ice-cold deionized water to remove salt. For determination of 5-HT_{2A} receptor binding, the 5-HT_{2C} agonist binding protocol was employed with the following exceptions: spiperone was excluded from the incubation buffer, and non-specific binding was determined in the presence of 1 µM ketanserin instead of 5 µM methysergide. Thoroughly dried slides and standards were apposed to X-ray film for 30 h ([¹²⁵I]DOI autoradiography) or 21 days ([³H]mesulergine autoradiography) at +4°C in an X-ray film cassette wrapped in plastic for protection from humidity.

Analysis of autoradiographic images

Autoradiographic images were analyzed on a computer-assisted image analyzer (camera, Hamamatsu C3077, Hamamatsu Photonics, K.K., Hamamatsu City, Japan; software, MCID, Imaging Research, St Catharines, Ontario, Canada). For each sample, analysis began by digitizing an image of the total ligand binding. Image densities were then linearized by calibration against the densities produced by the known radioactivities in the standards (American Radiolabeled Chemicals, St Louis, Miss., USA). In the 5-HT_{2C} receptor autoradiograms, total binding was considered specific, as non-specific binding produced a negligible signal on the film. In the 5-HT_{2A} autoradiograms, the non-specific signal was subtracted from the total signal on adjacent sections.

Receptor occupancy measurement

For 5-HT_{2C} receptor occupancy experiments, the above-described autoradiography protocol was used with the following exceptions to achieve optimal conditions for this type of assay: preincubation was omitted to maintain the in vivo drug concentrations, the slides were incubated for 15 min and subsequently wiped off into scintillation vials. For 5-HT_{2A} receptor occupancy experiments, 4 nM [³H]ketanserin (New England Nuclear, Boston, Mass., USA) was used as antagonist radioligand and the assays were carried out similarly to the 5-HT_{2C} occupancy experiments, with the exception of the incubation time which, in the 5-HT_{2A} assay, was 20 min. In both receptor occupancy assays, for each section, non-specific binding was determined for an adjacent section and then subtracted from total binding. The radioactivity in the scintillation vials was measured by beta-counting (Wallac LKB 1219 Rackbeta, Finland) using OptiFluor-O (Packard, Zurich, Switzerland) scintillation fluid at 50% efficiency, except in the 5-HT_{2A} receptor binding assay where OptiPhase "Hisafe" 3 (LKB Wallac, Loughborough, UK) scintillation fluid was used. Computer processing of the binding data (EBDA (equilibrium binding data analysis)/LIGAND) was performed as previously described (Hietala et al. 1990).

Statistical analysis

Statistical analysis of the data was carried out by one-way analysis of variance followed by Tukey's test for post-hoc analyses. *P* value lower than 0.05 was considered significant.

Results

5-HT-stimulated phosphoinositide hydrolysis

Results of phosphoinositide hydrolysis studies in choroid plexus are summarized in Fig. 1a and b. 100 nM 5-HT stimulated the accumulation of inositol monophosphate (IP) 4- to 5-fold over basal values of about 5000 DPM. Deramciclanc antagonized 5-HT (100 nM)-stimulated phosphoinositide hydrolysis, with an IC₅₀ value of 168 nM (Fig. 1a). Deramciclanc did not stimulate phosphoinositide hydrolysis itself, but inhibited basal PI hydrolysis by up to 33% with an EC₅₀ value of 90 nM, indicating that deramciclanc is a 5-HT_{2C} receptor inverse agonist (Fig. 1b).

Effect of chronic treatment with deramciclanc on 5-HT_{2A/2C} receptors

The results of autoradiography are summarized in Fig. 2. Chronic treatment with either dose (10 or 0.5 mg/kg) of deramciclanc did not alter [¹²⁵I]DOI or [³H]mesulergine binding to 5-HT_{2C} receptors in the choroid plexus. In comparison, clozapine treatment decreased [¹²⁵I]DOI binding to 5-HT_{2C} receptors in the choroid plexus by 32% (*P* = 0.012) and [³H]mesulergine binding by 53% (*P* < 0.001). The binding of [¹²⁵I]DOI to 5-HT_{2A} receptors in layer IV of the cerebral cortex was not significantly altered by chronic treatment with either dose regimen of deramciclanc,

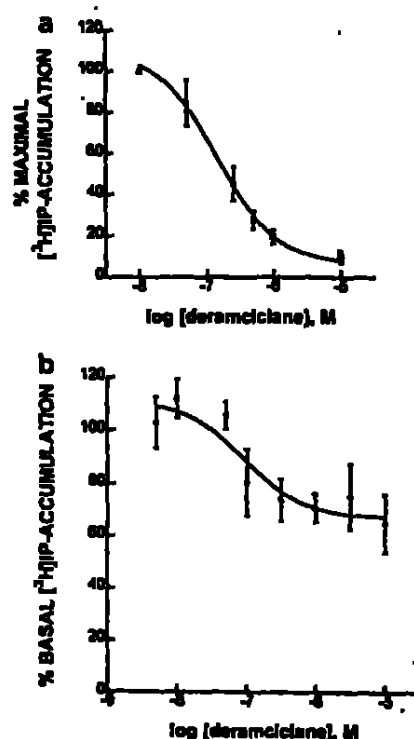


Fig. 1 a Inhibition of maximal 5-HT stimulated [³H]inositol monophosphate ([³H]IP) accumulation in the rat choroid plexus by deramciclanc. Points represent mean $\pm$ SEM of one to three individual experiments. b Effect of deramciclanc on basal [³H]IP production in the rat choroid plexus. Points represent mean $\pm$ SEM of five to eight individual experiments

whereas clozapine treatment elicited a 71% decrease in [¹²⁵I]DOI binding to 5-HT_{2A} receptors (*P* < 0.001). However, a trend towards reduced 5-HT_{2A} binding following deramciclanc treatment was observed; binding was decreased by 21% (*P* = 0.074) and 16% (*P* = 0.23) by treatment with 0.5 mg/kg and 10 mg/kg, respectively.

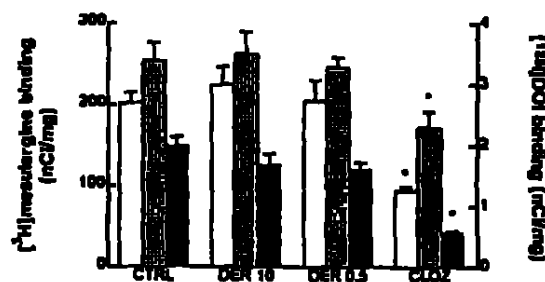


Fig. 2 Effects of chronic treatments (14 days) with deramciclanc (DER, 10 mg/kg and 0.5 mg/kg SC once daily) and clozapine (CLOZ, 25 mg/kg SC once daily) on the binding of [³H]mesulergine and [¹²⁵I]DOI to 5-HT_{2C} receptors in rat choroid plexus (open and dotted bars, respectively), and of [¹²⁵I]DOI to 5-HT_{2A} receptors in rat frontal cortex (solid bars). **P* < 0.05 compared to the control group (CTRL)

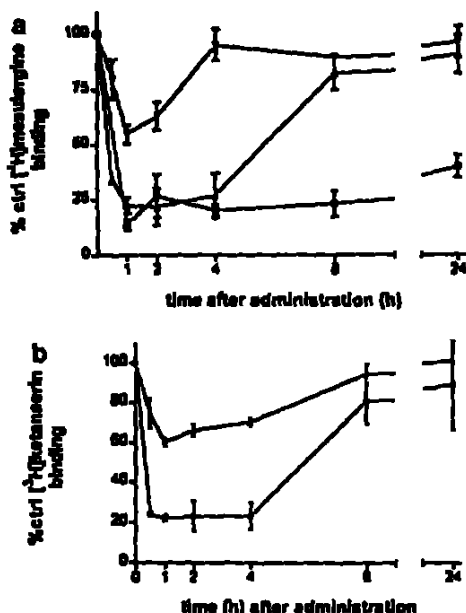


Fig. 3 a 5-HT_{2C} receptor occupancy by deramciclane and clozapine: a time-course plot of the effects of 0.5 mg/kg (Δ) and 10 mg/kg (▽) deramciclane and 25 mg/kg clozapine (■) on [³H]mesulergine binding to rat choroid plexus 5-HT_{2C} receptors. b 5-HT_{2A} receptor occupancy by deramciclane: a time-course plot of the effects of 0.5 mg/kg (Δ) and 10 mg/kg (▽) deramciclane on [³H]ketanserin binding to rat frontal cortex 5-HT_{2A} receptors

5-HT_{2C} receptor occupancy after single-dose administration of deramciclane and clozapine, and 5-HT_{2A} receptor occupancy after single-dose administration of deramciclane

Total binding per slide (three sections) in the 5-HT_{2C} assay was 1500–2000 DPM, out of which approximately 75% was displaceable by 5 μM methysergide. Total binding in the 5-HT_{2A} assay was 4000–6000 DPM/slide, out of which about 60% was displaceable by 1 μM ketanserin. 5-HT_{2A} and 5-HT_{2C} receptor occupancy by deramciclane is demonstrated in Fig. 3a and b. Administration of 0.5 mg/kg and 10 mg/kg deramciclane caused a maximal 5-HT_{2C} occupancy of 45% and 79%, respectively, and the effect was dose-related. The same doses caused a maximal 5-HT_{2A} receptor occupancy of 40% and 78%, respectively. The effect was dose-related. The reference drug clozapine (25 mg/kg) elicited a marked and persistent occupancy of 5-HT_{2C} receptors.

Discussion

Deramciclane is a predicted anxiolytic drug undergoing further research in early human studies. Deramciclane binds to the rat 5-HT_{2C} receptor with a K_i value of 27 nM for inhibition of [³H]mesulergine

binding, and to the rat 5-HT_{2A} receptor, with a K_i value of 11 nM for inhibition of [³H]ketanserin binding (Gacsályi et al., submitted). The present results demonstrate that deramciclane is a 5-HT_{2C} receptor antagonist with an IC₅₀ value of 168 nM for inhibition of 5-HT stimulated phosphoinositide hydrolysis. Deramciclane also inhibited basal PI hydrolysis in the choroid plexus, indicating that it possesses inverse agonist properties at this receptor. Other inverse agonists of the 5-HT_{2C} receptor include the atypical antipsychotic clozapine (Kuoppamäki et al. 1994), spiperone, mesulergine, ketanserin and the antidepressant mianserin (Barker et al. 1994). The significance of inverse agonism is unclear (for review, see Milligan et al. 1995), but reduction of constitutive activity by inverse agonists may contribute to their effects when, for instance, increased constitutive activity results in disease (e.g. Shenker et al. 1993). Consistent with our previous studies on other 5-HT_{2C} receptor antagonists (Kuoppamäki et al. 1995, Pälvimäki et al. 1996), the efficacy of deramciclane in displacing antagonist radioligand was 5- to 10-fold higher than its inhibition potency of PI hydrolysis.

The fact that a compound has high affinity for its receptor in vitro is not alone indicative of the functional significance of this interaction in vivo upon treatment. The ligand must pass through the blood-brain barrier and reach its target in sufficient concentrations to interact directly with its receptor. In the present study, we treated rats with two different doses of deramciclane and determined the consequent in vivo 5-HT_{2A} and 5-HT_{2C} receptor occupancies in cortical and choroid plexus coronal sections, respectively, at various time points.

Deramciclane caused marked occupancy of both 5-HT_{2A} and 5-HT_{2C} receptors in vivo after administration of doses that are active in anxiolytic tests in rodents (0.5 and 10 mg/kg), suggesting that antagonism at these receptors may be involved in its anxiolytic-like properties. The behavioral effects of mCPP have been suggested to be mediated by a combination of its agonist activity at 5-HT_{2C} receptors and antagonist activity at 5-HT_{2A} receptors (Fiorella et al. 1995). Moreover, selective 5-HT_{2A} receptor antagonists have been reported not to have an anxiolytic effect nor to block the anxiogenic action of mCPP (Gardner 1986; Kennett 1992; Gibson et al. 1994). Therefore, the 5-HT_{2C} receptor seems a likely candidate for mediating the anxiolytic activity of deramciclane. On the other hand, as the anti-anxiety models are not categorically selective of drugs targeting 5-HT_{1A}, 5-HT_{2A} or 5-HT_{2C} receptors (for review, see Griebel 1995), the 5-HT_{2A} receptor cannot be ruled out in this context.

As a rule, chronic treatment with agonists or antagonists of the 5-HT_{2A/2C} receptors leads to receptor down-regulation (Peroutka and Snyder 1980; Sanders-Bush and Breeding 1988, 1990; Pranzatelli 1991; Hietala et al. 1992; Barker and Sanders-Bush 1993; Pranzatelli

et al. 1993). However, many potent antagonists fail to elicit this effect (e.g. Kuoppamäki et al. 1994) and some have an opposite effect (Rinaldi-Carmona et al. 1993; Rocha et al. 1994). Furthermore, the regulation of 5-HT_{2C} receptors can be region-specific (e.g. Rocha et al. 1994) and may be brought about by indirect drug effects (Laakso et al. 1996). In the present study, we found that chronic treatment with deramciclane did not alter 5-HT_{2A} or 5-HT_{2C} receptor agonist or antagonist binding characteristics in the rat brain, whereas the reference drug clozapine elicited a marked decrease of both agonist and antagonist binding to 5-HT_{2A} and 5-HT_{2C} receptors. The effects of clozapine are in accordance with previous studies, and thus validate the methods used in the present investigation (Kuoppamäki et al. 1994).

Altered receptor gene expression is apparently not involved in drug-induced 5-HT_{2C} receptor regulation (Roth et al. 1990; Barker and Sanders-Bush 1993), but phosphorylation of the 5-HT_{2C} receptor may be necessary for receptor desensitization. Increased basal phosphorylation leads to 5-HT_{2C} receptor desensitization (Westphal et al. 1995) and dephosphorylation of the receptor may counteract protein kinase C-mediated desensitization (Boddeke et al. 1993). Therefore, direct effects on receptor phosphorylation might account for the ability of certain drugs to induce 5-HT_{2C} receptor down-regulation. However, mianserin has no effect on 5-HT_{2C} receptor phosphorylation on its own, although it does block the serotonin-induced increase (Westphal et al. 1995). Whether clozapine has direct effects on 5-HT_{2C} receptor phosphorylation is unknown. Since the inverse agonists clozapine and mianserin induce 5-HT_{2C} receptor down-regulation in vivo (Roth et al. 1991; Kuoppamäki et al. 1994; Rocha et al. 1995), it might be suggested that inverse agonism is predictive of 5-HT_{2C} receptor down-regulation. However, Labrecque et al. (1995), using 5-HT_{2C} receptor-transfected cells, found that inverse agonism is not predictive of the ability of a compound to cause 5-HT_{2C} receptor down-regulation. Furthermore, the present data provide evidence that marked receptor occupancy in vivo during chronic treatment by an inverse agonist is not sufficient to induce 5-HT_{2C} receptor down-regulation in the choroid plexus.

Since the occupancy of 5-HT_{2C} receptors by deramciclane is relatively short-lived, it might be conceived that in order to see down-regulation, a more sustained occupancy should be acquired. Indeed, the present data show that the 5-HT_{2C} occupancy after administration of 25 mg/kg clozapine is more persistent compared to that after deramciclane administration. Clozapine 25 mg/kg was, however, included solely as a positive control in this study, and no attempt was made to find doses resulting in similar receptor occupancy. In a previous study, we found that the 10 mg/kg clozapine dose administered once daily for 14 days was sufficient to cause a 49% down-regulation of the 5-HT_{2C} receptor,

as measured by methods identical to those used in the present investigation (Kuoppamäki et al. 1994). Baldessarini et al. (1993) found that the elimination half-life of clozapine in the rat brain is 1.5 h, and that 12 h after administration of a single dose of 10 mg/kg IP, neither clozapine nor its main metabolites were detectable in rat brain. This suggests that after 10 mg/kg clozapine, the receptor occupancy may be shorter-lived. We propose that the differential regulation of the 5-HT_{2C} receptor by clozapine and deramciclane is unlikely to be explained by pharmacokinetics, but further occupancy determinations and perhaps more frequent administration of deramciclane upon chronic treatment are needed to conclude on this matter.

In summary, the principle findings of the present investigation are the following: 1) The putative anxiolytic deramciclane is a 5-HT_{2C} receptor antagonist with inverse agonist properties; 2) single-dose administration of deramciclane at anxiolytic doses results in marked in vivo occupancy of 5-HT_{2C} (and 5-HT_{2A}) receptors in the rat brain; 3) chronic treatment (14 days, once daily, SC) with deramciclane (10 mg/kg and 0.5 mg/kg) does not alter the quantity of 5-HT_{2C} receptor agonist or antagonist radioligand binding in the rat choroid plexus.

Acknowledgements The technical assistance of Ms. Eija Lehtovirta is acknowledged. E.-P.P. was supported by a grant from the Science and Research Foundation of Farnos.

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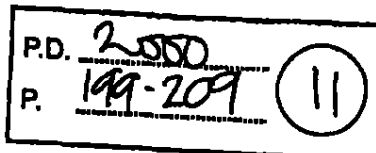
CITED IN THE PCT SEARCH REPORT

Current Medicinal Chemistry, 2000, 7, 199-209

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Inhibitors of Mammalian Central Nervous System Selective Amino Acid Transporters

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XP-000937809

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Abstract: Central nervous system (CNS) selective amino acid transporters provide an important function in maintaining tonic extracellular levels of amino acids that act as neurotransmitters, synaptic modulators or neurotransmitter precursors. Small molecule inhibitors of these transporters have been postulated and in some cases demonstrated to be useful in the treatment of a range of CNS driven disorders such as epilepsy, anxiety, psychosis, depression, pain and neurodegenerative disease. Although much of the research to date in this field has focussed on inhibition of the γ -amino butyric acid (GABA) transporters more recent reports have also generated interest in modulation of glycine, glutamate and proline transporters. This article will review the current medicinal chemistry literature and structure activity relationships known for mammalian CNS selective amino acid transporters.

Introduction

The uptake of amino acids into mammalian cells is mediated by energy dependent and passive transporters. These transporters are characterised by their general ability to transport amino acids in a stereoselective fashion with broad substrate specificity. Amino acid transporters in the central nervous system (CNS) maintain tonic extracellular levels of amino acids that act as neurotransmitters (or precursors to neurotransmitters) or synaptic modulators. Their role in maintaining intra and extra cellular CNS amino acid homeostasis is critical in preventing neurotoxicity by removing neurotransmitter-amino acids from synaptic clefts and also by providing a supply of precursors in the CNS.

Recent advances in the molecular biological aspects of plasma membrane amino acid transporters have been extensively reviewed [1, 2]. More information about their structure, function and tissue distribution is discovered on a regular basis. Breakthrough's in the identification of subtypes of classes of CNS amino acid transporters has fuelled further medicinal chemistry research to find selective inhibitors as potential new medicines for a range of CNS driven diseases such as epilepsy, anxiety, psychosis, depression, pain and neurodegenerative disease.

The aim of this review is to provide the reader with an overview of key medicinal chemistry advances in the development of CNS amino acid transport inhibitors. Each class of transporter will be reviewed in turn covering those that are expressed in CNS tissue – γ -amino butyric acid (GABA) transporters, glutamate transporters, glycine transporters, proline transporter, taurine transporter and the brain specific cationic amino acid transporter.

γ -Amino butyric acid (GABA) Transporters

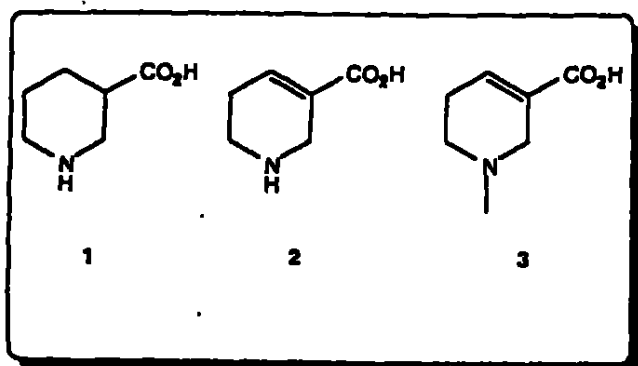
The GABA transporters form part of the superfamily of sodium and chloride dependent neurotransmitter transporters. The rat and human brain GABA transporter (GAT-1) was one of the first transporters of this family to be cloned in 1990 and was the starting point for the isolation of related cDNA constituting this superfamily [1]. The GABA transporters, like all transporters of this superfamily, are proteins with a 12-membrane spanning domain topology. Four mammalian GABA transporters have been cloned to date from mouse, rat and human brain [2]. They possess a high level of sequence homology but vary in their tissue distribution and pharmacological properties. The murine clones are referred to as mGAT-1, mGAT-2, mGAT-3 and mGAT-4, however, they correspond to the human (and rat) homologues hGAT-1, hBGT-1, hGAT-2 and hGAT-3 respectively in nomenclature terms [3]. mGAT-1 (hGAT-1) and mGAT-4 (hGAT-3) are high affinity neuronal GABA transporters and are found

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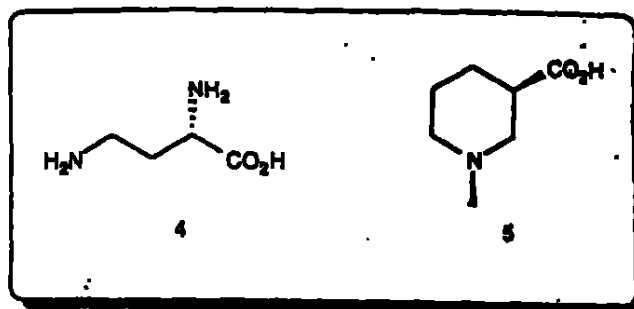
specifically in the CNS. mGAT-3 (hGAT-2) is a moderate affinity GABA transporter with primarily a glial distribution in the brain but is also expressed in liver and kidney. mGAT-2 (hBGT-1) is a low affinity GABA transporter that also transports the osmolyte betaine. mGAT-2 (hBGT-1) has primarily a glial distribution in the brain but is also expressed in liver and kidney [1].

Inhibition of GABA transporters has been a successful strategy in the treatment of epilepsy as demonstrated with the launch of Tiagabine (14) (Novo Nordisk) in 1996. GABA transporter inhibitors have also been implicated for use as analgesics, anxiolytics, and hypnotics and in the treatment of muscle movement disorders, depression and psychosis [4, 5, 6]. Recent studies have also indicated their potential as anti-tussive agents [7].

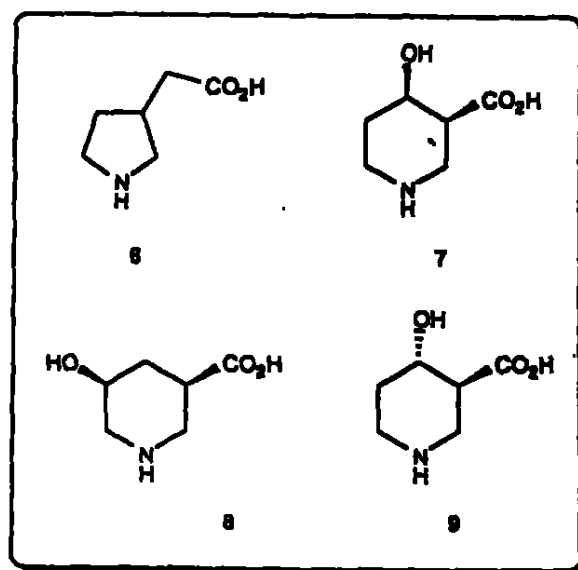
Medicinal chemistry research into GABA transporters is the most established of all the amino acid transporter classes and has periodically been covered in general GABA medicinal chemistry review articles [8, 9, 10]. The first major breakthrough in GABA transport research came when it was found that the heterocyclic amino acids, nipecotic acid (1) and guvacine (2) were potent inhibitors of GABA uptake (IC_{50} = 9 and 8 μ M respectively) whilst the simple N-methyl analogue of guvacine, arecaldine (3), was considerably less potent (IC_{50} = 122 μ M) [11]. Nipecotic acid and guvacine have subsequently been shown to be inhibitors of both neuronal and glial GABA uptake with some selectivity for the glial system [8] and no affinity for GABA receptors. Whilst (1) and (2) are inhibitors of GABA transport they are also substrates which can complicate interpretation of pharmacological data [10].



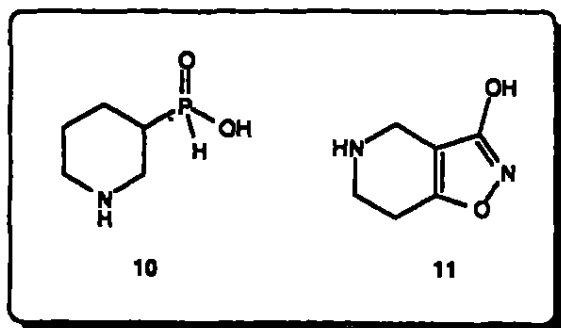
SAR studies with simple acyclic analogues of GABA lead to inhibitors of GABA uptake in rat brain slices, however, they showed little selectivity for transport inhibition over inhibition of GABA binding in rat brain [12]. The GABA analogues DABA (4) and ACHC (5) are selective inhibitors of neuronal GABA uptake and there is some evidence that they also act as substrates [8, 13].



SAR studies have also been carried out based on variation of the heterocyclic ring structure found in (1) and (2). Attempts were made to produce irreversible transport inhibitors to further probe biochemical function by introducing thiol and epoxide functionality to the heterocyclic ring, however, this led to only very weak inhibitors [14]. Attempts to improve inhibitory potency by decreasing or increasing heterocyclic ring size were unsuccessful, as was the introduction of amino, methyl or phenyl groups onto (1) [9, 15, 16, 17, 18]. Transposing the carboxylic acid moiety from C-3 to C-4 of the heterocyclic ring also proved to be an unsuccessful strategy [9, 15, 16, 17], however, appending an acetic acid group to pyrrolidine to give homo- β -proline (6) yielded a potent inhibitor of GABA uptake (IC_{50} = 2.5 μ M) which also had significant affinity for GABA_A receptors [9, 19]. One compound worthy of mention from this series is *cis*-4-hydroxypiperidine-3-carboxylic acid (7). This compound retains potent inhibitory properties (IC_{50} = 11 μ M) [17] of GABA uptake and has been shown to be selective for the glial system [9]. Interestingly, the related *cis*-5-hydroxy analogue (8) and the *trans*-4-hydroxy analogue (9) were inactive. X-ray crystallography and NMR studies subsequently showed that (7) possesses an essential axial hydroxyl group whilst (8) and (9) have equatorially aligned hydroxyl groups [9, 19].



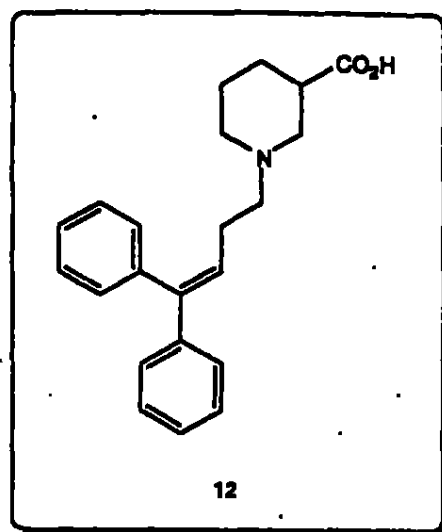
Bioisosteric replacements for the carboxylic acid functionality in (1) have recently been reported [20] with the phosphinic acid analogue (10) retaining uptake inhibitory activity ($IC_{50} = 24 \mu M$). Bioisosteric carboxylic acid replacements have also been used to address the pharmacokinetic problems associated with (1) and (2). Nipecotic acid (1) and guvacine (2) both suffer from poor blood brain barrier penetration upon systemic administration due to the fact that at physiological pH they exist in fully zwitterionic forms [8, 10]. The bioisosteric analogue THPO (11) overcomes this problem to some extent as it penetrates the brain upon systemic administration [8]. THPO (11) is highly selective glial uptake inhibitor ($IC_{50} = 160 \mu M$) that has been shown not to act as a substrate at the transporter [8, 13, 21]. The corresponding isothiazole to (11) is less active as are regioisomeric analogues of (11) [21]. Classical approaches to improve blood brain barrier penetration using lipophilic ester prodrugs of (1) met with limited success. The ethyl and pivaloyloxymethyl esters of (1) demonstrated *in vivo* activity in seizure models upon systemic administration, however, the weak cholinergic side-effects seen with the ethyl ester of (1) seem to have precluded their further development [6, 8].



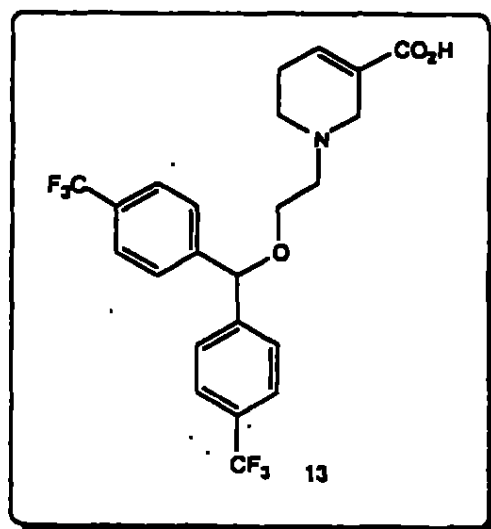
Exciting advances in medicinal chemistry GABA transport research were made in the early 1980's when workers at Smith Kline and French discovered that appending bulky lipophilic side chains to the nitrogen atoms of known GABA uptake inhibitors such as (1) and (2) led to marked increases in inhibitory potency *in vitro* [22]. SKF 89976-A (12) has an inhibitory IC_{50} of $0.2 \mu M$ in a brain synaptosome assay and does not act as a substrate at the transporter or have affinity for GABA receptors [10, 22]. It has improved pharmacokinetic properties by virtue of increased lipophilicity ($\log D$ 1.14 compared to -2.66 for (1)) and appears to readily enter the CNS as it protects rodents from bicuculline induced seizures when dosed orally [22]. SAR studies on (12) indicated that varying the side chain length or removing the double bond led to losses in inhibitory activity [22].

Subsequently, several groups have appended lipophilic side chains to known GABA uptake inhibitors to try and improve potency and pharmacokinetic

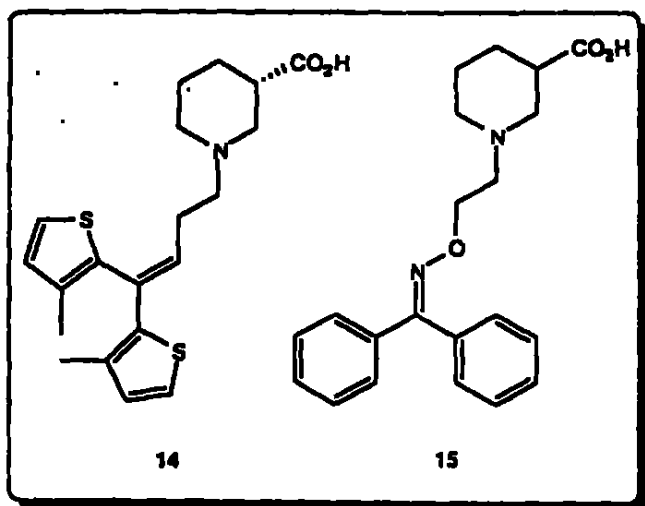
parameters. The 4,4-diphenyl-3-butenyl (DPB) substituted THPO derivatives showed improved potency over THPO ($IC_{50} = 7 \mu M$ compared to $160 \mu M$ for THPO) [13] and whilst DPB substituted GABA ($IC_{50} = 18.5 \mu M$) is weaker as an inhibitor than GABA [22] this potency can be regained by subsequent N-methylation of DPB GABA ($IC_{50} = 4 \mu M$) [23].



Workers at Parke-Davis reported on a series of N-(benzhydryl ethyl ether) derivatives of (1) and (2) [24]. Extensive SAR studies were carried out to probe chain length, spacer groups and aromatic substitution patterns. This work led ultimately to the discovery of the potent lipophilic inhibitor CI-966 (13) ($IC_{50} = 0.34 \mu M$) which like (12) also inhibited seizures *in vivo* upon oral dosing [24, 25]. The compound was progressed to the clinic where unfortunately it demonstrated severe adverse CNS symptoms such as memory deficits, myoclonus and tremors, unresponsiveness and subsequent severe psychological disturbances [22, 26]. For these reasons clinical trials with CI-966 (13) were terminated.

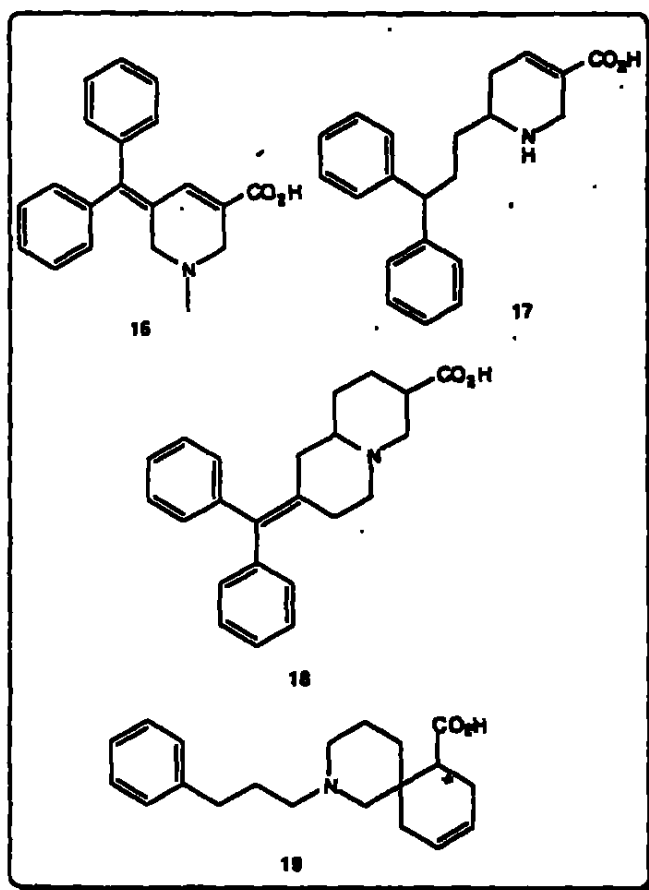


The most successful compound to come out of GABA transport research to date arose from work carried out at Novo Nordisk. Once again lipophilic N-substituents were added to nipecotic acid (1) to give a range of 4,4-diaryl or 4,4-diheteroarylbutenyl derivatives [27]. One of the most potent analogues, Tiagabine (NO-328) (14) ($IC_{50} = 0.067 \mu M$), demonstrated anticonvulsant activity in mice following i.p. administration at low doses. One key observation from the SAR study carried out at Novo Nordisk was the requirement for an ortho or pseudo ortho substituent in the aryl or heteroaryl rings to give potent levels of inhibition [27]. Tiagabine (14) has subsequently been progressed through the clinic and was launched in 1996 for the treatment of specific types of epilepsy. Tiagabine has been shown to be 90% orally bioavailable in man with a half-life of 3.8-9 hours requiring 2-4 times daily administration [28]. Novo Nordisk have also recently patented a range of lipophilic N-substituted ethers [29] and oxime ethers [30] of (1) and (2) and N-substituted lipophilic carboline derivatives of 4-phenyl piperidines [31] as GABA uptake inhibitors, some of which are believed to be in preclinical development. The oxime ether NNC-711 (15) is one of the most potent known inhibitors to date ($IC_{50} = 0.047 \mu M$) and demonstrates protective efficacy against seizures *in vivo* [32].

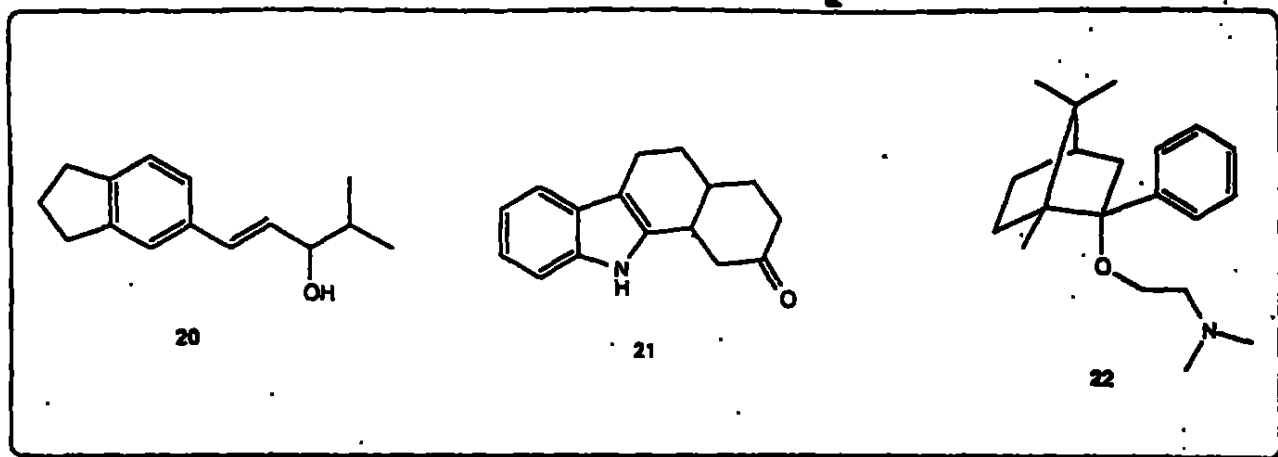


Inspired by the advances made in affinity for lipophilic N-substituted derivatives of (1) and (2), several groups investigated the effects of appending lipophilic functionality to other positions on the heterocyclic amino acid core. A range of 5-substituted derivatives of (3) have been reported [6, 33] with the 5-benzhydrylidene analogue (16) retaining reasonable inhibitory activity ($K_i = 13 \mu M$). Interestingly the N-demethylated version of (16) was considerably less active ($K_i = 104 \mu M$) [6]. The 6-(1,1-diphenylpropyl) substituted guvacine analogue (17) was synthesised after a pharmacophore model based on all known GABA inhibitors at that time had predicted it would be a

potent inhibitor of GABA transport [34]. The model proved successful as (17) is a potent inhibitor ($IC_{50} = 0.1 \mu M$), however, it lacks any *in vivo* activity in seizure models. This lack of *in vivo* activity has been attributed to the inability of (17) to cross the blood brain barrier due to its increased hydrogen bonding capability compared to tertiary amino analogues [34]. Attempts to improve potency by appending lipophilic ethers at the 6 position of (2) were unsuccessful [35]. Workers at Synaptic reported on a series of conformationally restricted analogues of (1), which were designed using the previously described pharmacophore model [34, 36]. The quinolizidine compounds such as (18) were shown to only be weak inhibitors indicating that the lipophilic side chains present in (12-14) may bind to the transporter in their fully extended conformation [36]. Spirocyclic analogues of (1) retain moderate affinity as GABA transport inhibitors with (19) having an $IC_{50} = 42 \mu M$ [37].



Several non-heterocyclic amino acid derivatives have been reported to be inhibitors of GABA transport. The anticonvulsant, stiripentol (20) is a moderate potency inhibitor ($IC_{50} = 50 \mu M$) [38] as are the quinolizidine derivatives exemplified by (21) ($IC_{50} = 8.5 \mu M$) [39]. The bicycloheptane derivative EGYT 3886 (22) acts as an anticonvulsant *in vivo* and displays moderate non-competitive inhibition of GABA transport



(IC_{50} = 19-44 μ M) [40]. EGYT 3886 is apparently in phase III trials.

Recent advances in GABA transport inhibition have focussed on the search for selective inhibitors of the cloned mammalian transporters in an attempt to further unravel their potential as therapeutic targets. Workers at Synaptic have described the potency of many known inhibitors at cloned mammalian GABA transporters [41, 42] (Table 1). The lipophilic derivatives, (12, 13, 14), are all highly selective for hGAT-1 suggesting that their anticonvulsant activity is mediated *via* inhibition of this transporter. The Synaptic team went on to discover (S)-SNAP-5114 (23), a selective inhibitor of hGAT-3 (Table 1), from a set of compounds designed around (22), and SNAP-5294 (24), a selective inhibitor of rGAT-2 (Table 1) [41,42]. Workers at Novo Nordisk have reported a selective mGAT-2 (hBGT-1) inhibitor, NNC 05-2090 (25) [3], and have patented a series of mGAT-

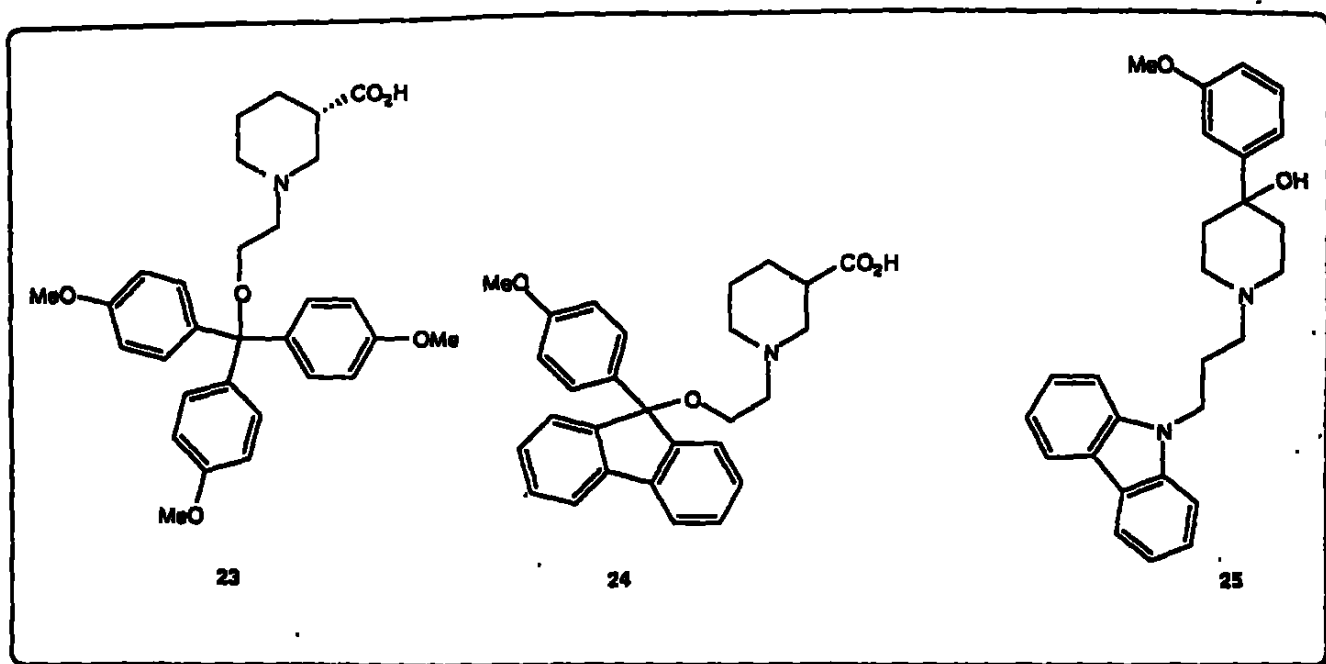
4 (hGAT-3) inhibitors based on similar structures to (25) [31]. NNC 05-2090 (25) has a K_i = 1.4 μ M at mGAT-2 and is 10-30 fold selective over the other GABA transporters [3]. Future *in vivo* studies utilising subtype selective GAT inhibitors will allow their pharmacological and physiological role to be probed further.

Glycine Transporters

The glycine transporters also form part of the superfamily of sodium and chloride dependent neurotransmitter transporters. Two glycine transporter genes have been identified to date which give rise to two transporters with approximately 50% amino acid sequence homology. GLYT1 has three variants (1a, 1b, 1c) arising from alternative splicing and human GLYT2 exists only in one form [1]. GLYT1 and GLYT2 transporters are distinct from the classical system A,

Table 1. Potency of Compounds at Cloned GABA Transporters (IC_{50} μ M) [41, 42]

Compound	hGAT-1 (mGAT-1)	rGAT-2 (mGAT-3)	hGAT-3 (mGAT-4)	hBGT-1 (mGAT-2)
GABA	5	5	7	38
(R, S)-(1)	8	39	105	2370
(R)-(1)	5	19	51	2310
(S)-(1)	118	783	2320	8410
(2)	14	58	119	1870
(12)	0.13	550	1990	7210
(13)	0.26	1280	333	300
(14)	0.07	1410	917	1670
(22)	28	30	46	39
(23)	388	21	5	140
(24)	133	51	142	27% at 100 μ M

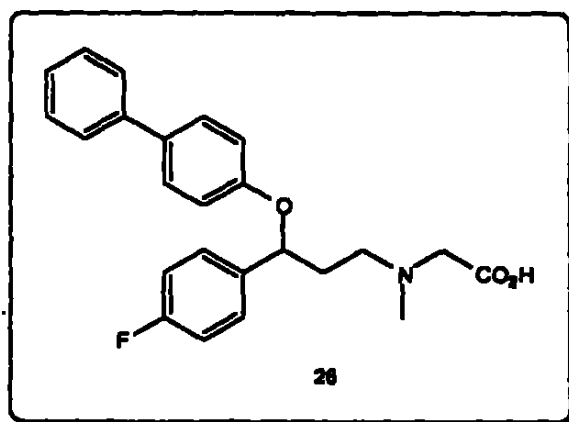


ASC and 'imino' transporters which have broad substrate affinity and selectivity whereas GLYT transporters are more specific and are expressed in different regions of the body [2]. GLYT1 is expressed centrally and peripherally whilst GLYT2 has a CNS specific distribution [1]. The expression of GLYT2 is consistent with the distribution of the inhibitory glycine receptor and neurones of high glycine content (spinal cord, auditory system). GLYT2 is located axonally at presynaptic terminals whereas GLYT1 has predominantly a glial distribution [1]. GLYT1 is not only expressed in areas corresponding to strychnine-sensitive and strychnine-insensitive glycine receptors but also outwith these areas. The role of GLYT1 in these areas has been postulated to involve modulation of NMDA receptor function or non-strychnine-sensitive glycine receptors [1]. Inhibition of the glycine transporter has been hypothesised to be useful in the treatment of epilepsy and pain directly and of schizophrenia and dementia *via* modulation of NMDA receptor function [43, 44].

There are only a few reports of glycine transport inhibitors in the literature. Simple substrate specificity and potency has been reported by several groups and has been summarised [1]. Glycine acts as a moderate affinity substrate at both GLYT1 and GLYT2 ($K_m = \sim 20\text{--}50\ \mu\text{M}$). GLYT1 is inhibited by sarcosine (*N*-methyl glycine) ($K_i < 100\ \mu\text{M}$) but alanine, GABA, serine, glycine esters, glutamate and proline have no affinity [1, 45]. In contrast GLYT2 is not inhibited by sarcosine, alanine, β -alanine, serine, cysteine, glycine esters or *N*-methylaminoisobutyric acid [1, 46].

Trophix pharmaceuticals have recently patented two series of glycine transport inhibitors based on lipophilic

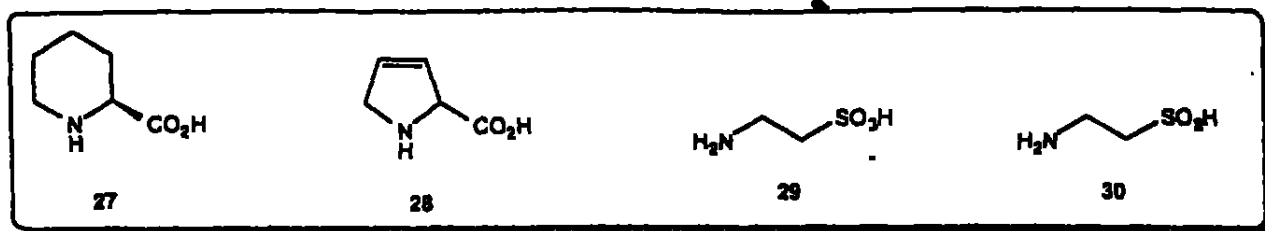
analogues of sarcosine and lipophilic piperidine *N*-acetic acid derivatives [43, 44]. The lipophilic sarcosine derivative (26) has reported to be a potent ($K_i = 0.005\ \mu\text{M}$) and selective GLYT1 inhibitor [47].



Proline Transporter

The proline transporter (PROT) is another member of the superfamily of sodium and chloride dependent neurotransmitter transporters. It has a CNS specific tissue distribution being found in the olfactory bulb, cerebral cortex and hippocampus [1]. The PROT transporter is distinct from the classical system 'imino' transporters which have broad substrate affinity and selectivity. It has approximately 45% sequence homology to the related glycine transporters [1].

Only simple inhibitors and substrates for PROT have been reported to date. L-Proline is a high affinity substrate ($K_m = \sim 10\ \mu\text{M}$) whilst L-pipecolic acid (27) ($K_i = 14\ \mu\text{M}$), sarcosine ($K_i = 30\ \mu\text{M}$) and 3,4-



dehydroproline (28) ($K_i = 31 \mu\text{M}$) act as inhibitors [48]. Other moderately potent inhibitors of PROT include norleucine, phenylalanine, histidine and cysteine, but many other amino acids show no affinity [48]. Interestingly, *N*-methyl and *N*-acetyl derivatives of proline are inactive as are the carboxylic ester derivatives [48,49]. More recently enkephalins and their analogues have been shown to be potent inhibitors of PROT with des-Tyr-Leu-enkephalin having an $\text{IC}_{50} = 0.26 \mu\text{M}$ [50]. It is still unclear as to the role of PROT in the CNS, however, circumstantial evidence implicates L-proline as a putative synaptic regulatory molecule [1].

Taurine Transporter

The final member of the superfamily of sodium and chloride dependent neurotransmitter transporters worthy of mention is the Taurine transporter, TAUT. Although the expression of TAUT is widespread throughout the body it is found in the brain along with an abundance of taurine which may suggest a specific function of TAUT in the CNS [1, 2]. TAUT has a high sequence homology (~60-70%) with BGT-1 and has a high affinity for the transport of taurine (29) ($K_m = <10 \mu\text{M}$). TAUT has moderate affinity for the transport of β -alanine ($K_m = -60 \mu\text{M}$) and is inhibited by hypotaurine (30) ($K_i = <10 \mu\text{M}$). GABA, alanine, glycine and proline have all been shown to lack affinity for TAUT [1, 51].

Cationic Amino Acid Transporters

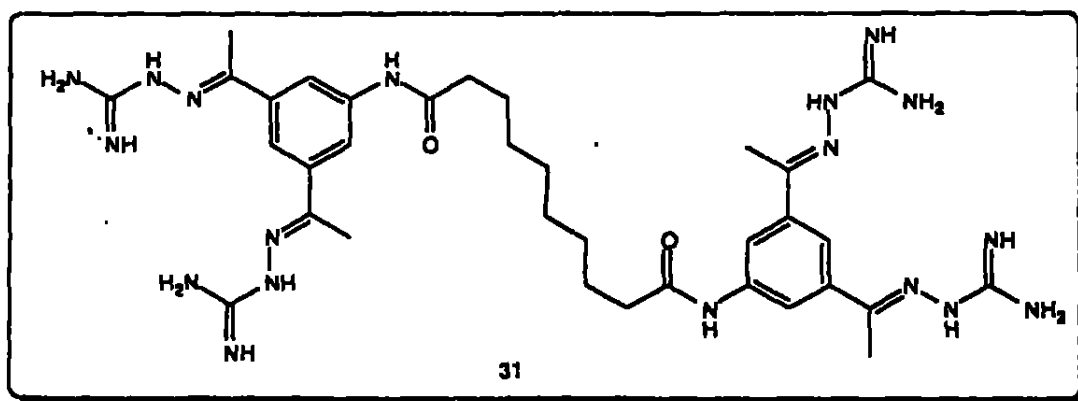
Four mammalian genes defining a family of cationic amino acid transporters (CAT-1, -2, -3, -4) have been

identified. CAT-1, CAT-2a, CAT-2b and CAT-4 have been reported to have a widely expressed tissue distribution, however, the remaining transporter CAT-3 has been shown to have a brain specific expression in rat and mouse [1]. CAT-3 exhibits a 53-58% amino acid sequence homology with CAT-1, CAT-2a and CAT-2b. It is related to the common cationic amino acid transporter group γ^+ and is believed to exist structurally in a similar fashion to mCAT-1 that has been postulated to exist with a 12 or 14 trans-membrane topology [1]. CAT-3 transports L-arginine ($K_m = -100 \mu\text{M}$) as a substrate in a sodium and chloride independent manner. It also transports L-lysine ($K_m = -150 \mu\text{M}$) and L-ornithine ($K_m = -150 \mu\text{M}$) [1, 52]. Although the specific role of CAT-3 in the CNS is as yet undefined, it is known that cationic amino acids are required for protein and urea synthesis (arginine) and as precursors for the synthesis of bioactive molecules such as nitric oxide, urea and polyamines (arginine and ornithine respectively) [1]. Hence, CAT-3 may have a CNS specific role in the provision of these key biochemical entities.

No CAT-3 specific inhibitors have been reported to date, however, moderately potent general inhibitors of arginine transport such as the guanilylhydrazone (31) ($\text{IC}_{50} = 10\text{-}50 \mu\text{M}$) have been disclosed [53].

Glutamate Transporters

CNS glutamate transporters, their substrates and competitive inhibitors have recently been reviewed in detail [54]. Glutamate transporters form part of the superfamily of sodium dependent transporters for anionic and zwitterionic amino acids which have been

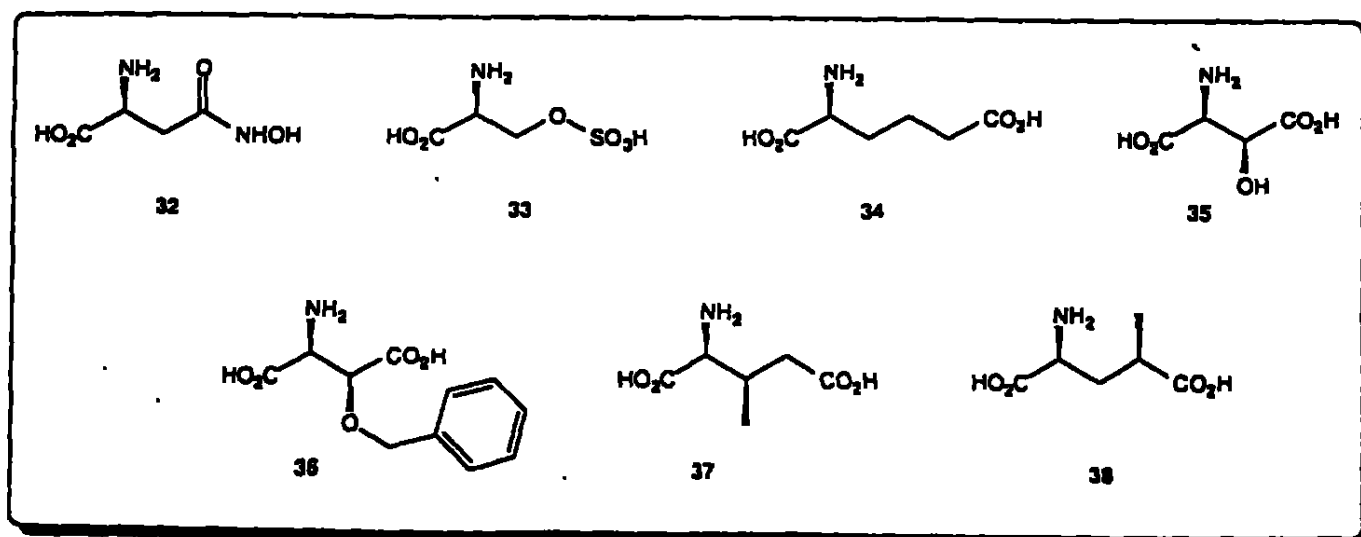


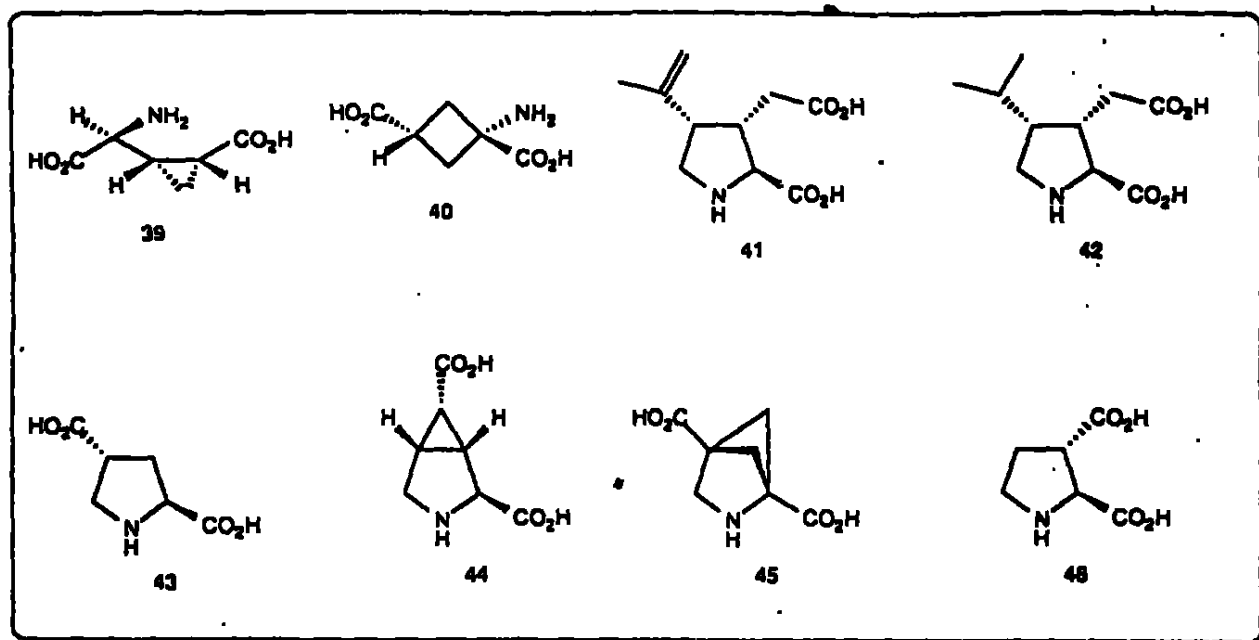
postulated to have a substrate (amino acid and sodium)-gated chloride channel mode of action in addition to their transport role [1]. Five glutamate or anionic amino acid transporters have been identified and labelled excitatory amino acid transporters (EAAT 1-5) [1, 54]. EAATs 1-5 show a sodium and potassium dependent transport and are likely to be neural and nonneural variants of the anionic amino acid transport system X⁻_{AG}. With the possible exception of EAAT-5 the transport mechanism has been shown to be electrogenic with glutamate being co-transported with 2 or 3 sodium ions and an optional proton with counter-transport of a potassium ion and an optional hydroxyl anion [1]. Their topology is not fully defined with models suggesting 6-10 transmembrane domains consisting of α -helices and β -sheets. EAAT 1-5 show 36-65% amino acid homology with each other and have varying tissue distributions. Human EAAT-1, 3 and 4 are expressed in the brain and other tissues, EAAT-2 is brain specific whilst EAAT-5 is expressed in the retina. EAAT-1 and 2 are present in astroglial cells whereas EAAT-3 is present in neurons. [1]. In terms of application to the treatment of CNS disease, inhibition of glutamate transport may prove useful in the treatment of schizophrenia by improving glutamatergic function [55], however the excitotoxic potential in raising glutamate levels is always present [54].

Simple substrates for EAATs 1-5 are the anionic amino acids L-glutamate and L- or D-aspartate. EAATs 1, 2, 3, and 5 have moderate affinity ($K_m = 10$ -64 μ M) and EAAT-4 has a high affinity for these substrates ($K_m = 2$ -3 μ M) [1]. Simple structure activity relationships indicated that α -amino acids with a second acidic group 2-4 carbons away from the α -carboxyl moiety were competitive inhibitors [54]. The nature of the second acidic group has been varied and led to some selective inhibitors being identified. L-aspartate- β -hydroxamate (32) has some selectivity for neuronal EAAT-3 ($K_i = 133$ μ M compared to 389 and 184 μ M for EAAT 1 and 2

respectively), L-serine-O-sulfate (33) has some selectivity for EAAT-1 ($K_i = 107$ μ M compared to 1157 and 150 μ M for EAAT 2 and 3 respectively) and L- α -aminoadipic acid (34) has good selectivity for EAAT-4 as a substrate ($K_m = 168$ μ M at EAAT-4 and >1mM at EAAT 1-3) [54]. The addition of substituent groups to the β and γ positions of the side chain in aspartate and glutamate has yielded some interesting analogues. L- β -Threo-hydroxyaspartate (35) (and the corresponding D- isomer) are substrates for EAAT-1-4 but act as an inhibitor at EAAT-5 ($K_i \sim 1$ μ M). Hydroxy ester and ether derivatives of (35) have been reported which vary in their pharmacological profile [56, 57]. Alkyl substituted hydroxy esters of (35) act as substrates whereas the benzoate and 1-naphthoate esters are moderately potent inhibitors of EAAT-1. The benzyl ether derivative (36) is a potent inhibitor of EAAT-1 and 2 ($K_i = 9$ and 0.1 μ M respectively) that also has some weak affinity for NMDA and kainate receptors [54, 56, 57]. The β -methyl derivative L-threo-3-methyl glutamate (37) shows no affinity for EAAT-1 but acts as an inhibitor at EAAT-2 ($K_i = 18$ μ M) whereas the corresponding erythro isomer has no affinity for either transporter [54, 58]. The γ -methyl derivative (2S, 4R)-4-methylglutamate (38) acts as a substrate at EAAT-1 but as an inhibitor at EAAT with $K_i = 3.4$ μ M, however, it is also a potent kainate receptor agonist [54, 59].

Conformationally constrained cyclic glutamate derivatives have also been investigated as potential inhibitors of glutamate transport. The cyclopropyl analogue (39) is a potent inhibitor of glutamate transport (EAAT-1 $K_i = 7.5$ μ M, EAAT-2 $K_i = 2.5$ μ M, EAAT-3 $K_i = 10$ μ M) whilst related stereoisomers tend to be less potent [54, 59]. The cis-cyclobutyl analogue (40) shows moderate inhibitory action (EAAT-1 $K_i = 120$ μ M, EAAT-2 $K_i = 660$ μ M, EAAT-3 $K_i = 244$ μ M) whilst the corresponding trans isomer is inactive [54]. Pyrrolidine dicarboxylate derivative medicinal chemistry research has yielded some of the most interesting





Compounds to date. Kainic acid (41) and the reduced analogue dihydrokainate (42) act as specific inhibitors ($K_i \sim 10\text{--}80 \mu\text{M}$) of EAAT-2 with no appreciable affinity for other EAATs and more interestingly (42) has reduced affinity for AMPA and kainate receptors compared to (41) [1, 54]. The 2,4 dicarboxylate derivative, L-*trans*-2,4-PDC (43) acts as a substrate at EAAT-1-4 with similar potency to L-glutamate, however, it acts as a potent inhibitor of EAAT-5 ($K_i = 6.2 \mu\text{M}$) [54, 60]. In an attempt to constrain the somewhat flexible pyrrolidine ring in (43), the bicyclic analogues (44) and (45) were synthesised [54, 61]. Both (44) and (45) show promise as potent modulators of glutamate transport with (44) showing potent inhibition of EAAT-2 ($K_i = 1.8 \mu\text{M}$) whereas (45) acts as a moderately potent substrate for EAAT-2 ($K_m = 45 \mu\text{M}$). The related 2,3 pyrrolidine dicarboxylate analogue (46) is a potent inhibitor of EAAT-2 ($K_i \sim 10 \mu\text{M}$) but has little activity at EAAT-1 or 3; however the use of (46) as a pharmacological tool may be hampered by its excitotoxic side effects mediated through excitatory amino acid ionotropic receptors [54].

Conclusions

The use of amino acid transport inhibitors as effective medicines has now been demonstrated by Tiagabine (14). With the recent advances in molecular biology, medicinal chemists will be able to target transporter subtypes to generate potent and selective chemical entities to probe pharmacological and physiological function of CNS specific amino acid transporters. Improvements in pharmacokinetic parameters will in turn hopefully provide suitable candidates to progress into the clinic with the aim of

treating a wide range of psychiatric, neurodegenerative and CNS driven disorders.

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ORION CORPORATION

Oficio N° **07045**

ASUNTO:	Radicación N°	02-82770
	Solicitud internacional	PCT/FI01/00266
	Fecha inicio fase nacional	17/10/2002
	Trámite	011
	Evento	379
	Actuación	430
	Folios	001

Como resultado del examen de forma, realizado con fundamento el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

- De ser el caso, la solicitud debe contener la traducción de los anexos del reporte de examen preliminar internacional (Art. 36.2.b) del tratado PCT).
- La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486).
- El solicitante debe presentar un nuevo título que permita identificar la materia de la cual trata la solicitud, ya que el título relacionado no resulta lo suficientemente descriptivo como para identificar claramente el objeto de la solicitud de patente. (Regla 4.3 PCT, Art 26-27 D-486, Circular Única 5.2.9).

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.



ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

29 AGO. 2003

El anterior requerimiento fue notificado por fijación en lista de fecha _____.

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