

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 13-2648- -4	FECHA: 2013-12-13 17:33:08
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 19
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
LUZ CLEMENCIA DE PAEZ

Asunto: Radicación: 13-2648- -4
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 23099
 Folios: 19

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/GB2011/000863				
Fecha de Presentación Internacional	08/06/2011				

1. Documentos estudiados

Descripción:	Radicado N°13-002648-00000-0000	Enero 8 de 2013
Reivindicaciones	Radicado N°13-002648-00000-0000	Enero 8 de 2013
Prioridad:	GB 1009603.0	Junio 8 de 2010

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

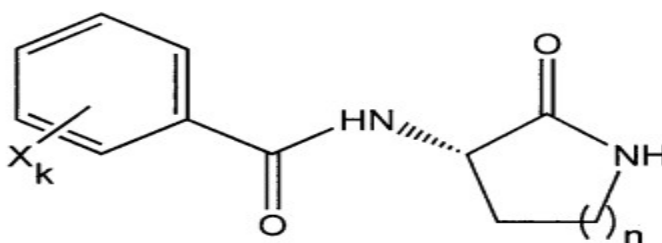
De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Por lo tanto, el capítulo reivindicatorio presente en el Radicado N°13–002648–00000–0000 está compuesto por 20 reivindicaciones será estudiado en su totalidad, porque se incluye el pago por las reivindicaciones adicionales 11 a 20, de acuerdo con el recibo presente en la página 94 del Radicado N°13–002648–00000–0000,.

Título de la solicitud: “Agentes anti inflamatorios”

El problema planteado en esta solicitud consiste en la obtención de compuestos alternativos con actividad antiinflamatoria.

Para resolver este problema técnico, la solicitud en estudio plantea la obtención de compuestos derivados de 3 – aminolactama aril sustituida, con actividad antiinflamatoria..



El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K31/45; A61K31/4523; A61K31/55; A61P17/06; A61P29/00; A61P37/00; C07D211/56; C07D223/12; C07D401/12.

4. Excepción a la Patentabilidad (Art 20 D 486, literal d)

El objeto de las reivindicaciones 18 y 19 no es patentable, de acuerdo con el artículo citado.

5. Reivindicaciones relativas al uso o a un segundo uso (Arts. 14 y 21 D 486)

El uso de las reivindicaciones 3, 4, 8, 9, 11 a 17 y 20 no es patentable, de acuerdo con el artículo 14.

6. De la Solicitud de la Patente

Título

El título de la presente solicitud se considera muy general y ambiguo, porque al mencionar “agentes antiinflamatorios” se define el objeto de la invención por su actividad más no por su conformación. Además, dentro de este rubro se incluye una cantidad indefinida de compuestos los cuales no hacen parte del objeto de la presente invención. Se observa que los compuestos de la presente invención son derivados de N – (2 – oxo azepin – 3 – il) benzamida y derivados de N – (2 – oxo piperidin – 3 – il) benzamida. Se solicita modificar el título de la presente invención de acuerdo con las observaciones anteriores.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicación 1 no es clara ni concisa, porque se mencionan sustituyentes de manera muy general e indefinida tales como “alquilo, haloalquilo, hidroxialquilo, alcoxi, aminoalquilo, aminodialquilo, halógeno”, los cuales pueden ser cualquier

sustituyente posible, de tal manera que a partir de los sustituyentes planteados, se obtiene una cantidad indefinida de posibles compuestos, de tal manera que se incrementa la complejidad de la presenta solicitud y por lo tanto, no es posible definir de forma certera el verdadero alcance de lo que se desea proteger. Por lo tanto, se solicita realizar una restricción muchísimo más razonable de los sustituyentes de la presente invención, de tal forma que a partir de estos se obtengan los compuestos sintetizados, probados y que representan la solución al problema técnico planteado dentro de la presente invención. Se solicita mencionar específicamente los sustituyentes empleados (por ejemplo, en vez de mencionar alquilo, mencionar si es metil, etil, isopropil, butil dodecil, 2, 5, 9 – trimetiltetradecanoil, etc...). Es cierto que un inventor esta en el pleno derecho de proteger su invención en la extensión que desee siempre y cuando se conserve un único concepto inventivo, sin embargo, el artículo 30 de la Decisión 486 determina que las reivindicaciones que definen una invención deben ser claras, concisas y estar plenamente detalladas en el capítulo descriptivo. Se sugiere al solicitante realizar dicha restricción, de acuerdo a lo que se muestra en la siguiente tabla:

Sustituyente	Soportado en la descripción
X	F, CF ₃ , CH ₃ , CH ₂ CH ₃ , CH ₂ CH ₂ CH ₃ , C(CH ₃) ₃ , (CH ₂) ₅ CH ₃ , (CH ₂) ₇ CH ₃
k	1, 2
n	2, 3

Adicionalmente, cuando se mencionan las condiciones de los sustituyentes C1 a C5, la explicación es confusa y por lo tanto no es clara. Por lo tanto, se solicita mencionar las sustituyentes para cada uno de los sustituyentes C1 a C5 y se sugiere modificar el sustituyente X_k por C1, C2, C3, C4 y C5 en los carbonos correspondientes para entender con más claridad las salvedades planteadas en la reivindicación 1. También se solicita eliminar la expresión “para uso en el tratamiento de un trastorno inflamatorio”, ya que el uso no se considera como material susceptible de patentabilidad.

De igual manera, se solicita eliminar la expresión “para uso en el tratamiento de un trastorno inflamatorio” de la reivindicación 2, ya que el uso no se considera como material susceptible de patentabilidad.

Para la reivindicación 5, se solicita realizar la restricción razonable solicitada para la reivindicación 1, dado que al mencionar “alquilo C7 o superior, hidroxialquilo C7 o superior, alcoxi C7 o superior, aminoalquilo C4 o superior, dialquilamino C4 o superior”, se sigue obteniendo una posible cantidad indefinida de compuestos. Se recomienda especificar los sustituyentes específicos para cada uno de los grupos anteriormente mencionados.

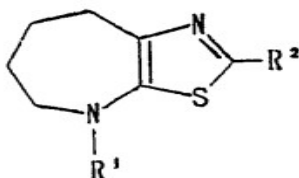
Para la reivindicación 20, la cual se afecta por uso, no es clara la mención del término “biblioteca”, porque este término no es claro desde el punto de vista técnico de la invención y además no hace parte del concepto inventivo de las reivindicaciones 1 a 19, por lo cual se recomienda eliminar esta reivindicación.

6. Determinación del Estado de la Técnica

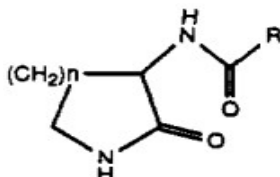
El Estado de la Técnica se determinó con base en la fecha de presentación de la solicitud de la cual se invoca prioridad, es decir, el 8 de junio de 2010. Consultadas las fuentes de información con que se cuenta, se encontró los siguientes documentos que anticipan el objeto de esta solicitud.

Item	Documento	Título	Fecha de Publicación
D1	EP0351856	"Thiazolo [5,4-b] azepino compounds"	Enero 24 de 1990
D2	<i>Journal of Medicinal Chemistry</i> . 1993 . 36 (11); 1511 – 1519	"Synthesis and Amnesia – Reversal Activity of a Series of 7 – and 5 – Membered – 3 – Acylamino Lactams"	Mayo 28 de 1993
D3	<i>Tetrahedron Letters</i> . 1995 . 36 (3); 439 – 440	"A Novel Synthetic Route to Enantiomers of ϵ – Hydroxynorleucine and ϵ – Chloronorleucine from L – and D, L - Lysine "	1995
D4	<i>Journal of Heterocyclic Chemistry</i> . 1994 . 31 (4); 877 – 887	"Aminothiazole derivatives. I. A convenient synthesis of monocyclic and condensed 5-aminothiazole derivatives"	Agosto 31 de 1994

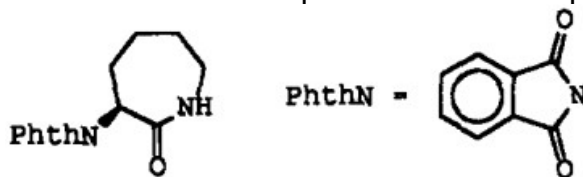
El documento D1¹ divulga compuestos derivados de tiazolo [5,4-b] azepina, como inhibidores de lipooxigenasa, para el tratamiento de enfermedades inflamatorias



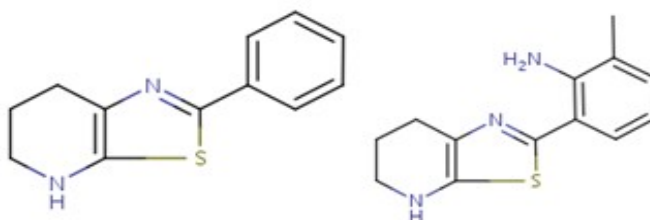
El documento D2² divulga compuestos derivados de 3 – Acilamino – ϵ – Caprolactamas para el tratamiento de la amnesia inducida por Escopolamina



El documento D3³ divulga la obtención de compuestos ϵ – Hidroxinorleucina y ϵ – Cloronorleucina, como intermediarios para la síntesis de péptidos



El documento D4⁴ divulga la síntesis de aminotiazoles a partir de α – aminoácidos y pentasulfuro de fosforo, mediante la reconfiguración de Lawesson



7. Examen de Patentabilidad.

Novedad (Art 16 D 486)

La reivindicación 1 divulga un compuesto de fórmula general I o una sal farmacéuticamente aceptable, caracterizado por la estructura, donde... (Página 70

¹[http://worldwide.espacenet.com/publicationDetails/originalDocument?](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=EP&NR=0351856A2&KC=A2&FT=D&ND=3&date=19900124&DB=worldwide.espacenet.com&locale=es_LP)

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²<http://pubs.acs.org.ezproxy.unal.edu.co/doi/pdf/10.1021/jm00063a001>

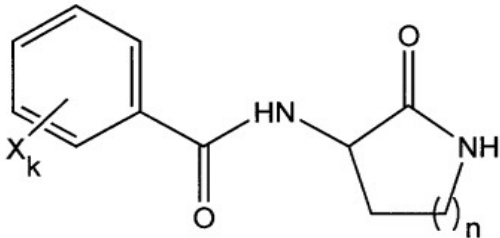
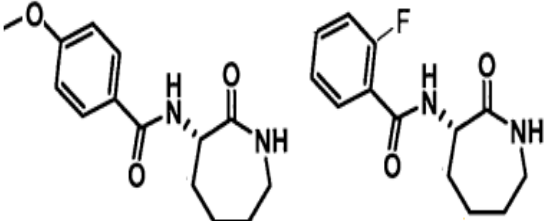
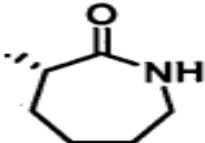
³http://ac.els-cdn.com/004040399402278J/1-s2.0-004040399402278J-main.pdf?_tid=71cf1480-5d01-11e3-bea0-00000aacb362&acdnat=1386174833_aea661a8cb23010489263f5d94b9f688

⁴[http://onlinelibrary.wiley.com/doi/10.1002/jhet.5570310432/abstract?](http://onlinelibrary.wiley.com/doi/10.1002/jhet.5570310432/abstract?systemMessage=Wiley+Online+Library+will+be+disrupted+on+7+December+from+10%3A00-15%3A00+BST+%2805%3A00-10%3A00+EDT%29+for+essential+maintenance)

[systemMessage=Wiley+Online+Library+will+be+disrupted+on+7+December+from+10%3A00-15%3A00+BST+%2805%3A00-10%3A00+EDT%29+for+essential+maintenance](http://onlinelibrary.wiley.com/doi/10.1002/jhet.5570310432/abstract?systemMessage=Wiley+Online+Library+will+be+disrupted+on+7+December+from+10%3A00-15%3A00+BST+%2805%3A00-10%3A00+EDT%29+for+essential+maintenance)

y 71 del radicado N°13-02648-00000-0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D1
Compuesto de la fórmula I 	Compuestos 
X _k , donde X es halógeno o alcoxi, k es 1 n es 3 para obtener el anillo de 2 – oxo azepina	OCH ₃ o F sustituyen el grupo benzamida 

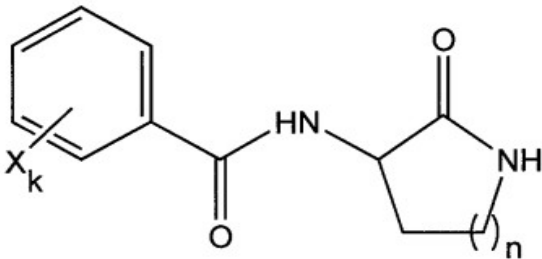
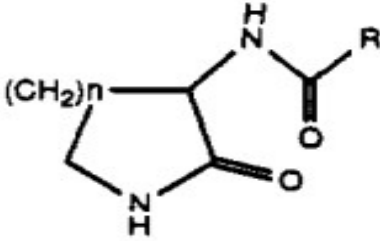
Las características técnicas esenciales de los compuestos de la fórmula I de acuerdo con la reivindicación 1, se han divulgado previamente en D1 (D1: Página 23, línea 45; página 30, línea 42), en donde se muestran los compuestos 3 – (4 – metoxi benzoilamino) – 2 – oxo azepina y 3 – (2 – fluoro benzoilamino) – 2 – oxo azepina, como intermediarios para la síntesis de compuestos derivados de tiazolo [5,4-b] azepina como inhibidores de lipooxigenasa, con las características técnicas esenciales descritas en la tabla anterior.

En consecuencia, la reivindicación 1, de acuerdo a lo divulgado en D1, carece de Novedad.

Las reivindicaciones 2 y 5 a 7, dependientes de la reivindicación 1, no divulgan características adicionales que otorguen novedad a los compuestos de la reivindicación 1. Por lo tanto, estas reivindicaciones también carecen de Novedad.

La reivindicación 1 divulga un compuesto de fórmula general I o una sal farmacéuticamente aceptable, caracterizado por la estructura, donde... (Página 70 y 71 del radicado N°13-02648-00000-0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D2
Compuesto de la fórmula I 	Compuestos de la Tabla I 
n es 1 o 3	n es 1 o 3
X _k , donde X es haloalquilo o alcoxi, k es 1 o 3	R es fenil sustituido por CF ₃ , OCH ₃

Las características técnicas esenciales de los compuestos de la fórmula I de acuerdo con la reivindicación 1, se han divulgado previamente en D2 (D2: Página

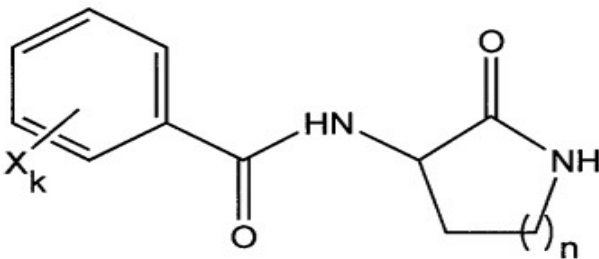
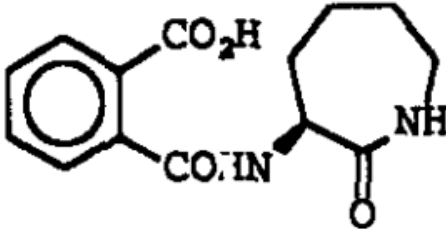
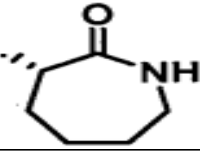
1513, tabla 1, compuestos 13, 14, 15, 16 y 21), en donde se muestran compuestos derivados de 3 – benzoilamino – 2 – oxo azepina y de 3 – benzoilamino – 2 – oxo pirrolidina para el tratamiento de la amnesia inducida por Escopolamina, con las características técnicas esenciales descritas en la tabla anterior.

En consecuencia, la reivindicación 1, de acuerdo a lo divulgado en D2, carece de Novedad.

Las reivindicaciones 2 y 7, dependientes de la reivindicación 1, no divulgan características adicionales que otorguen novedad a los compuestos de la reivindicación 1. Por lo tanto, estas reivindicaciones también carecen de Novedad.

La reivindicación 1 divulga un compuesto de fórmula general I o una sal farmacéuticamente aceptable, caracterizado por la estructura, donde... (Página 70 y 71 del radicado N°13-02648-00000-0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D3
Compuesto de la fórmula I 	Compuesto 3 
n es 3 para obtener el anillo de 2 – oxo azepina	
X_k, donde carboxi, k es 1	COOH en la posición 2 del grupo benzamida

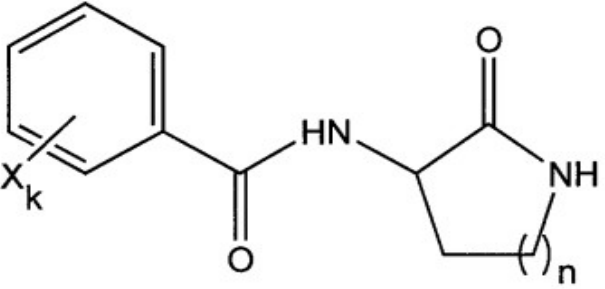
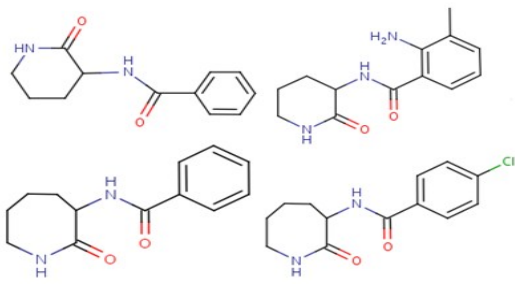
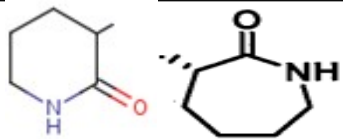
Las características técnicas esenciales de los compuestos de la fórmula XI de acuerdo con la reivindicación 12, se han divulgado previamente en D3 (D3: Página 439, compuesto 3), en donde se muestra el compuesto ácido 2 – [(2 – oxo azepin – 3 – il) aminocarbonil] benzoico, como intermediario en la síntesis de péptidos enantioméricamente puros, con las características técnicas esenciales descritas en la tabla anterior.

En consecuencia, la reivindicación 1, de acuerdo a lo divulgado en D3, carece de Novedad.

Las reivindicaciones 2, 5 y 7, dependientes de la reivindicación 1, no divulgan características adicionales que otorguen novedad a los compuestos de la reivindicación 1. Por lo tanto, estas reivindicaciones también carecen de Novedad.

La reivindicación 1 divulga un compuesto de fórmula general I o una sal farmacéuticamente aceptable, caracterizado por la estructura, donde... (Página 70 y 71 del radicado N°13-02648-00000-0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D4
Compuesto de la fórmula I 	Compuesto 
n es 2 o 3 para obtener el anillo de 2 – oxo pirrolidina y de 2 – oxo azepina	
X _k , donde X es halógeno, alquilo, amino; k es 0, 1, 2	Grupo benzamida opcionalmente sustituido por halógeno, alquilo o amino

Las características técnicas esenciales de los compuestos de la fórmula XI de acuerdo con la reivindicación 12, se han divulgado previamente en D4 en donde se muestran compuestos derivados de 3 – benzoilamino – 2 – oxo azepina y de 3 – benzoilamino – 2 – oxo pirrolidina para la síntesis de aminotiazoles a partir de α – aminoácidos y pentasulfuro de fósforo, mediante la reconfiguración de Lawesson, con las características técnicas esenciales descritas en la tabla anterior.

En consecuencia, la reivindicación 1, de acuerdo a lo divulgado en D4, carece de Novedad.

Las reivindicaciones 2 y 5 a 7, dependientes de la reivindicación 1, no divulgan características adicionales que otorguen novedad a los compuestos de la reivindicación 1. Por lo tanto, estas reivindicaciones también carecen de Novedad.

8. Conclusión

Los requisitos de Patentabilidad de esta solicitud se ven afectados de la siguiente manera:

Item	Documento	Reivindicaciones	Requisitos que afecta
D1	EP0351856	1, 2 y 5 a 7	Novedad
D2	Journal of Medicinal Chemistry. 1993. 36 (11); 1511 – 1519	1, 2, 5 y 7	Novedad
D3	Tetrahedron Letters. 1995. 36 (3); 439 – 440	1, 2, 5 y 7	Novedad
D4	Journal of Heterocyclic Chemistry. 1994. 31 (4); 877 – 887	1, 2 y 5 a 7	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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 No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-12-17

Elaboró: Dayron Alexander Ramirez Reyes
Revisó: Gloria Jacqueline Alfonso Roza

JOURNAL OF MEDICINAL CHEMISTRY

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Articles

Synthesis and Amnesia-Reversal Activity of a Series of 7- and 5-Membered 3-Acylamino Lactams[†]

Luciano Angelucci,[‡] Pina Calvisi,[§] Roberto Catini,[§] Ugo Cosentino,[⊥] Roberto Cozzolino,^{*||} Paolo De Witt,[§] Orlando Ghirardi,^{||} Fabio Giannessi,[§] Alessandro Giuliani,^{||} Donatella Guaraldi,^{||} Domenico Misiti,[©] Maria Teresa Ramacci,^{||} Carlo Scolastico,[‡] Maria Ornella Tinti[§]

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Received June 25, 1992

A series of 3-(acylamino)- ϵ -caprolactams and 3-(acylamino)-2-pyrrolidinones was synthesized. Some of these compounds reversed at different degrees electroconvulsive shock- and Scopolamine-induced amnesia, using a step-through passive avoidance in mice. Classical nootropic drugs, i.e., Aniracetam, Oxiracetam, and Piracetam, were used as reference compounds. Within the analyses of data performed, we introduced a new parameter, the confrontation index (CI), which is a function of Mann-Whitney's *U* statistic. The CI permits a common scale of activity of substances to be generated, independently of probabilistic hypotheses, with higher scores representing higher activities. The most active compounds were characterized by the formylamino and [3-(trifluoromethyl)benzoyl]amino groups in the 3-position of the ring. None of the substances assayed showed any effect on spontaneous behavior and neurovegetative system.

Over the last two decades, an ever growing interest has been devoted to the search for new drugs that might be useful for prevention or treatment of human cognitive disorders. Although cognitive impairments may occur in people of any age, the increase in life expectancy has made treatment of age-related cognitive disorders one of today's scientific, medical, and social targets.

Cognition enhancing agents include a wide range of

different substances,^{1,2} characterized by different and, so far, not fully understood mechanisms of action.

In this context, nootropic drugs are substances known to be able to ameliorate cognitive functions by acting directly and selectively on the highest telencephalic integrative activity. The absence of the pharmacological effects usually exerted by neuropsychotropic drugs and low toxicity are among the major characteristics of these substances which have also been shown to enhance resistance to brain insults.³ The most representative nootropic agents are those belonging to the class of 2-pyrrolidinones, such as Piracetam (1), Oxiracetam (2), Pramiracetam (3), Aniracetam (4), Rolziracetam (5), etc.⁴

It has recently been reported that some amino acid derivatives, for example (*Z*)-prolylprolynal (6),⁵ SUAMM 1221 (7),⁶ and [(4-phenylbutanoyl)prolyl]prolynal (8)⁷ possess anti-amnesic properties and the ability to strongly

[†] This paper has been presented in part at the Congresso Interdivisionale della Società Chimica Italiana, San Benedetto del Tronto, Italy, September 30–October 5, 1990, Abstr. p 115, and at the Fourth Meeting of the Italian Society of Neuroscience, Palermo, Italy, 4–5 December 1990, Abstr. *Neurosci. Lett.* 1990, 39, S62.

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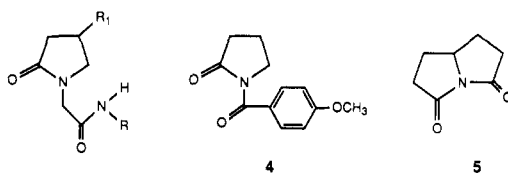
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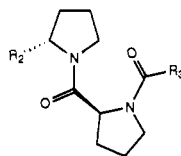
^{||} Istituto di Ricerca sulla Senescenza, Sigma-Tau S.p.A.

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- 1: R = H, R₁ = H
 2: R = H, R₁ = OH
 3: R = CH₂CH₂N[CH(CH₃)₂]₂, R₁ = H



- 6: R₂ = CHO, R₃ = OCH₂Ph
 7: R₂ = H, R₃ = (CH₂)₃Ph
 8: R₂ = CHO, R₃ = (CH₂)₃Ph

inhibit mammalian prolyl endopeptidase (PEP). Also, some nootropics such as Aniracetam and Pramiracetam have also been found to inhibit brain PEP,⁸ suggesting the hypothesis of a common mechanism of action. In fact, PEP inhibitors and nootropics might influence memory by prolonging the lifetime of endogenous neuropeptides that modulate cholinergic neurotransmission.⁹

In a previous study, the conformational features of 10 known amnesia-reversal compounds and two potent PEP inhibitors showing strong anti-amnesic activity have been analyzed by molecular mechanics calculations¹⁰ in order to identify a common spatial disposition of the polar functional groups present in all the molecules (an NC=O amidic group and an XC=O group, with X = O, N). These polar groups, included in the pharmacophore, are supposed to be involved in the biological interaction.

Minimum-energy conformations of investigated compounds were calculated by the MM2 force field.¹¹ Principal component analysis (PCA) led to the identification of three interatomic distances (D_{O-O} , D_{C-C} , and D_{N-X}) which provide all the information necessary to describe the relative spatial disposition of the two functional groups.

Cluster analysis was then performed to group minimum-energy conformations according to the values of those three distances. Clusters were checked to single out the ones containing conformations of the maximum number of active compounds. This procedure allowed us to point out two acceptable pharmacophore models, characterized by the distance values 3.57, 3.14, and 3.07 Å, and by 3.65, 3.24, and 3.75 Å, respectively.

Based on the hypothesis that common stereoelectronic factors produce a similar biological behavior, we sought for structures having a spatial disposition of polar functional groups according to the pharmacophore models and found that 3-(acylamino)- ϵ -caprolactam and, to a lesser extent, 3-(acylamino)-2-pyrrolidinone presented a satisfactory fitting. These findings prompted us to synthesize a series of (*R*)- and (*S*)-3-(acylamino)- ϵ -caprolactams and (*S*)-3-(acylamino)-2-pyrrolidinones, derivatized at will at the nitrogen atom of the ring, in order to evaluate their anti-amnesic activity in the electroconvulsive shock (ECS)- and Scopolamine-induced amnesia models in mice.

Chemistry

Derivatives 11–22 (Table I) were readily synthesized, as outlined in Scheme I, from 9A ((*S*)-3-amino- ϵ -capro-

lactam), 9B ((*R*)-3-amino- ϵ -caprolactam), 10 ((*S*)-3-amino-2-pyrrolidinone), and the relative carboxylic acids, using carbonyldiimidazole (CDI) or 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ) as condensing agents. As an alternative, the relative acyl chloride was used.

All the other compounds, substituted at the nitrogen atom of the lactam (Table II), were obtained as outlined in Scheme II.

(Acylamino)- ϵ -caprolactams 11 and 20 were alkylated with BrCH₂COOMe and NaH to give the methyl esters 24 and 27, which were reduced to give the corresponding alcohols 25 and 28, respectively. Amides 23 and 26 were obtained by bubbling gaseous NH₃ into the MeOH solution of 24 and 27, respectively. Compound 30 was obtained by hydrolysis of the intermediate dimethyl acetal 29, which was obtained by reacting compound 20 with BrCH₂CH(OMe)₂ and KH.

Compounds 11 and 12 are chemically known substances.^{12,13}

Molecular Modeling

A molecular modeling study was performed in order to verify whether different candidate compounds might fit the pharmacophore models previously defined.¹⁰ For each compound, minimum-energy conformations were calculated by a systematic search for the accessible conformational space. Analysis of the three distance D_{O-O} , D_{C-C} , and D_{N-X} values in the conformational minima pointed out that 3-(acylamino)- ϵ -caprolactams (12 for instance) and, to a lesser extent, 3-(acylamino)-2-pyrrolidinones (22 for instance) presented a satisfactory fitting to both the pharmacophore models.

Table III reports the relative energies, with respect the global minima and the pharmacophore distance values for the most interesting conformations of compounds 12 and 22. These conformations were selected because, as regards the six atoms of the pharmacophore, they provide the best fitting to the "active conformations" of compound 8 possessing the *S* configuration.¹⁰ The goodness of the fit is reported in the table as the root mean square (RMS) value.

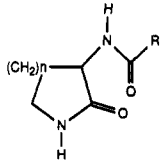
Pharmacology

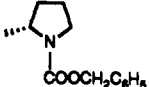
The most commonly used screening method to investigate the effect of nootropic drugs consists of behavioral tests on learning and memory in which the amnesia-reversal activity (AA) is evaluated in relation to amnesic treatments, such as Scopolamine,¹⁴ ECS,¹⁵ or hypoxia.³ In this study, amnesia was induced in mice by either ECS or Scopolamine administration, and AA of the various substances was determined by a step-through passive avoidance. The two different amnesic models were chosen to investigate for each substance an effect related to the cholinergic system (i.e., against Scopolamine administration¹⁶) or a general protective effect on CNS (i.e., against ECS treatment¹⁷).

Piracetam, Aniracetam, and Oxiracetam, which are considered to be major enhancers of cognitive functions among 2-pyrrolidinones derivatives, were used as reference compounds.

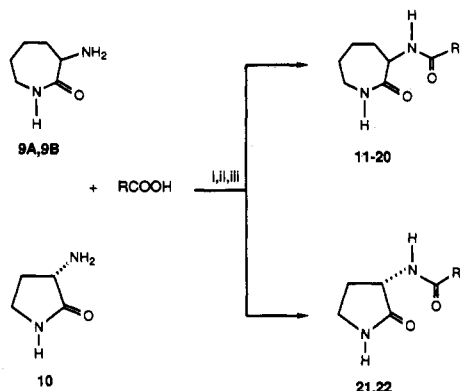
In order to find possible low-dose nootropic effects in our compounds, they were tested at the low dose-effect levels where the reference drugs have been reported to be scarcely effective.^{14,15,18–20} This choice was made because nootropic drugs should be given chronically and it would

Table I. (S)-3-Acylamino Lactams (except 14, R Form)



no.	n	R	method	mp, °C	yield, ^a %	formula	anal.	[α] _D ²⁵ , ^b
11	3	H	A ^c	176	47	C ₇ H ₁₂ N ₂ O ₂	C, H, N	+83 ^d
12	3	CH ₃	A ^e	147–149	62	C ₈ H ₁₄ N ₂ O ₂	C, H, N	+13.7
13	3	3-CF ₃ C ₆ H ₄	A	218–219	74	C ₁₄ H ₁₅ F ₃ N ₂ O ₂	C, H, F, N	+26.9
14 ^f	3	3-CF ₃ C ₆ H ₄	A	218–219	74	C ₁₄ H ₁₅ F ₃ N ₂ O ₂	C, H, F, N	-26.3
15	3	4-OCH ₃ C ₆ H ₄	A	170–172	50	C ₁₄ H ₁₈ N ₂ O ₃	C, H, N	+42.2
16	3	3-(OCH ₃) ₃ C ₆ H ₂	A	95–97	51	C ₁₆ H ₂₂ N ₂ O ₅	C, H, N	+35.7
17	3	C ₅ H ₄ N	B	177	57	C ₁₂ H ₁₆ N ₃ O ₂	C, H, N	+88 ^d
18	3		A	245–247 ^g	50	C ₁₂ H ₁₉ N ₃ O ₃	C, H, N	+7.1
19	3		B	156–158	70	C ₁₉ H ₂₅ N ₃ O ₄	C, H, N	-69.7
20	3	(CH ₂) ₃ C ₆ H ₅	A	106–108	90	C ₁₆ H ₂₂ N ₂ O ₂	C, H, N	+10.2
21	1	3-CF ₃ C ₆ H ₄	A	133–135	40	C ₁₂ H ₁₁ F ₃ N ₂ O ₂	C, H, F, N	-31.5 ^h
22	1	H	A	177–179	44	C ₅ H ₈ N ₂ O ₂	C, H, N	-86.25 ^h

^a Yields were not optimized. ^b (c = 1% MeOH.) ^c Reference 12. ^d (c = 1% CHCl₃.) ^e Reference 13. ^f R form. ^g Crystallized from CH₃CN. ^h (c = 0.5% MeOH.)

Scheme 1^a

^a (i) CDI, method A; (ii) EEDQ, method B; (iii) RCOOH → RCOCl, method C. 9A = S form. 9B = R form.

be important to restrict the amount of active principle, so as to minimize side effects. To confirm the validity of the testing procedure, here we report also the results of tests performed in the ECS model using the reference compounds at a dose level which enabled a significant reversal amnesia to be obtained.

Neuropharmacological tests in mice were also performed to see that the new compounds had no sedative, analgesic, or other effects on CNS. Their tolerability was also ascertained.

Data from the passive avoidance tests were analyzed through nonparametric statistical inferences. Furthermore, a novel parameter was introduced, the confrontation index (CI),²¹ which is a function of *U* statistic and permits a common scale of activity of substances to be generated, with higher scores representing higher activities.

Lastly, the most active new substances, the standard compounds, as well as the *S* and *R* forms of the original 3-amino-ε-caprolactam, were evaluated for the ability to inhibit in vitro acetyl cholinesterase activity.

Results and Discussion

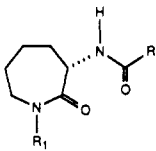
As expected, amnesia induced by either ECS or Scopolamine decreased retention latency of step-through

passive avoidance in mice. Inferential statistical analysis revealed for each experiment significant differences between Sham and the respective control groups (Mann-Whitney *U* two-tailed test, confidence interval with *p* < 0.05 at least). Latencies of Sham and control groups from each experiment are reported as a whole data set in Table IV, for both ECS- and Scopolamine-induced amnesia models. The resulting overall CI for Sham versus control groups was 68 and 75% for the ECS and the Scopolamine models, respectively.

Table IV reports the efficacy of the substances. Compound 25 significantly reversed ECS-induced amnesia (*p* < 0.05; AA = 61) in the equimolar dose of 10 mg/kg of Piracetam, whereas compound 13 significantly reversed Scopolamine-induced amnesia (*p* < 0.001; AA = 65) in the equimolar dose of 1 mg/kg of Piracetam. Strikingly, compound 11 significantly reversed both ECS- (*p* < 0.01; AA = 71) and Scopolamine-induced amnesia (*p* < 0.05; AA = 49) in equimolar doses of 1 mg/kg of Piracetam. Relevant effects tending to statistical significance were exhibited by other tested substances, such as compounds 21 (AA = 58), 23 (AA = 55), 22 (AA = 53), 17 (AA = 50), 16 (AA = 42), 15 (AA = 37), 9A (AA = 37), in the ECS model; and 9A (AA = 47), 9B (AA = 44), 12 (AA = 39), in the Scopolamine model. In Figure 1, the activity of each compound in both amnesic treatments is summarized graphically.

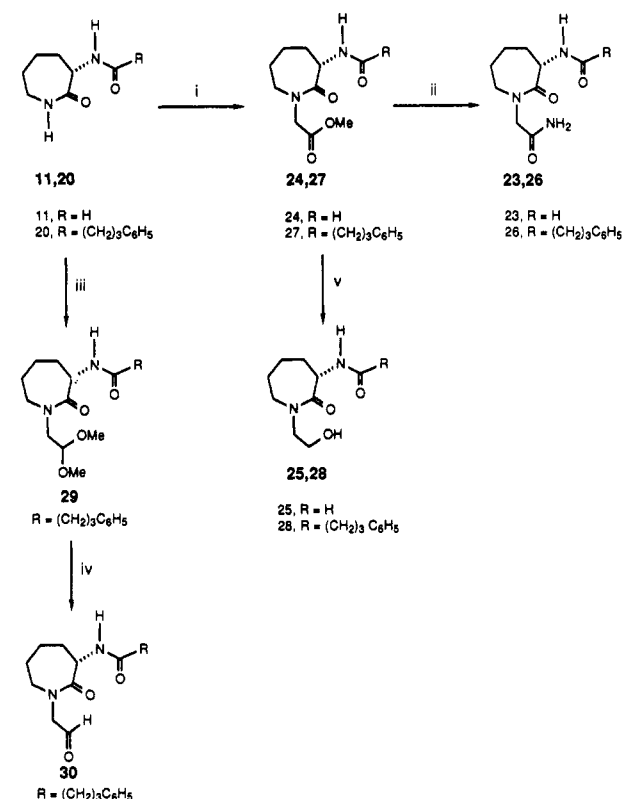
In accord with several authors,^{14,15,18–20} the reference compounds used were not effective in reversing ECS- or Scopolamine-induced amnesia at the low dose levels used. However, the respective AA values reveal some activities of those substances. On the other hand, the notable activity exerted by Aniracetam (*p* < 0.05; AA = 83) and Oxiracetam (*p* < 0.05; AA = 57) at the equimolar dose of 100 mg/kg of Piracetam in the ECS model allow us to assert the validity of our experimental paradigm.

In general, AA values contribute to better evaluate the effect of a substance through the construction of scales of activities. Variations in the ring size suggested that 5-membered compounds were less active than 7-membered

Table II. (S)-3-(Acylamino)- ϵ -caprolactam Derivatives


no.	R	R ₁	mp, °C	yield, %	formula	anal.	[α] _D ²⁵
23	H	CH ₂ CONH ₂	208–210	80	C ₉ H ₁₅ N ₃ O ₃	C, H, N	+12.4 ^a
24	H	CH ₂ COOCH ₃	60–62	70	C ₁₀ H ₁₆ N ₂ O ₄	C, H, N	+1.2 ^b
25	H	CH ₂ CH ₂ OH	89–92	55	C ₉ H ₁₆ N ₂ O ₃	C, H, N	+6.5 ^a
26	(CH ₂) ₃ C ₆ H ₅	CH ₂ CONH ₂	153–155	85	C ₁₈ H ₂₅ N ₃ O ₃	C, H, N	+8.2 ^a
27	(CH ₂) ₃ C ₆ H ₅	CH ₂ COOCH ₃	c	74	C ₁₉ H ₂₆ N ₂ O ₄	C, H, N	–9.7 ^b
28	(CH ₂) ₃ C ₆ H ₅	CH ₂ CH ₂ OH	c	57	C ₁₈ H ₂₆ N ₂ O ₃	C, H, N	–1 ^d
30	(CH ₂) ₃ C ₆ H ₅	CH ₂ CHO	c	35	C ₁₈ H ₂₄ N ₂ O ₃	C, H, N	+0.9 ^a

^a (c = 1% CHCl₃). ^b (c = 1% MeOH). ^c Not determined, oil. ^d (c = 2.2% MeOH).

Scheme II^a

^a (i) BrCH₂COOMe, NaH, CH₃CN; (ii) NH₃, MeOH; (iii) BrCH₂CH(OMe)₂, KH, THF; (iv) CF₃COOH, H₂O/CH₃CN; (v) NaBH₄, (CH₃)₃COH, MeOH.

Table III. Relative Energies (ΔE , kcal mol^{–1}) and Interatomic Distances D_{O-O} , D_{C-C} , D_{N-X} (Å) for Conformations of Compounds 12 and 22 Presenting the Lowest RMS Values for the Fitting with the "Active Conformations" of Compound 8^a

compd	ΔE	D_{O-O}	D_{C-C}	D_{N-X}	RMS	pharm model
8	0.00	3.61	3.15	3.13		1
	2.69	3.52	3.22	3.79		2
12	1.65	3.22	3.04	3.23	0.14	1
	1.43	3.83	3.32	3.79	0.15	2
22	0.78	4.15	3.53	3.23	0.76	1
	0.92	3.46	3.11	3.61	0.16	2
pharm mod 1		3.57	3.14	3.07		
pharm mod 2		3.65	3.24	3.75		

^a For comparison, the pharmacophore distance values of the active conformations of compound 8 and of the two pharmacophore models are reported.

ones. In fact, (S)-3-amino-2-pyrrolidinone 10 was less active than 3-amino- ϵ -caprolactams 9A (S form) and 9B (R form), especially in the Scopolamine-induced amnesia

reversal test. Compound 9A was a little more active than 9B in the ECS test and almost equipotent in the Scopolamine test. (S)-3-(Formylamino)- ϵ -caprolactam 11 was more potent than (S)-3-(formylamino)-2-pyrrolidinone 22 in both ECS and Scopolamine tests. Furthermore, 3-[(trifluoromethyl)benzoyl]- ϵ -caprolactams 13 (S form) and 14 (R form) were more active than the 5-membered ring analogue 21 in the Scopolamine test. By contrast, 13 and 14 exhibited a lower activity compared with 21 in the ECS test.

Alkylation of N-1 atom with the hydroxyethyl group retained (25 versus 11) or enhanced (28 versus 20) ECS-induced amnesia reversal activity. On the contrary, in the Scopolamine test 11 was markedly more active than 25, whereas no changes in activity were observed between 28 and 20. The formylmethyl substitution gave a more potent compound in both ECS and Scopolamine tests (30 compared with 20), whereas (aminocarbonyl)methyl and (methoxycarbonyl)methyl substituents did not improve the activity in (3-phenylbutanoyl)amino derivatives (26, 27 versus 20), and lowered the anti-amnesic activity in 3-formylamino derivatives (23, 24 versus 11).

Acylation of 9A with aromatic acids either maintained or enhanced the activity of compounds 15, 16, 17, only in the ECS test, whereas the activity of compound 13 was enhanced only in the Scopolamine test.

The amnesic effects of Scopolamine have been attributed to a central cholinergic action.^{14,16} Thence, those 3-amino- ϵ -caprolactam derivatives (e.g., compounds 11 and 13, and with slightly lower effects, 9A and 9B) that antagonized Scopolamine-induced amnesia might be suspected to exert some action on the cholinergic system.

Substances presenting the highest amnesia-reversal activity (11, 21, 22, 25), the S and R forms of the original 3-amino- ϵ -caprolactam (9A and 9B, respectively), and the standard compounds (Aniracetam, Oxiracetam, and Piracetam) did not inhibit AchE activity. These results are in accord with the inefficacy on AchE, as repeatedly reported for standard nootropic drugs.²² In this context, compound 13 was not tested, although it was highly active in the Scopolamine model, because it could not be solubilized at the required experimental conditions. Further investigations are planned for the most active compounds, such as an evaluation of their effects on N-methyl-D-aspartate (NMDA) receptors, long term potentiation (LTP), etc., taking in account that some of these compounds have some structural features (glycinamide moiety) in common with substances known to involve effects on NMDA receptors.^{23,24}

Table IV. Amnesia-Reversal Activity (AA) of New 7- and 5-Membered 3-Acylamino Lactams^a

drug	ECS model				Scopolamine model		
	dose, mg/kg	no. of animals	median (ir)	AA	no. of animals	median (ir)	AA
Sham		294	300 (204-300)	100	246	300 (174-300)	100
control		498	48 (22-140)	0	387	26 (19-115)	0
Aniracetam	100	14	300 (208-300)*	83			
	10	38	52 (13-154)	0			
	1	27	125 (55-242)	29	35	112 (30-189)	18
Oxiracetam	100	38	131 (38-242)*	57			
	10	32	90 (27-142)	8			
	1	31	84 (24-238)	31	23	135 (48-255)	0
Piracetam	100	13	145 (18-208)	39			
	10	30	55 (13-150)	0			
	1	27	59 (18-166)	0	34	68 (28-210)	25
9A	10	24	130 (22-190)	26			
	1	23	125 (18-224)	37	23	178 (30-300)	47
9B	10	24	94 (49-175)	25			
	1	23	25 (18-69)	0	23	70 (25-168)	44
10	10	12	116 (33-172)	0			
	1	10	249 (108-300)	24	11	88 (57-230)	0
11	10	12	83 (41-185)	55			
	1	24	88 (43-152)**	71	24	93 (31-202)*	49
12	10	11	74 (40-132)	14			
	1	12	86 (29-135)	7	22	122 (36-261)	39
13	10	24	115 (54-290)	17			
	1	35	147 (65-232)	18	36	184 (83-293)***	65
14	10	12	67 (25-159)	0			
	1	34	142 (31-300)	21	48	36 (16-88)	15
15	10	24	125 (19-191)	37			
	1	23	174 (20-300)	37	12	22 (13-58)	0
16	10	9	76 (34-237)	42			
	1	12	60 (47-141)	31	12	18 (12-36)	0
17	10	33	67 (30-182)	24			
	1	12	105 (60-140)	50	11	69 (18-113)	0
18	10	12	17 (16-30)	0			
	1	12	40 (16-169)	10	12	29 (22-40)	21
19	10	12	49 (14-244)	12			
	1	10	18 (12-118)	0	32	84 (27-170)	27
20	10	12	87 (37-164)	0			
	1	12	118 (45-196)	0	19	98 (18-136)	5
21	10	24	150 (69-231)	58			
	1	12	201 (82-243)	51	11	44 (24-106)	0
22	10	12	96 (17-228)	9			
	1	24	137 (50-280)	53	10	91 (46-281)	0
23	10	12	103 (28-277)	9			
	1	24	191 (53-300)	55	10	33 (23-290)	0
24	10	12	113 (52-285)	9			
	1	10	94 (35-210)	0	23	30 (20-67)	0
25	10	24	160 (123-233)*	61			
	1	24	143 (90-203)	46	12	82 (19-247)	7
26	10	11	39 (18-82)	0			
	1	12	27 (23-135)	0	12	73 (16-154)	0
27	10	20	66 (19-218)	0			
	1	18	51 (26-160)	0	12	34 (21-63)	0
28	10	11	28 (18-102)	20			
	1	23	30 (15-161)	7	12	25 (16-68)	12
30	10	24	59 (30-176)	25			
	1	12	110 (69-132)	0	48	36 (19-141)	26

^a Data for retention trial are reported as median latency (s), interquartile range (ir), and AA value for each group in both ECS- and Scopolamine-induced amnesia models. All substances were tested in equimolar doses of 1, 10, 100 mg/kg of Piracetam. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

All compounds proved to be ineffective on gross behavior, motor activity, and neurovegetative system in mice, except compound 20, which had convulsant effects and caused the loss of the animals' righting reflex at equimolar doses of 100 mg/kg ip of Piracetam.

These experimental results lead to the following considerations:

(1) CI proved to be a good parameter in evaluating the activity of substances, within a screening phase, allowing a meaningful scaling of activities independent of strictly probabilistic constraints. Through the AA values, substances can be easily classified in a rank with relation to

their own amnesia-reversal activity. CI's can be utilized as scores for parameterizing the biological activities of molecules including data distribution features. Thus, CI constitutes a good candidate for parameterization of the "biological score" in QSAR procedures, even in instances other than nootropic drugs.

(2) The encouraging data obtained by low-dose treatments with 7- and 5-membered 3-acylamino lactams, as well as their high tolerability, seemingly hold for the validity of our pharmacophore model and for these substances playing a role as a new class of nootropic agents.²⁵

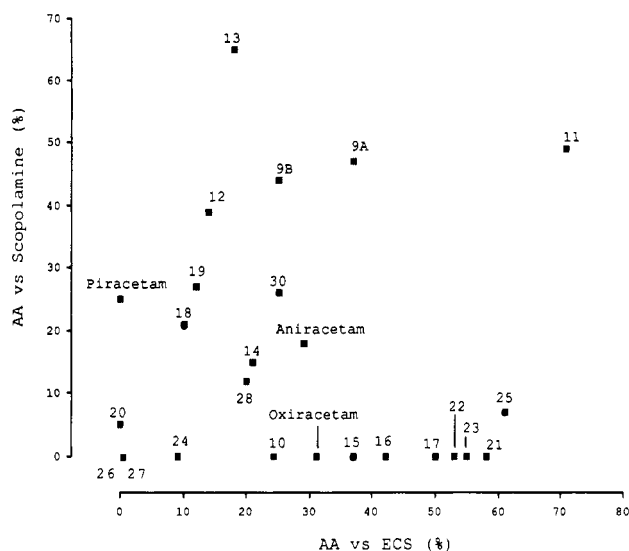


Figure 1. Graphic representation of the amnesia-reversal activity (AA) percent obtained for each substance tested in the ECS- and Scopolamine-induced amnesia models. For the ECS model, the highest scores obtained with either dose levels used are reported.

Experimental Section

Chemistry. Melting points were determined by the capillary method on an electrothermal apparatus (Buchi 535) and are uncorrected. ^1H NMR spectra were taken on a Varian VXR 300-MHz FT spectrometer; chemical shifts were expressed in δ values downfield from Me_4Si ; the coupling constants are expressed in hertz; exchange with D_2O was used, when necessary, to identify NH protons. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; m, multiplet(s); br, broad. Elemental analyses were performed by an EA 1108 elemental analyzer (Carlo Erba) apparatus and agreed with theoretical values within 0.40%. TLC was carried out with 0.25-mm silica gel 60 F254 (Merck) glass plates. Chromatographic separation was carried out by flash column chromatography using 40–63-mm silica gel (Merck) as stationary phase.

(S)-3-Amino-2-pyrrolidinone was prepared as described by Pellegata et al.²⁶ 3-Amino- ϵ -caprolactam, in both *R* and *S* forms, was supplied by Aldrich.

(S)-3-[(4-Phenylbutanoyl)amino]- ϵ -caprolactam (20). Method A. CDI (3.89 g, 24 mmol) was added to 4-phenylbutyric acid (3.28 g, 20 mmol) in CH_2Cl_2 (100 mL) and the resulting solution kept under stirring for 30 min at room temperature. Then, (S)-3-amino- ϵ -caprolactam (2.56 g, 20 mmol) was added and the resulting solution kept with stirring for 24 h at room temperature.

The organic phase was washed with 1 N NaOH (3 $\times$ 30 mL), 1 N HCl (3 $\times$ 30 mL), H_2O , and NaCl saturated solution, dried over anhydrous Na_2SO_4 , and filtered. The filtrate was concentrated at reduced pressure to yield 5 g (90%) of 20: mp 106–108 $^\circ\text{C}$; TLC eluant EtOAc R_f = 0.28; ^1H NMR (CDCl_3) δ 7.3–7.15 (m, 5 H, aromatics), 6.9 (br d, 1 H, CHNHCO), 6.55 (br t, 1 H, CH_2NHCO), 4.5 (m, 1 H, CHNCO), 3.35–3.15 (m, 2 H, CH_2NCO), 2.62 (t, J = 7 Hz, 2 H, CH_2Ph), 2.22 (t, J = 7 Hz, 2 H, $\text{NCOCH}_2\text{CH}_2$), 2.1–1.66 (m, 6 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHNCOCH}_2\text{CH}_2$), 1.55–1.28 (m, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHNCO}$).

(S)-3-[[3-(Trifluoromethyl)benzoyl]amino]- ϵ -caprolactam (13). Compound 13 was prepared according to method A. The crude product was chromatographed (EtOAc/MeOH, 8:2) to give 13 in 74% yield: mp 218–219 $^\circ\text{C}$; TLC eluant EtOAc/MeOH (5:5) R_f = 0.5; ^1H NMR ($\text{CDCl}_3/\text{DMSO}-d_6$) δ 8.15 (s, 1 H, aromatic), 8.05 (d, J = 7 Hz, 1 H, aromatic), 7.9 (br, 1 H, HNCOPh), 7.78 (d, J = 7 Hz, 1 H, aromatic), 7.6 (t, J = 7 Hz, 1 H, aromatic), 7.35 (br, 1 H, CH_2NHCO), 4.75–4.65 (m, 1 H, CHNCO), 3.40–3.22 (m, 2 H, CH_2NCO), 2.25–1.35 (m, 6 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHN}$).

(R)-3-[[3-(Trifluoromethyl)benzoyl]amino]- ϵ -caprolactam (14). Compound 14 was prepared as compound 13 starting from (*R*)-3-amino- ϵ -caprolactam.

(S)-3-[(4-Methoxybenzoyl)amino]- ϵ -caprolactam (15). Compound 15 was prepared according to method A. Chromatography was carried out on SiO_2 using EtOAc–MeOH (9:1) as eluant. The product thus obtained was taken up with CH_2Cl_2 and precipitated with Et_2O to give 15 in 50% yield: mp 170–172 $^\circ\text{C}$; TLC eluant EtOAc R_f = 0.3; ^1H NMR (CDCl_3) δ 7.82 (d, J = 10 Hz, 2 H, aromatics), 7.58 (br d, 1 H, CHNHCO), 6.93 (d, J = 10 Hz, 2 H, aromatics), 6.58 (br t, 1 H, CH_2NHCO), 4.76–4.66 (m, 1 H, CHNCO), 3.85 (s, 3 H, OCH_3), 3.42–3.22 (m, 2 H, CH_2NCO), 2.3–1.35 (m, 6 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHN}$).

(S)-3-[(3,4,5-Trimethoxybenzoyl)amino]- ϵ -caprolactam (16). Compound 16 was prepared according to method A, yield 51%: mp 95–97 $^\circ\text{C}$; TLC eluant EtOAc–MeOH (95:5) R_f = 0.28; ^1H NMR (CDCl_3) δ 7.65 (br d, 1 H, HNCOPh), 7.10 (s, 2 H, aromatics), 6.75 (br t, 1 H, CH_2NHCO), 4.78–4.68 (m, 1 H, CHNCO), 3.94 (s, 6 H, 2 *m*- OCH_3), 3.88 (s, 3 H, *p*- OCH_3), 3.42–3.22 (m, 2 H, CH_2NHCO), 2.28–1.35 (m, 6 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHN}$).

(S)-3-(Formylamino)- ϵ -caprolactam (11). Compound 11 was prepared according to method A. After 24 h of stirring at room temperature, Et_2O was added until complete precipitation took place; the precipitate was collected by filtration and further purified by crystallization from $\text{CHCl}_3/\text{Et}_2\text{O}$ to give 11 in 48% yield: mp 176 $^\circ\text{C}$; TLC eluant EtOAc R_f = 0.1; ^1H NMR ($\text{DMSO}-d_6$) δ 8.25 (br, 1 H, HNCHO), 8.1 (s, 1 H, CHO), 7.9 (br, 1 H, HNCO), 4.6–4.45 (m, 1 H, CHNCO), 3.35–3.05 (m, 2 H, CH_2NCO), 2–1.60 (m, 4 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHN}$), 1.55–1.20 (m, 2 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHN}$).

(S)-3-[[2-Oxopyrrolidin-1-yl]acetyl]amino]- ϵ -caprolactam (18). Compound 18 was prepared according to method A. After 24 h of stirring, Et_2O was added to the mixture; the precipitate was collected by filtration and further purified by crystallization from CH_3CN to give 18 in 50% yield: mp 245–247 $^\circ\text{C}$; TLC eluant EtOAc/MeOH (7:3) R_f = 0.43; ^1H NMR (D_2O) δ 4.65 (d, J = 10 Hz, 1 H, CHNHCO), 4.05 (s, 2 H, $\text{COCH}_2\text{NCH}_2$), 3.5 (t, J = 6 Hz, 2 H, $\text{COCH}_2\text{NCH}_2$), 3.4–3.2 (m, 2 H, CH_2NHCO), 2.5 (t, J = 8 Hz, 2 H, $\text{CH}_2\text{NCOCH}_2$), 2.20–1.30 (m, 8 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CHN}$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CON}$).

(S)-3-[[3-(Trifluoromethyl)benzoyl]amino]-2-pyrrolidinone (21). Compound 21 was prepared according to method A. After workup, the organic phase was evaporated at reduced pressure and the residue crystallized from EtOAc/*n*-hexane to give 21 in 40% yield: mp 133–155 $^\circ\text{C}$; TLC eluant EtOAc/MeOH (9:1) R_f = 0.37; ^1H NMR (CDCl_3) δ 8.08 (s, 1 H, aromatic), 7.98 (d, J = 7 Hz, 1 H, aromatic), 7.8 (br d, 1 H, CHNHCO), 7.7 (d, J = 7 Hz, 1 H, aromatic), 7.5 (t, J = 7 Hz, 1 H, aromatic), 6.7 (s, 1 H, CH_2NHCO), 4.7–4.6 (m, 1 H, CHNCO), 3.5–3.38 (m, 2 H, CH_2NCO), 2.82–2.7 (m, 1 H, CHHCHN), 2.2–2 (m, 1 H, CHHCHN).

(S)-3-(Formylamino)-2-pyrrolidinone (22). Compound 22 was prepared according to method A. After 24 h of stirring, Et_2O was added to the mixture until complete precipitation took place; the precipitate was collected by filtration and chromatographed (EtOAc/MeOH, 8:2). The product thus obtained was further purified by treatment with refluxing CHCl_3 to give 22 in 44% yield: mp 177–179 $^\circ\text{C}$; TLC eluant EtOAc/MeOH (7:3) R_f = 0.4; ^1H NMR (D_2O) δ 8.15–8.05 (2 s, 1 H, NCHO), 4.62–4.4 (2 m, 1 H, CHNHCO), 3.48–3.3 (m, 2 H, CH_2NCO), 2.6–2.45 (m, 1 H, CHHCHN), 2.15–2 (m, 1 H, CHHCHN).

(S)-3-[(Benzyloxycarbonyl)-(S)-prolyl]amino]- ϵ -caprolactam (19). Method B. EEDQ (3.45 g, 13.95 mmol) was added with stirring to (S)-(benzyloxycarbonyl)proline (2.9 g, 11.63 mmol) in CH_3CN (100 mL) and (S)-3-amino- ϵ -caprolactam (1.49 g, 11.63 mmol) was added after 30 min. The mixture was kept at room temperature with stirring for 24 h, concentrated under vacuum, and chromatographed (EtOAc) to yield 2.9 g (70%) of 19: mp 156–158 $^\circ\text{C}$; TLC eluant EtOAc R_f = 0.23; ^1H NMR (CDCl_3) δ 7.5–7.2 (m, 6 H, aromatics, CH_2NHCO), 6.55 (br d, 1 H, CH_2NHCO), 5.25–5.05 (m, 2 H, OCH_2Ph), 4.55–4.20 (m, 2 H, NCOCHNCO , CHNCOO), 3.7–3.42 (m, 2 H, CH_2NCOO), 3.35–2.15 (m, 2 H, CH_2NHCO), 2.25–1.2 (m, 10 H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}$, $\text{CH}_2\text{CH}_2\text{CHN}$).

(S)-3-(Nicotinoylamino)- ϵ -caprolactam (17). Compound 17 was prepared according to method B. Chromatography was carried out (EtOAc/MeOH, 8:2) to give 17 in 57% yield: mp 177 $^\circ\text{C}$; TLC eluant EtOAc/MeOH (8:2) R_f = 0.38; ^1H NMR (CDCl_3) δ 9.08 (m, 1 H, aromatic), 8.75 (m, 1 H, aromatic), 8.15 (m, 1 H,

aromatic), 7.75 (br d, 1 H, CHNHCO), 7.40 (m, 1 H, aromatic), 6.40 (br t, 1 H, CH₂NHCO), 4.76–4.68 (m, 1 H, CHNCO), 3.42–3.22 (m, 2 H, CH₂NCO), 2.30–1.38 (m, 6 H, CH₂CH₂CH₂CHN).

(S)-3-(Acetylamino)- ϵ -caprolactam (12). Method C. A solution of acetyl chloride (1.35, 17.17 mmol) in CH₂Cl₂ (10 mL) was added to an ice-cold solution of (S)-3-amino- ϵ -caprolactam (2.2 g, 17.16 mmol) and triethylamine (1.91 g, 18.88 mmol) in CH₂Cl₂ (50 mL). After 24 h of stirring at room temperature, Et₂O was added until complete precipitation took place; the filtrate was evaporated at reduced pressure and the residue chromatographed (EtOAc) to yield 1.8 g (62%) of 12: mp 147–149 °C; TLC eluant EtOAc/MeOH (8:2) *R_f* = 0.47; ¹H NMR (CDCl₃) δ 7.2–7 (br, 2 H, NHCOCH₃), 4.6–4.5 (m, 1 H, CHNCO), 3.4–3.2 (m, 2 H, CH₂NCO), 2.15–1.75 (m, 7 H, CH₂-CH₂CH₂CHNCOCH₃), 1.60–1.3 (m, 2 H, CH₂CH₂CHNCO).

(S)-1-[(Methoxycarbonyl)methyl]-3-[(4-phenylbutanoyl)amino]- ϵ -caprolactam (27). NaH 80% in mineral oil (372.82 mg, 10.92 mmol) was added to a solution of 20 (1.5 g, 5.46 mmol) and BrCH₂COOCH₃ (1.672 g, 10.92 mmol) in CH₃CN (80 mL) in two aliquots over 1 h. The resulting mixture was kept with stirring at room temperature for 24 h and then filtered. The filtrate, brought to dryness, was chromatographed (EtOAc) to yield 1.4 g (74%) of 27 as an oily product: TLC eluant EtOAc *R_f* = 0.55; ¹H NMR (CDCl₃) δ 7.3–7.15 (m, 5 H, aromatics), 6.9 (br d, 1 H, CHNHCO), 4.72–4.62 (m, 1 H, CHNCO), 4.18 (s, 2 H, NCH₂CO), 3.78–3.65 (m, 4 H, OCH₃, CHHNCO), 3.25–3.15 (m, 1 H, CHHNCO), 2.65 (t, *J* = 7 Hz, 2 H, CH₂Ph), 2.2 (t, *J* = 7 Hz, 2 H, NCOCH₂CH₂), 2.1–1.75 (m, 6 H, CH₂CH₂CH₂-CHNCOCH₂CH₂), 1.7–1.45 (m, 2 H, CH₂CH₂CH₂CHN).

(S)-1-[(Aminocarbonyl)methyl]-3-[(4-phenylbutanoyl)amino]- ϵ -caprolactam (26). Gaseous NH₃ was bubbled for 30 min into a solution of 27 (1.6 g, 4.62 mmol) in MeOH (50 mL) cooled with an ice bath. The solution was kept under stirring at room temperature for 24 h, the solvent was evaporated, and the residue was chromatographed (EtOAc/MeOH, 9:1) to yield 1.3 g (85%) of 26: mp 153–155 °C; TLC eluant EtOAc/MeOH (9:1) *R_f* = 0.3; ¹H NMR (CDCl₃) δ 7.31–7.13 (m, 5 H, aromatics), 6.87 (br d, 1 H, CHNHCO), 6.42 (br s, 1 H, CONHH), 5.89 (br s, 1 H, CONHH), 4.76–4.66 (m, 1 H, CHNCO), 4.29 (d, *J* = 15 Hz, 1 H, NCHHCON), 3.82 (d, *J* = 15 Hz, 1 H, NCHHCON), 3.76–3.64 (m, 1 H, CH₂CHHNCO), 3.35–3.24 (m, 1 H, CH₂-CHHNCO), 2.63 (t, *J* = 7 Hz, 2 H, CH₂Ph), 2.2 (t, *J* = 7 Hz, 2 H, NCOCH₂CH₂), 2.08–1.74 (m, 6 H, CH₂CH₂CH₂CHNCOCH₂CH₂), 1.58–1.38 (m, 2 H, CH₂CH₂CHN).

(S)-1-(2-Hydroxyethyl)-3-[(4-phenylbutanoyl)amino]- ϵ -caprolactam (28). NaBH₄ (402 mg, 10.7 mmol) was added to 27 (1 g, 2.88 mmol) in *tert*-butyl alcohol (18 mL), the temperature was brought to 80 °C, and MeOH (3 mL) was added slowly. The resulting mixture was kept at the reflux temperature for 2 h. After cooling in an ice bath, H₂O was added and the alcohols were evaporated under vacuum; the aqueous phase was extracted with EtOAc, and the solvent was evaporated. The residue was chromatographed (EtOAc/MeOH, 9:1) to yield 530 mg (57%) of 28 as an oily product: TLC eluant EtOAc/MeOH (9:1) *R_f* = 0.4; ¹H NMR (CDCl₃) δ 7.32–7.14 (m, 5 H, aromatics), 6.82 (br d, 1 H, CHNHCO), 4.7–4.6 (m, 1 H, CHNHCO), 3.82–3.48 (m, 5 H, CHHNCH₂CH₂OH), 3.38–3.26 (m, 1 H, CHHNCH₂CH₂OH), 2.8 (br s, 1 H, OH), 2.64 (t, *J* = 7 Hz, 2 H, CH₂Ph), 2.23 (t, *J* = 7 Hz, 2 H, NCOCH₂CH₂), 2.08–1.76 (m, 6 H, CH₂CH₂CH₂CHNCOCH₂CH₂), 1.54–1.36 (m, 2 H, CH₂CH₂CHN).

(S)-1-(Formylmethyl)-3-[(4-phenylbutanoyl)amino]- ϵ -caprolactam (30). A solution of 20 (6 g, 21 mmol) in THF (90 mL) was added dropwise to a suspension of KH (35% in mineral oil, 3.726 g, 32 mmol, washed with pentane) in THF (90 mL). The resulting mixture was kept at reflux temperature until hydrogen development was over. Bromoacetaldehyde dimethyl acetal (7.38 g, 43 mmol) was added dropwise, and the resulting mixture was kept at the reflux temperature overnight. The mixture was evaporated under vacuum, and a saturated NH₄Cl solution (200 mL) was added thereto; the resulting mixture was extracted with CH₂Cl₂ (3 $\times$ 70 mL) and the organic phase dried over anhydrous Na₂SO₄.

The residue obtained following solvent evaporation was chromatographed using EtOAc/hexane (95:5) as eluant to obtain 4 g of (S)-1-(2,2-dimethoxyethyl)-3-[(phenylbutanoyl)amino]- ϵ -caprolactam 29 (TLC eluant EtOAc, *R_f* = 0.5) which was

dissolved in CH₃CN (100 mL) and H₂O (100 mL). Trifluoroacetic acid (2 mL) was added, the solution was stirred overnight at room temperature, the CH₃CN was evaporated under vacuum, and the residue was extracted with CHCl₃ (3 $\times$ 50 mL). The organic phase was washed with a saturated NaCl solution, dehydrated over Na₂SO₄, and evaporated to yield 2.3 g (35% overall) of 30: TLC eluant EtOAc *R_f* = 0.31; ¹H NMR (CDCl₃) δ 9.55 (s, 1 H, CHO), 7.30–7.15 (m, 5 H, aromatics), 6.90 (br d, 1 H, NHCO), 4.75–4.65 (m, 1 H, CHNHCO), 4.25 (m, 2 H, NCH₂-CHO), 3.80–3.65 (m, 1 H, CHHNCO), 3.15–3.05 (m, 1 H, CHHNCO), 2.62 (t, *J* = 7 Hz, 2 H, CH₂Ph), 2.22 (t, *J* = 7 Hz, 2 H, NCOCH₂CH₂), 2.10–1.75 (m, 6 H, CH₂CH₂CH₂CHNCOCH₂CH₂), 1.65–1.45 (m, 2 H, CH₂CH₂CHNCO).

(S)-1-[(Methoxycarbonyl)methyl]-3-(formylamino)- ϵ -caprolactam (24). Compound 24 was prepared as compound 27 (starting from 11 instead of 20) in 70% yield: mp 60–62 °C; TLC eluant EtOAc *R_f* = 0.24; ¹H NMR (CDCl₃) δ 8.15 (s, 1 H, NCHO), 7.10 (br, 1 H, NHCHO), 4.78–4.68 (m, 1 H, CHNCO), 4.18 (s, 2 H, NCH₂CO), 3.78–3.62 (m, 4 H, OCH₃, CH₂CHHNCO), 3.25–3.15 (m, 1 H, CH₂CHHNCO), 2.15–1.48 (m, 6 H, CH₂CH₂CH₂-CHN).

(S)-1-[(Aminocarbonyl)methyl]-3-(formylamino)- ϵ -caprolactam (23). Compound 23 was prepared as compound 26 (starting from 24 instead of 27). As chromatography eluant, EtOAc/MeOH (7:3) was used: yield 80%; mp 208–210 °C; TLC eluant EtOAc/MeOH (7:3) *R_f* = 0.46; ¹H NMR (D₂O) δ 8.08 (s, 1 H, NCHO), 4.9 (m, 1 H, CHNCO), 4.39 (d, *J* = 16.6 Hz, 1 H, NCHHCON), 3.98 (d, *J* = 16.6 Hz, 1 H, NCHHCON), 3.85–3.75 (m, 1 H, CH₂CHHNCO), 3.42–3.3 (m, 1 H, CH₂CHHNCO), 2.05–1.48 (m, 6 H, CH₂CH₂CH₂CHN).

(S)-1-(2-Hydroxyethyl)-3-(formylamino)- ϵ -caprolactam (25). Compound 25 was prepared as compound 28 (starting from 24 instead of 27). The aqueous phase was evaporated under vacuum and the residue chromatographed (EtOAc/MeOH, 95:5) to give 25 in 55% yield: mp 89–92 °C; TLC eluant EtOAc/MeOH (95:5) *R_f* = 0.33; ¹H NMR (CDCl₃) δ 8.1 (s, 1 H, NCHO), 7.1 (br s, 1 H, NHCHO), 4.8–4.65 (m, 1 H, CHNCO), 3.9–3.45 (m, 5 H, CHHNCH₂CH₂OH), 3.4–3.26 (m, 1 H, CHHNCH₂CH₂OH), 2.85 (br s, 1 H, OH), 2.14–1.76 (m, 4 H, CH₂CH₂CH₂CHN), 1.58–1.36 (m, 2 H, CH₂CH₂CH₂CHN).

Molecular Modeling. Conformational analysis of candidate compounds was carried out using the MM2¹¹ force field included in the molecular modeling software MACROMODEL,²⁷ running on a Personal IRIS 4D/25 workstation. The MM2 force field was selected on the basis of our previous study;²⁸ a comparison made with *ab initio* calculations showed that it was more reliable, in the study of conformational properties of compounds containing a pyrrolidinonic ring, than the AMBER²⁹ and OPLS³⁰ force fields and the quantum mechanical semiempirical AM1 method.³¹

For each compound, conformational analysis was performed generating conformational isomers by the MULTICONF³² option of MACROMODEL: ring conformational isomers were generated choosing a breaking bond in the ring and systematically increasing the ring torsions by 30° in the 0–360° range; all the structures presenting values of the ring closure distance, i.e. of the broken bond, within a predefined range (1.0–2.0 Å) were retained. Relevant torsional angles of the side chains were increased by 30° while amidic bonds were increased by 180°. The resulting structures were completely optimized by the Block Diagonal Newton Raphson procedure using as convergence criteria a gradient variation lower than 0.01 kcal mol⁻¹ Å⁻¹; the nature of the stationary points was tested by computing eigenvalues of the second-derivative matrix.

Pharmacology. (A) Amnesia-Reversal Testing. (1) Experimental Procedure. Experiments were carried out on male CD1 mice (Charles River Italia), weighing 24–28 g. The animals were housed in transparent makrolon cages and received tap water and standard laboratory diet (4RF21, Mucedola S.r.l. Italia) *ad libitum*. The animal rooms were maintained under standard conditions of temperature (22 $\pm$ 1 °C) and relative humidity (55 $\pm$ 10%), with a 12:12-h light/dark cycle, and 12–15 filtered air changes/h.

The method used was similar to the one described by Ader et al.³³ envisaging acquisition followed by retention trials. The apparatus for the passive avoidance test consisted of a dark plastic

box (42 × 42 × 40 cm high), provided with a stainless steel grid floor. Against the front wall was leaned a smaller white chamber (30 × 10 × 12 cm high), connected to the box through a sliding door (6 × 6 × 6 cm high). A 60-W lamp was fixed 40 cm above the center of the white chamber, while the box was kept dark.

For the acquisition trial, each mouse was placed for 1 min in the light compartment and then allowed to enter the dark box, recording the time it did so in seconds. Mice whose latency was greater than 60 s were discarded. Once the mouse entered the dark box with all four paws, the sliding door was closed, and 3 s afterward a scrambled electric foot-shock (0.21 mA for 2 s) was delivered through the grid floor. The animal was then returned to its home cage until the retention trial, which was carried out at 24 h after the acquisition trial: each mouse was placed back into the light chamber and the time taken to enter the dark box was recorded. End point of retention latency was set at 300 s.

In the passive avoidance test with ECS- or Scopolamine-induced amnesia, the animals were distributed into three groups as follows:

(1) Sham group, to measure the normal retention in the passive avoidance paradigm. The animals were injected vehicle (ip) at 30 min and at 30 and 15 min before the acquisition trial in the ECS- and in the Scopolamine-induced amnesia test, respectively.

(2) Control group, to measure amnesia caused by either ECS or Scopolamine. The animals received ECS (delivered through auricular electrodes; rectangular positive pulses: current = 20 mA, pulse width = 0.6 ms, shock duration = 0.5 s, frequency = 50 Hz) immediately after the acquisition trial, or Scopolamine hydrobromide (1.5 mg/kg sc; Merck) 15 min before the acquisition trial. In both amnesic procedures, each mouse was injected vehicle (ip) 30 min before the acquisition trial.

(3) Treated group, to measure AA of each compound as compared with the amnesic treatment. The animals received either ECS or Scopolamine as described for the control group. The substances were administered 30 min before the acquisition trial.

For each substance, two doses (equimolar to 10 and 1 mg/kg ip of Piracetam) and one dose (equimolar to 1 mg/kg ip of Piracetam) were tested for ECS- and Scopolamine-induced amnesia, respectively. The reference compounds, i.e. Aniracetam, Oxiracetam, and Piracetam, were also tested at the equimolar dose of 100 mg/kg ip of Piracetam in the ECS model. Saline (NaCl, 0.9%) was the vehicle used for water-soluble substances; otherwise, it was a solution of dimethyl sulfoxide–2% Tween 80 (Merck), 1:4 (v:v).

(2) **Statistics.** Data were reported as median retention latency and interquartile range per group. For each experiment the amnesic effect of Scopolamine or ECS was verified by comparing control groups with Sham groups through the Mann-Whitney's *U* test. When no significant differences were found ($p > 0.05$), the experiment was discarded. For the accepted experiments, the Mann-Whitney's *U* test was used to compare the retention latency of treated and control animals. All probabilities were two-tailed.

Results for each compound were also expressed as percent of AA to enable comparison of the activity of each substance. AA is described as follows:

$$AA = (CI_T/CI_S) \times 100$$

where CI_T = confrontation index for the treated group and CI_S = confrontation index for Sham group.

Confrontation Index. This new parameter was introduced for the evaluation of data. The CI is calculated as follows:

$$\frac{U' - U}{n_i n_j} = CI$$

with $U = n_i n_j + n_i(n_i + 1)/2 - R_i$, $U' = n_i n_j - U$, n_i = number of elements of group I (Sham or treated), n_j = number of elements of group J (control), R_i = sum of the ranks assigned to the group I. Throughout this formula, that is a function of the Mann-Whitney's *U* parameters,³⁴ it is possible to obtain a descriptive index (confrontation index) which permits a common scale of activities to be made among substances, independently of the number of tested animals. In other words, through the CI, the

degree of discrimination between two experimental groups as measured by *U* statistics can be easily converted into a numerical value which can be inserted in a score scale.

The CI reaches a maximum when $U' = 0$ ($CI = 1$), corresponding to the total separation of the two experimental groups, and a minimum when $U = U'$ ($CI = 0$), i.e., complete overlapping.

(B) Observational Tests for CNS Signs. Male CD1 mice (Charles River Italia), weighing 22–24 g, were used and housed under standard conditions as described in A. Compounds were administered as reported in A, at 4 equimolar doses up to 100 mg/kg ip of Piracetam, each to 10-mice groups, whereas the respective control groups were treated with vehicle alone. All the animals were food-deprived starting 18 h before testing. Each test was performed using different animals.

(1) **Irwin Test.**³⁵ From drug administration until the successive 6 h, each animal was rated by an expert observer, unaware of the treatment, for the presence or absence of the signs and symptoms of alteration of normal behavioral, neurological, and autonomic conditions, such as changes in motor activity, mood, CNS excitation, motor coordination, muscle tone, reflexes, effects on eyes, secretions/excretions, etc.

(2) **Hot-Plate Test.**³⁶ The animals from each dose group were placed on a metal plate, at constant 56 °C, at 30, 60, 120, and 180 min after administration of the compounds. The increased latency (in seconds) of reaction (jumping or licking paws) in comparison with the basal and/or control groups was considered a measure of the analgesic effect. Unpaired two-tailed Student's *t* test was used as statistical inference for comparison between treated and control groups.

(C) Evaluation of in Vitro Acetyl Cholinesterase (AChE) Inhibition. One male Fischer 344 rat (Charles River Italia) brain was homogenized in 15 mL of Sørensen 66.7 mM, pH 7.2, by addition of a 1% solution of Triton x-100 (2 mL/1 mL of homogenate). The supernatant was used as AChE source. Substances were suspended in H₂O or DMSO and diluted in Sørensen buffer, pH 7.2, dissolved at final concentrations ranging from 10⁻³ to 10⁻¹¹ M. AChE activity was determined as described by Ellman et al.³⁷ at 37 °C. A Test-Combination Cholinesterase (Boehringer Mannheim Gmgh) kit was used and suited to an autoanalyzer (Cobas Mira S, Roche). Physostigmine was used as reference inhibitor. For this known AChE agonist the concentration causing 50% inhibition (IC_{50}) was about 1.15 × 10⁻⁸ M.

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A Novel Synthetic Route to Enantiomers of ϵ -Hydroxynorleucine and ϵ -Chloronorleucine from *L*- and *D,L*-Lysine

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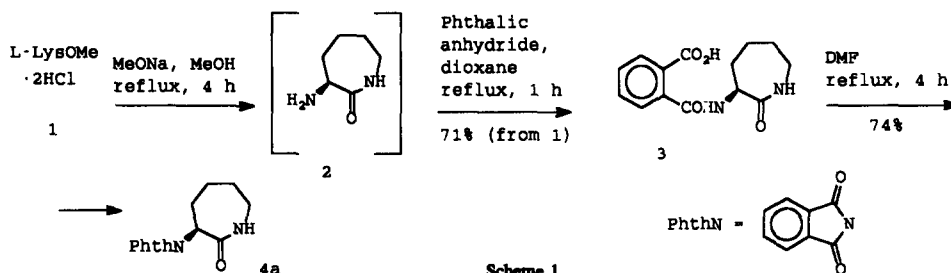
Abstract: Thermal rearrangement of (*S*)- or (*R*)-hexahydro-1-nitroso-3-phthalimido-2H-azepin-2-one to the corresponding lactone followed by nucleophilic ring opening of the latter gives ϵ -chloro- or ϵ -hydroxynorleucine.

The non-proteinogenic α -amino acid *L*- ϵ -hydroxynorleucine (**7a**) has been widely used for the synthesis of modified peptides¹⁻³, siderophores aerobactin and mycobactin^{4,5}, and *L*- α -amino adipic acid derivatives^{3,6}. In most cases, **7a** was obtained by enzymatic resolution of the racemic *N*-acylated derivatives with acylase¹⁻⁵. Non-crystalline crude *N*-Cbz-*L*- ϵ -hydroxy-norleucine was prepared as an intermediate for the synthesis of *L*- α -amino adipic acid derivative by treatment of *N*²-Cbz-*L*-lysine with sodium nitroprusside⁶. Alkylation of dienolate derived from (*5S,6R*)- or (*5R,6S*)-4-(benzyloxycarbonyl)-5,6-diphenyl-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one with 1,4-diiodobutane followed by nucleophilic substitution of the second iodine atom gave derivatives of *L*- or *D*- ϵ -hydroxynorleucine, which were used for the synthesis of 2-amino-6-[(*p*-methoxybenzyl)-thio]hexanoic acid in both enantiomeric forms⁷.

Recently, we reported the preparation of *N*⁶-methyl-*L*-lysine⁸ and *L*- α -amino adipic acid⁹ via the common intermediate - (*S*)-hexahydro-3-phthalimido-2H-azepin-2-one (**4a**). We now describe the simplified synthesis of the above lactam **4a** from *L*-lysine and its use for preparation of **7a** and *L*- ϵ -chloronorleucine (**8**).

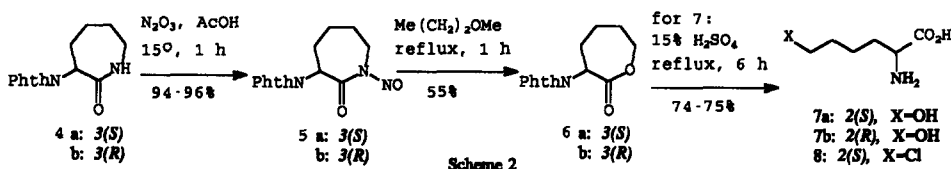
Lactam **4a** was prepared earlier by treatment of (*S*)-3-amino-2H-azepin-2-one (**2**) with *N*-carbomethoxyphthalimide⁸. The relatively high cost of the latter prompted us to look for a more economical pathway to **4a**. For this purpose, *L*-lysine methyl ester (**1**) was cyclized in the presence of sodium methoxide and, without isolation of the lactam **2**, acylated by phthalic anhydride. Phthalamic acid **3** thus obtained gave lactam **4a** upon heating in dimethylformamide solution (Scheme 1). No racemization was observed in this reaction sequence. Although yields were not very high, low cost of the starting materials and easy workup made this synthetic route satisfactory.

Unsubstituted ϵ -caprolactam can be *N*-nitrosated by nitrous gases in acetic acid¹⁰, nitrosyl tetrafluoroborate



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in pyridine¹¹ or dinitrogen tetroxide/sodium acetate in dichloromethane¹². *N*-Nitrosolactam thus obtained was shown to undergo thermal rearrangement in the carbon tetrachloride solution at the room temperature to give the corresponding lactone^{11,12}. Treatment of the phthalimido lactam **4a** with nitrous gases in acetic acid gave *N*-nitrosolactam **5a** in quantitative yield (mp 118° C (dec); $[\alpha]_D^{20} +185.0^\circ$ (C=1, CHCl₃)). Nitroso derivative **5a** appeared to be more stable as compared with unsubstituted *N*-nitroso- ϵ -caprolactam. Thus, heating of **5a** in dichloromethane solution under reflux for eight hours was shown by TLC to produce only traces of the desired lactone. The highest yield of the lactone **6a** was obtained upon heating of nitroso lactam **5a** in dry 1,2-dimethoxyethane under reflux for one hour (Scheme 2)¹³. Low concentration (ca. 3.4 mmol/L) of the nitroso lactam **5a** as well as lactone **6a** in the reaction mixture was critical for the yield of the latter. Thus, the increase of the **5a** concentration up to 34 mmol/L or slow addition of the concentrated lactam **5a** solution to the boiling dimethoxyethane with the same (ca. 34 mmol/L) final theoretical concentration of lactone **6a** lead to the formation of a very complex mixture of unidentified substances. Hydrolysis of the lactone **6a** in aqueous hydrochloric acid gave the mixture of **7a** and **8**, the latter can be isolated by crystallization from ethanol/water. The use of diluted sulfuric acid afforded pure **7a** (Scheme 2).



(*R*)-Enantiomer of the lactam **2** can be readily prepared by resolution of racemic **2** with *L*-pyroglutamic acid or by kinetically controlled crystallization¹⁴. It is also commercially available. Application of the above procedure to (*R*)-phthalimido lactam **4b**, which was prepared from (*R*)-**2** similarly to **4a**⁸, allowed us to obtain *D*- ϵ -hydroxynorleucine (**7b**) via *N*-nitrosolactam **5b** and lactone **6b** (Scheme 2).

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(*S*)- and (*R*)-3-Phthalimidooxepan-2-one (**6a,b**): A solution of nitrosolactam **5a** or **5b** (0.5 g, 1.7 mmol) in dimethoxyethane (500 mL) was heated under reflux for one hour and evaporated under reduced pressure to dryness. The residue was recrystallized from 85% aq EtOH (10 mL) to give **6a** or **6b**. For **6a**: Yield: 0.25 g (55%); mp 203° C; $[\alpha]_D^{20} +95.0^\circ$ (C=1, CHCl₃). ¹H-NMR (CDCl₃/TMS): δ = 1.2-2.9 (m, 6H, CH₂CH₂CH₂), 4.42 (m, 2H, OCH₂), 5.12 (dd, 1H, J=11.7, 1.6 Hz, CHCO), 7.80 (m, 4H_{arom}). MS (70 eV): m/z (relative intensity)= 259 (M, 23), 186 (31), 173 (59), 148 (100), 130 (23), 104 (33), 76 (28), 67 (16).
For **6b**: Yield: 0.25 g (55%); mp 201° C; $[\alpha]_D^{20} -95.0^\circ$ (C=1, CHCl₃).
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