

Synthesis and Pharmacological Activity of a Series of Dihydro-1*H*-pyrrolo[1,2-*a*]imidazole-2,5(3*H*,6*H*)-diones, a Novel Class of Potent Cognition Enhancers

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A series of dihydro-1*H*-pyrrolo[1,2-*a*]imidazole-2,5(3*H*,6*H*)-diones were synthesized. These bicyclic derivatives contain both the 2-pyrrolidinone and 4-imidazolidinone nuclei, already recognized as important for cognition enhancing activity. In addition, these structures maintain the backbone of piracetam and oxiracetam with the acetamide side chain restricted in a folded conformation. Their ability to reverse scopolamine-induced amnesia was assessed in a one trial, step-through, passive avoidance paradigm. The main features observed are a potent antiamnesic activity after ip administration (minimal effective dose being between 0.3 and 1 mg/kg ip for most compounds), the presence of a bell-shaped dose-response curve and, generally, a reduction of biological activity after po administration. However, the unsubstituted compound (15, dimiracetam) shows no evidence of a bell-shaped dose-response curve and completely retains activity when given orally, being 10-30 times more potent than the reference drug oxiracetam.

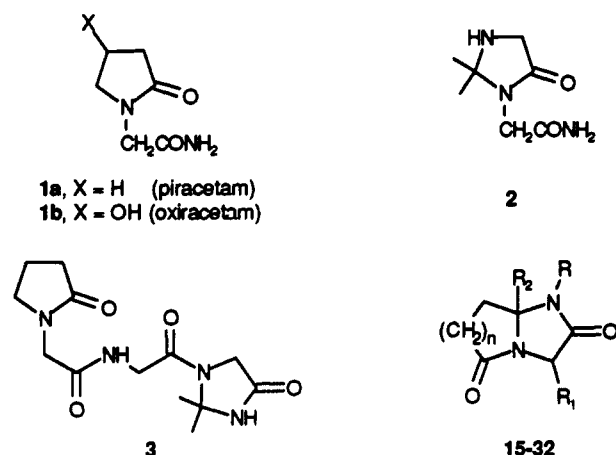
A large number of compounds belonging to many different chemical classes have been proposed as cognition enhancers.¹⁻⁴ Indeed, most of these compounds were found to be effective in mild forms of cognition impairment, while their efficacy in more severe forms of dementia still remains questionable.

Among them, pyrrolidinone cognition enhancers like piracetam (1a, Chart I) and oxiracetam (1b) form a group apart due to their unique selectivity for brain areas involved in learning and memory processes and their exceptionally safe profile.⁵⁻⁷ These features make them suitable for chronic treatment in elderly patients. Though the exact mechanism of action of pyrrolidinone cognition enhancers has not yet been elucidated, it has recently been demonstrated that they exert a variety of biochemical and electrophysiological effects related to learning and memory processes. These include the enhancement of cholinergic and glutamatergic hippocampal and cortical systems and the induction of hippocampal long-term potentiation in rodent brain slices.⁸⁻¹¹

Thus, taking oxiracetam^{12,13} as the model drug, we continued research in the field with the aim of finding compounds endowed with increased potency and efficacy and with a similar profile in terms of safety. Within this framework we had already shown that the 2-pyrrolidinone nucleus, which can be considered a mimic of a dipeptide with a restricted conformation,¹⁴ can be isosterically replaced by the 4-imidazolidinone ring, to give, for example, compound 2, which retains antiamnesic activity.¹⁵ Combination of the two features, namely 2-pyrrolidinone and 4-imidazolidinone, in a single molecule, such as in compound 3, further enhanced the activity.¹⁶

The finding of an antiamnesic activity confirmed that the 4-imidazolidinone nucleus possessed the required properties of stability and brain penetration and retained a similar and complementary pharmacological activity to that of 2-pyrrolidinone.

Chart I



On the basis of these results, we decided to combine, in the most compact way and with possible advantages for stability, the two γ -lactamic heterocycles into a novel fused bicyclic structure. In addition, the designed structure maintains the backbone of piracetam and oxiracetam with the acetamide side chain restricted in a folded conformation.

Thus, a series of variously substituted dihydro-1*H*-pyrrolo[1,2-*a*]imidazole-2,5(3*H*,6*H*)-diones 15-32 and the homologous compounds 33 and 34, where the 2-pyrrolidinone was enlarged to six- and seven-membered rings, were prepared, and their ability to reverse the scopolamine-induced amnesia in rats was assessed.

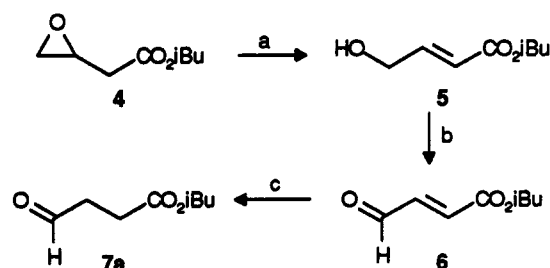
Chemistry. Compounds 15-27, 33, and 34 were prepared by condensation of the suitable α -amino amides with oxoesters 7. All starting materials have been previously described in the literature, apart from isobutyl 4-oxobutanoate (7a), which was obtained (Scheme I) from isobutyl 3,4-epoxybutanoate (4)¹⁷ by base-catalyzed rearrangement to isobutyl γ -hydroxycrotonate (5), oxidation with pyridinium chlorochromate to 6, and, finally, selective hydrogenation of the double bond.

Reaction in refluxing water (method A) of 7a with glycineamide (8a), DL-alanineamide (DL-8b), and L-serina-

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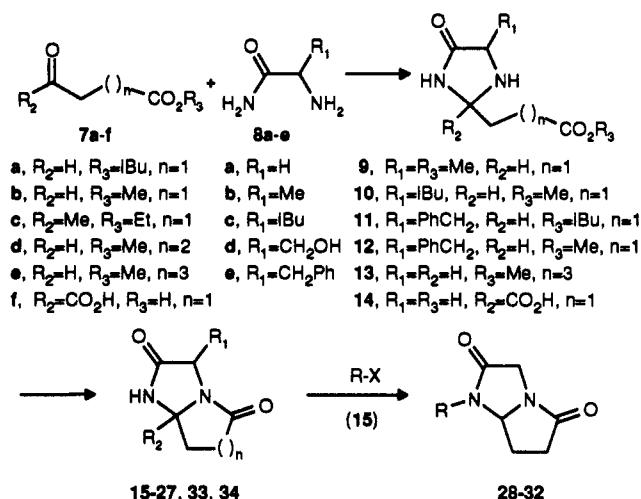
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Scheme I^a

^a Reagents: (a) NaH, toluene; (b) pyridinium chlorochromate, CH_2Cl_2 ; (c) H_2 , 5% Pd/C, EtOH.

Scheme II

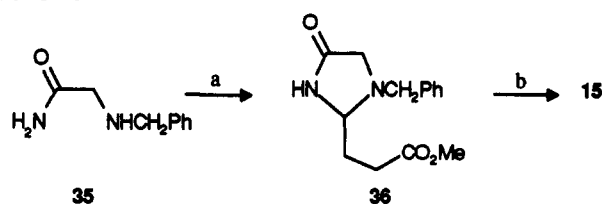


mide (L-8d) and of ethyl levulinate (7c) with 8a,b (Scheme II) gave directly the bicyclic compounds 15, 17–19, 21, and 24 (Table I) without isolation of the intermediate 4-imidazolidinones. The homologous piperidone derivative 33 was prepared from glycineamide (8a) and methyl 5-oxovalerate (7d). This method was very quick and straightforward, but yields were generally low (19–30%) and the reaction mixtures contained several side products from which the desired compounds were obtained by tedious chromatographic purifications. Moreover, when L-phenylalaninamide (L-8e) was used or ethyl 6-oxohexanoate (7e) was employed as the oxoester, only the 4-imidazolidinonic intermediates 11 and 13 could be obtained in low yields.

When the methyl ester 7b¹⁸ was used in place of the isobutyl ester 7a, the reaction did not occur, probably due to a faster degradation of 7b under the reaction conditions.

Thus, to overcome the above difficulties, a two-step synthesis was set up, involving condensation in refluxing methanol to the 4-imidazolidinone and subsequent cyclization. In this manner, L-alaninamide (L-8b), L-leucinamide (L-8c), and D-phenylalaninamide (D-8e) with 7b in methanol afforded exclusively the 4-imidazolidinones 9, 10, and 12, respectively. Generally, further cyclization of 4-imidazolidinones to the final compounds required drastic conditions and occurred with low yields: compounds 9 and 10 were cyclized to 16 and 20 by heating without solvent and under vacuum at 120 °C (method B) while compounds 11–13 were refluxed in xylene (method C) to give 22, 23, and 34, respectively.

Compounds 25, 26, and 27 (Table I) were obtained through the corresponding 4-oxoimidazolidinedicarboxylic acid 14, prepared, in turn, by reacting glycineamide (8a)

Scheme III^a

^a Reagents: (a) 7b, MeOH; (b) ammonium formate, 5% Pd/C; MeOH– H_2O .

with 2-oxoglutaric acid (7f). In this case, cyclization was accomplished by treatment with hexamethyldisilazane and trimethylchlorosilane.¹⁹ The obtained 7a-carboxy derivative 25 was converted into the ester 26 and finally into the corresponding amide 27. Compound 15 was alkylated under phase-transfer-catalysis conditions either with ethyl bromide or ethyl bromoacetate to give 28 and 29. The ethyl ester 29, treated with methanolic ammonia, yielded the corresponding amide 30. Compound 15 was also acylated either with acetic anhydride or benzyl chloroformate to afford the imides 31 and 32.

Since, during the continuation of our study, substantial amounts of compound 15 were required for biological and toxicological studies, and yields of the above described methods were not satisfactory for that purpose, an alternative synthesis was developed. We reasoned that the main causes of the low yields obtained with method A were the instability of the reagents under reaction conditions and the rather high basicity of glycineamide ($\text{pK}_\text{B} = 8.05$), leading to polymerization of the aldehyde group and/or hydrolysis of the ester group as well as the possibility of an equilibrium between the cyclic (imidazolidinone) and open (Schiff base) form. Therefore, we studied the reaction of the less basic and more stable 2-(benzylamino)acetamide²⁰ (35, $\text{pK}_\text{B} = 7.2$), with methyl 4-oxobutanoate (7b) and we found that it proceeds very smoothly in refluxing methanol, affording methyl 1-benzyl-4-oxo-2-imidazolidinepropanoate (36) in very good yields (Scheme III).

Surprisingly, when hydrogenolytic debenzylation was performed with ammonium formate as the hydrogen source, the desired bicyclic compound 15 was obtained directly in greater than 80% yield. Such an easy cyclization was most probably due to the catalytic action of ammonia produced (as ammonium carbonate) during the reaction; in fact, we confirmed that added ammonium hydroxide as well as other bases, such as triethylamine or 4-(dimethylamino)pyridine, were capable of accelerating the cyclization of the intermediate methyl 4-oxo-2-imidazolidinepropanoate (37, obtained from 36 by replacing ammonium formate with cyclohexene or hydrogen). The catalytic efficacy of ammonia in the intramolecular aminolysis was so strong that, even in the presence of a very large excess of ammonium hydroxide, no evidence of amide formation by ester ammonolysis was observed.

Pharmacology. Since the mechanism of action of 2-pyrrolidinone cognition enhancers is still under investigation, the search for new drugs in this area generally relies on behavioral tests,^{21,22} although their predictive validity has been debated.²³ Among them, the reversal of the experimentally-induced amnesia evaluated in a passive avoidance test has been widely used for screening purposes,^{24–26} and in particular, scopolamine-induced amnesia has been considered to mimic, in animals²⁷ and humans,^{28,29} the cholinergic impairment associated with

Table I. Substituted Dihydro-1*H*-pyrrolo[1,2-*a*]imidazole-2,5(3*H*,6*H*)-diones 15–32 and Homologues 33 and 34

no.	R	R ₁	R ₂	n	config at C-3	method (% yield)	mp (°C)	formula ^a	reversal (%) of scopolamine-induced amnesia ^b							
									mg/kg ip				mg/kg po			
									0.1	0.3	1.0	10	0.3	1.0	10	30
15	H	H	H	1	—	A (22.6) (or 81 ^c)	155–157	C ₈ H ₈ N ₂ O ₂		16	68	70	28	75	60	40
16	H	Me	H	1	<i>S</i> ^d	B (51.2)	129–132	C ₇ H ₁₀ N ₂ O ₂		0	52	3				
17	H	Me	H	1	<i>RS</i>	A (24)	84–86	C ₇ H ₁₀ N ₂ O ₂			38	38		9	33	
18	H	Me	Me	1	<i>S</i> ^e	A (19.1)	228–230	C ₈ H ₁₂ N ₂ O ₂		10	65	44		21	26	37
19	H	Me	Me	1	<i>RS</i>	A (22.8)	184–192	C ₈ H ₁₂ N ₂ O ₂		30	38	0		17	42	
20	H	iBu	H	1	<i>S</i> ^f	B (28)	156–157	C ₁₀ H ₁₆ N ₂ O ₂			3	0				
21	H	CH ₂ OH	H	1	<i>S</i> ^g	A (19)	150–162	C ₇ H ₁₀ N ₂ O ₃	3	30	38			0	42	0
22	H	CH ₂ Ph	H	1	<i>S</i> ^h	C (50)	141–145	C ₁₃ H ₁₄ N ₂ O ₂	0	53	53	0	0	42	0	7
23	H	CH ₂ Ph	H	1	<i>R</i> ⁱ	C (42.9)	134–138	C ₁₃ H ₁₄ N ₂ O ₂	15	42	19	0				
24	H	H	Me	1		A (21)	187–189	C ₇ H ₁₀ N ₂ O ₂		0	40	46			0	26
25	H	H	CO ₂ H	1		(50) ^c	207 dec	C ₇ H ₈ N ₂ O ₄			14	53		1	33	
26	H	H	CO ₂ Et	1		(49) ^c	116–117	C ₈ H ₁₂ N ₂ O ₄			25	62		0	0	
27	H	H	CONH ₂	1		(77) ^c	295 dec	C ₇ H ₈ N ₃ O		38	62	38		0	0	
28	Et	H	H	1		(57.3) ^c	53–56	C ₈ H ₁₂ N ₂ O ₂			0	0				
29	CH ₂ CO ₂ Et	H	H	1		(92) ^c	75–80	C ₁₀ H ₁₄ N ₂ O ₄			3	0				
30	CH ₂ CONH ₂	H	H	1		(75) ^c	182–185	C ₈ H ₁₁ N ₃ O ₃			49	62		0	9	
31	COMe	H	H	1		(78.4) ^c	68–72	C ₈ H ₁₀ N ₂ O ₃			0	0				
32	CO ₂ CH ₂ Ph	H	H	1		(29) ^c	120–121	C ₁₄ H ₁₄ N ₂ O ₄			31	12				
33	H	H	H	2		A (30)	170–174	C ₇ H ₁₀ N ₂ O ₂			27	27		0	0	
34	H	H	H	3		C (23)	175–176	C ₈ H ₁₂ N ₂ O ₂			22	12				
1b	oxiracetam										12	53			27	69

^a For all compounds, elemental analyses were within $\pm 0.4\%$ of the theoretical values. ^b Values reported in boldface are significantly different from those of the scopolamine group ($p < 0.05$) in the Fisher's exact probability test. For statistical calculations, see Experimental Section. ^c See Experimental Section for methodology. ^d $[\alpha]_D = +85.8$ ($c = 2$, MeOH). ^e $[\alpha]_D = +50.7$ ($c = 3$, water). ^f $[\alpha]_D = +35.5$ ($c = 1$, MeOH). ^g $[\alpha]_D = +10.3$ ($c = 1$, MeOH).

the aging of the brain and senile dementias.³⁰ Moreover, tacrine, which antagonizes the antiamnesic effect of scopolamine in the passive avoidance test,³¹ was shown, by means of extensive clinical trials, to be effective in patients with Alzheimer's disease.³²

Therefore, we evaluated the ability of new compounds to protect rats from scopolamine-induced amnesia in a one-trial passive avoidance test. All compounds were administered intraperitoneally and those showing a good profile in terms of potency were also tested orally. Most of them were also evaluated up to very high doses in the Irwin test on the mouse³³ to rule out in a preliminary way other important pharmacological or toxic effects.

Results and Discussion. The antiamnesic activity of all the new compounds is reported in Table I, in comparison to that of oxiracetam, assumed as the standard drug.

As far as the intraperitoneal administration is considered, most of the new compounds are able to reverse scopolamine-induced amnesia at a level of between 20 and 68% at 1 mg/kg whereas oxiracetam shows only a 12% reversal of this deficit at the same dose. The unsubstituted derivative 15 is active from the dose of 1 mg/kg; substitution at position 3, *i.e.* replacement of the glycine moiety with other natural amino acids such as alanine, serine and phenylalanine, in general slightly increases the potency. However, compounds 19, 21, 22, and 23 show a narrow, inverted U-shaped dose-response curve. An exception is the complete lack of activity, at the tested doses, of the derivative of L-leucine, 20. All the compounds bearing a substituent at position 3 are diastereoisomeric mixtures since the chiral center at 7a is formed during the reaction. Referring to the chirality at position 3, it does not seem to be of great importance for activity even if the comparison between compounds 22 and 23 (derivatives of L- and

D-phenylalanine, respectively) indicates that compounds derived from natural amino acids might have some advantages.

Substitution at position 1, *i.e.* alkylation or acylation of the imidazolidinone NH, generally removes activity with the exception of compound 30, which features the acetamide side chain typical of piracetam-like cognition enhancers. The effect of substitution at 7a was checked with the derivatives of levulinic acid 18, 19, and 24 and with those of 2-oxoglutaric acid, 25, 26, and 27; all the considered compounds exhibited some antiamnesic activity, but again, only the amide 27 showed an increased potency, being active from 0.3 to 10 mg/kg. The marked antiamnesic activity of 27 and 30 suggests that an increase in hydrophilic character, within certain limits, positively influences cognition-enhancing activity, as already observed for oxiracetam in comparison with the nonhydroxylated piracetam. This fact appears rather surprising for centrally active compounds. Also the importance of the 2-pyrrolidinone moiety has been confirmed: homologues 33 and 34, with enlarged six and seven-membered lactamic rings, completely lose antiamnesic activity.

When oral administration is considered it is readily apparent that while compound 15 maintains the activity over a wide dose range, any kind of substitution produces negative effects since either antiamnesic activity disappears or a very narrow, inverted U-shaped dose-response curve becomes evident. Such a reduction of activity after oral administration was frequently encountered during our work on cognition enhancers and this could not have been predicted purely on the basis of the chemical structure.

When compounds 15, 18, 19, 21, 22, 24–27, and 30 were submitted to the Irwin test,³³ no mortality or evident modification of gross behavior was noted. These results

Table II. Effect of Scopolamine and Compound 15 Plus Scopolamine in the Radial Maze in the Rat^a

treatment	no. of rats	day before treatment		day of treatment		day after treatment	
		efficiency in responding (%)	running time (s)	efficiency in responding (%)	running time (s)	efficiency in responding (%)	running time (s)
scopolamine ^b	21	87.3 ± 3.0	14.3 ± 0.9	63.9 ± 4.1 ^c	34.6 ± 6.4 ^c	83.9 ± 2.8	19.6 ± 3.9
15 (1 mg/kg sc) + scopolamine ^b	17	96.3 ± 1.7	16.6 ± 2.7	75.6 ± 4.1 ^{c,d}	34.6 ± 6.4 ^c	88.6 ± 4.3	19.6 ± 3.9

^a Values (reported as mean ± SEM) for the days preceding and following a pharmacological treatment were used as controls. ^b Scopolamine was given at the dose of 50 µg/kg sc. ^c *p* < 0.05 against control days. ^d *p* < 0.05 against scopolamine.

suggest good selectivity and a safe profile for this novel class of dihydro-1*H*-pyrrolo[1,2-*a*]imidazole-2,5(3*H*,6*H*)-diones.

On the whole, the unsubstituted derivative 15 exhibits a good bioavailability after oral administration and the highest potency/efficacy combination within the narrow, inverted U-shaped dose response-curve noticed with other cognition enhancers.^{9,25a} Moreover, it is practically devoid of any significant acute toxic effect.

To corroborate and better characterize the antiamnesic properties, compound 15, the most potent of the series, was evaluated with the same model of scopolamine-induced memory impairment in an eight-armed-radial-maze test.³⁴ This task provides measures of working memory which are known to be impaired in dementia. Compound 15 at 1 mg/kg partially antagonized the reduction of efficiency produced by scopolamine in solving the maze (Table II), while it was completely unable to counteract the increase of running time produced by scopolamine. This finding suggests that cholinergic mechanisms involved in learning and memory can be selectively enhanced without affecting motor behavior.

On these bases, 15 (dimiracetam) was selected for further studies and its evaluation will be extended to other multitrial behavioral tests on several animal models and to neurochemical investigations in order to confirm its suitability as a candidate cognition enhancing drug and to identify its exact mechanism of action.

Experimental Section

Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 197 spectrophotometer. Proton nuclear magnetic resonance spectra (NMR) were recorded on a Varian FT-80A spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a Varian MAT 112 instrument via direct inlet (EI, 70 eV, 1.5 mA). Elemental analyses were within 0.4% of the theoretical value for the indicated elements. Column chromatographies were performed with Merck 0.063–0.200 mm silica gel. Thin-layer chromatographies were performed on Merck 60 F 254 silica gel plates and revealed by UV or iodine vapors or by chlorine vapors followed by spraying with a KI-starch solution.

Starting α -amino amides 8a–e were either commercially available or were prepared from the corresponding α -amino acids as described in the literature.³⁵ Oxoesters 7c,f were commercially available while 7b,d,e were prepared according to the literature procedures from methyl 4-chloro-4-oxobutanoate,¹⁸ valerolactone,³⁶ and caprolactone,³⁷ respectively.

Chemistry. Isobutyl (*E*)-4-Hydroxy-2-butenate (5). To an ice-cold solution of isobutyl 3,4-epoxybutanoate¹⁷ (4; 300 g, 1.9 mol) in toluene (2.5 L) was added 55% sodium hydride in oil (3 g, 0.07 mol) portionwise. The solution was stirred at 0–5 °C for 1 h, then 55% sodium hydride (3 g, 0.07 mol) was added again. After stirring at room temperature for 1 h the solution was washed with brine (0.4 L) containing 10% hydrochloric acid (60 mL) and then twice with brine (300 mL each). The organic solution was dried over anhydrous sodium sulfate and evaporated to dryness. Distillation of the residue afforded 175 g (58.3%) of 5 as a colorless oil: bp 89–90 °C (0.5 mmHg); NMR (CDCl₃) δ

7.05 (dt, *J* = 15, 4 Hz, 1H, CH=CHCO), 6.15 (dt, *J* = 15, 2 Hz, 1H, CH=CHCO), 4.35 (m, 2H, CH₂OH), 3.95 (d, *J* = 6 Hz, 2H, CO₂CH₂), 2.20–1.75 (m, 1H, CH(CH₃)₂), 1.85 (br s, 1H, OH), 0.95 (d, *J* = 6 Hz, 6H, CH(CH₃)₂); MS *m/z* 127 (M – CH₂OH), 85 (M – C₃H₅O₂). Anal. (C₈H₁₄O₃) C, H.

Isobutyl (*E*)-4-Oxo-2-butenate (6). To a suspension of pyridinium chlorochromate (100 g, 0.463 mol) in dichloromethane (350 mL) was added a solution of isobutyl (*E*)-4-hydroxybutanoate (5; 50 g, 0.316 mol) in dichloromethane (150 mL). The internal temperature gradually rose to 40 °C and stirring was continued for 2 h without cooling. Diethyl ether (0.9 L) was added and the supernatant was decanted from the black gum. The insoluble residue was washed twice with 300-mL portions of diethyl ether. The combined organic solutions were filtered through a short pad of Florisil and the solvent was removed by distillation, to yield 45.3 g (91.6%) of 6 as a pale yellow oil: *R*_f = 0.5 (cyclohexane–ethyl acetate 6:4); NMR (CDCl₃) δ 9.80 (ABX, *J* = 6, 2 Hz, 1H, CHO), 6.98 and 6.75 (ABX, *J*_{AB} = 16 Hz, 2H, CH=CHCHO), 4.00 (d, *J* = 6 Hz, 2H, CO₂CH₂), 2.20–1.80 (m, 1H, CH(CH₃)₂), 0.95 (d, *J* = 6 Hz, 6H, CH(CH₃)₂); MS *m/z* 155 (M – H), 85 (M – C₃H₅O₂). Anal. (C₈H₁₂O₃) C, H.

Isobutyl 4-Oxobutanoate (7a). To a solution of isobutyl (*E*)-4-oxo-2-butenate (6; 97 g, 0.62 mol) in 96% ethanol (700 mL) was added 5% palladium on charcoal (9.7 g) and hydrogen was bubbled through the solution at 5–10 °C for 20 h. Removal of the catalyst and evaporation of the solvent gave an oil which was distilled through a short Vigreux column (90–95 °C, 3 mbar) to afford 66 g (67.3%) of 7a; *R*_f = 0.41 (cyclohexane–ethyl acetate 6:4; iodine vapors); NMR (CDCl₃) δ 9.85 (t, *J* = 1 Hz, 1H, CHO), 3.90 (d, *J* = 6 Hz, 2H, CO₂CH₂), 2.90–2.40 (m, 4H, CH₂CH₂), 2.25–1.75 (m, 1H, CH(CH₃)₂), 0.90 (d, *J* = 6 Hz, 6H, CH(CH₃)₂); MS *m/z* 101 (M – C₄H₉), 85 (M – C₃H₅O₂), 57 (M – C₄H₅O₃). Anal. (C₈H₁₄O₃) C, H.

General Procedure for the Synthesis of Alkyl 4-Oxo-2-imidazolidinealkanoates 9–13. To a mixture of α -amino amide hydrochloride 8 (0.1 mol) in methanol (100 mL) adjusted to pH 8.2 with 2 N NaOH in methanol was added oxoester 7 (0.1 mol) and the solution was refluxed for 2.5 h. After cooling, the solvent was evaporated under vacuum and the residue was chromatographed over silica gel (dichloromethane–methanol 9:1).

Methyl (2*RS*,5*S*)-5-methyl-4-oxo-2-imidazolidinepropanoate (9) was prepared, according to the general procedure, from L-alaninamide hydrochloride (12.5 g, 0.1 mol) and 7b (11.6 g, 0.1 mol) to afford 6.54 g (36%) of 9 as an oil, which was characterized as the hydrochloride: mp 136–142 °C dec (acetone–diisopropyl ether); [α]_D = +14.5 (c = 1, MeOH); NMR (DMSO-*d*₆) δ 10.75 (br s, 2H, NH₂⁺), 9.25 (br s, 1H, CONH), 4.97 and 4.87 (t, *J* = 5 Hz, 1H, NHCHNH), 4.3–3.9 (m, 1H, COCHNH), 3.62 (s, 3H, CO₂CH₃), 2.75–2.50 (m, 2H, CH₂CO₂), 2.40–1.85 (m, 2H, CHCH₂), 1.39 (d, *J* = 6 Hz, CHCH₃); MS *m/z* 186 (M), 171 (M – CH₃), 127 (M – CO₂CH₃).

Methyl (2*RS*,5*S*)-5-isobutyl-4-oxo-2-imidazolidinepropanoate (10) was prepared, according to the general procedure, from L-leucinamide hydrochloride (16.7 g, 0.1 mol) and 7b (11.6 g, 0.1 mol) to afford 3.88 g (17%) of 10 as an oil. The hydrochloride salt had mp 184–189 °C dec; [α]_D = –24.7 (c = 1.15, H₂O); NMR (DMSO-*d*₆) δ 10.8 (br s, 2H, NH₂⁺), 9.20 (br s, 1H, CONH), 4.95 and 4.85 (t, *J* = 6 Hz, 1H, NHCHNH), 4.20–3.90 (m, 1H, COCHNH), 3.64 (s, 3H, CO₂CH₃), 2.80–2.45 (m, 2H, CH₂CO₂), 2.40–2.00 (m, 2H, CHCH₂), 2.00–1.50 [m, 3H, CH₂CH(CH₃)₂], 0.94 [d, *J* = 5 Hz, 6H, CH(CH₃)₂]. MS *m/z* 228 (M), 197 (M – OCH₃), 141 (M – C₄H₇O₂).

Isobutyl (2*RS*,5*S*)-5-benzyl-4-oxo-2-imidazolidinepropanoate (11) was prepared, according to the general procedure, from L-phenylalaninamide hydrochloride (20 g, 0.1 mol) and **7a** (16 g, 0.1 mol) in isobutyl alcohol to afford **6 g** (20%) of **11**, as an oil, which was characterized as the hydrochloride: mp 152–155 °C dec (ethanol-diethyl ether); $[\alpha]_D = -40$ ($c = 0.125$, MeOH); NMR (DMSO- d_6 + CDCl $_3$) δ 11.0 (br s, 2H, NH $_2^+$), 9.25 (br s, 1H, CONH), 7.6–7.1 (m, 5H, PhH), 4.90 (t, $J = 6$ Hz, 1H, NHCHNH), 4.17 (t, $J = 6$ Hz, 1H, COCHNH), 3.84 (d, $J = 7$ Hz, 2H, CO $_2$ CH $_2$), 3.35 (m, 2H, CH $_2$ Ph), 2.50–1.60 (m, 5H, CH $_2$ CH $_2$ and CH(CH $_3$) $_2$), 0.90 (d, $J = 6$ Hz, 6H, CH(CH $_3$) $_2$); MS m/z 304 (M), 213 (M – C $_7$ H $_7$), 84 (C $_3$ H $_4$ N $_2$ O).

Methyl (2*RS*,5*R*)-5-benzyl-4-oxo-2-imidazolidinepropanoate (12) was prepared, according to the general procedure, from D-phenylalaninamide hydrochloride (5 g, 25 mmol) and **7b** (2.9 g, 25 mmol) to afford 4.26 g (65%) of **12**, as an oil: $[\alpha]_D = +21.5$ ($c = 2$, MeOH); NMR (CDCl $_3$) δ 7.25 (s, 5H, PhH), 7.20 (br s, 1H, CONH), 4.65 and 4.42 (t, $J = 5$ Hz, 1H, NHCHNH), 4.00–3.50 (m, 1H, COCHNH), 2.65 and 2.62 (s, 3H, CO $_2$ CH $_3$), 3.25–2.75 (m, 2H, PhCH $_2$), 2.5–2.20 (m, 2H, CH $_2$ CO $_2$), 2.13 (br s, 1H, NH), 2.0–1.7 (m, 2H, CHCH $_2$); MS m/z 175 (M – C $_4$ H $_7$ O $_2$), 171 (M – C $_7$ H $_7$).

Methyl 4-oxo-2-imidazolidinepentanoate (13) was prepared, according to the general procedure, from glycineamide hydrochloride (6.73 g, 61 mmol) and methyl 6-oxohexanoate³⁶ (**7e**; 8.8 g, 61 mmol) to afford 3.95 g (32.5%) of **13**: mp 58–60 °C; NMR (DMSO- d_6) δ 8.15 (br s, 1H, CONH), 4.40 (m, 1H, NHCHNH), 3.60 (s, 3H, CO $_2$ CH $_3$), 3.10 (s, 2H, COCH $_2$ NH), 2.45–2.15 (m, 2H, CH $_2$ CO $_2$), 1.75–1.10 (m, 6H, CH $_2$ CH $_2$ CH $_2$); MS m/z 201 (M + H), 169 (M – OCH $_3$), 141 (M – C $_2$ H $_3$ O $_2$).

Method A. Dihydro-1*H*-pyrrolo[1,2-*a*]imidazole-2,5-(3*H*,6*H*)-dione (15). To a solution of glycineamide hydrochloride (**8a**; 18.4 g, 0.166 mol) in water (200 mL), adjusted to pH 9.5 with 10% sodium hydroxide (about 60 mL) was added **7a** (22.2 g, 0.14 mol). The solution was refluxed for 20 h. Water was evaporated under vacuum and the residue was chromatographed over silica gel (dichloromethane-methanol 9:1) to afford 4.44 g (22.6%) of **15**: mp 155–157 °C; NMR (DMSO- d_6) δ 8.65 (br s, 1H, CONH), 5.20 (t, $J = 6$ Hz, 1H, NCHNH), 3.80 and 3.40 (AB q, $J = 15$ Hz, 2H, COCH $_2$ NH), 2.85–1.55 (m, 4H, CH $_2$ CH $_2$); MS m/z 140 (M), 97 (M – CONH).

Method B. (3*S*,7*aRS*)-Dihydro-3-methyl-1*H*-pyrrolo[1,2-*a*]imidazole-2,5-(3*H*,6*H*)-dione (16). Compound **9** (4.85 g, 0.026 mol) was heated at 120 °C under vacuum (20–30 mmHg) for 5 h. The crude residue was chromatographed over silica gel (dichloromethane-methanol 9:1), the appropriate fractions were evaporated and the residue was crystallized from acetone-diisopropyl ether to afford 2.05 g (51.2%) of **16** as a white powder: mp 129–132 °C; $[\alpha]_D = +85.8$ ($c = 2$, MeOH); NMR (CDCl $_3$) δ 8.02 (br s, 1H, CONH), 5.35 (t, $J = 5$ Hz, 1H, NCHNH), 4.30 (q, $J = 8$ Hz, 1H, NCHCO), 2.9–1.8 (m, 4H, CH $_2$ CH $_2$), 1.38 (d, $J = 8$ Hz, 3H, CHCH $_3$); MS m/z 154 (M), 139 (M – CH $_3$), 111 (M – CHNO), 98 (M – C $_3$ H $_4$ O).

Method C. (3*R*,7*aRS*)-3-Benzyl-dihydro-1*H*-pyrrolo[1,2-*a*]imidazole-2,5-(3*H*,6*H*)-dione (23). A solution of **12** (3.2 g, 12.2 mmol) in xylene (120 mL) was refluxed for 3 days. After evaporation, the crude residue was flash chromatographed over silica gel (dichloromethane-methanol 95:5). The collected fractions were evaporated, and the residue was triturated with diisopropyl ether to afford 1.2 g (42.9%) of **23**: mp 134–138 °C, $[\alpha]_D = -97.3$ ($c = 2$, MeOH); NMR (CDCl $_3$) δ 7.75 (br s, 1H, CONH), 7.25 (br s, 5H, PhH), 4.65 (t, $J = 4.5$ Hz, 1H, NCHCO), 4.45 (t, $J = 5$ Hz, 1H, NCHNH), 3.15 (d, $J = 4.5$ Hz, 2H, PhCH $_2$), 2.8–1.6 (m, 4H, CH $_2$ CH $_2$); MS m/z 230 (M), 139 (M – C $_7$ H $_7$).

2-Carboxy-4-oxo-2-imidazolidinepropanoic Acid (14). A solution of 2-oxoglutaric acid (10 g, 68.4 mmol), glycineamide hydrochloride (8.3 g, 75.2 mmol), and sodium hydroxide (8.2 g, 205.2 mmol) in water (120 mL) was refluxed for 4 h. After cooling, the solution was adjusted to pH 2.5 and the resulting precipitate was collected and dried under vacuum at 60 °C to afford 5.9 g (43%) of **14**: mp 202–205 °C dec; NMR (DMSO- d_6) δ 8.5 (s, 1H, CONH), 7.00–4.00 (br s, 3H, NH, CO $_2$ H), 3.22 and 3.18 (AB q, $J = 16$ Hz, 2H, COCH $_2$ NH), 2.40–1.75 (m, 4H, CH $_2$ CH $_2$); MS m/z 140 (M – H $_2$ O – CO $_2$), 84 (C $_3$ H $_4$ N $_2$ O).

2,5-Dioxohexahydro-1*H*-pyrrolo[1,2-*a*]imidazole-7*a*-carboxylic Acid (25). A mixture of **14** (2 g, 9.89 mmol), hexa-

methyldisilazane (20 mL), and trimethylchlorosilane (10 mL) in dry acetonitrile (50 mL) was refluxed under nitrogen for 4 h. After cooling, the precipitate was filtered off and the filtrate was evaporated under vacuum. The residue was dissolved in methanol (20 mL) containing a few drops of concentrated hydrochloric acid and stirred for 10 min. The insoluble material was filtered off and the filtrate was evaporated to dryness. The residue was triturated with acetonitrile and crystallized with tetrahydrofuran (250 mL) to yield 0.9 g (50%) of **25**: mp 207 °C dec; NMR (DMSO- d_6) δ 13.0 (br s, 1H, CO $_2$ H), 9.15 (br s, 1H, CONH), 3.82 and 3.46 (AB q, $J = 16$ Hz, 2H, NCH $_2$ CO); 2.9–1.8 (m, 4H, CH $_2$ CH $_2$); MS m/z 184 (M), 139 (M – CO $_2$ H), 83 (C $_3$ H $_3$ N $_2$ O).

Ethyl 2,5-dioxohexahydro-1*H*-pyrrolo[1,2-*a*]imidazole-7*a*-carboxylate (26). A suspension of **25** (0.8 g, 4.34 mmol) in dry tetrahydrofuran (100 mL) was cooled to 0 °C and treated with oxalyl chloride (0.55 g, 4.34 mmol) and a drop of dimethylformamide. After stirring for 2 h at 0 °C, the resulting solution was allowed to reach room temperature and vacuum was applied for 10 min. After cooling to 0 °C, 4-(dimethylamino)pyridine (0.53 g, 4.34 mmol) and dry ethanol (2 mL) were added. The suspension was stirred at 0 °C for 30 min and at room temperature for 30 min. The precipitate was filtered off and the filtrate was evaporated under vacuum. The residue was chromatographed over silica gel (ethyl acetate-methanol 95:5) to afford 0.45 g (49%) of **26**, mp 116–117 °C; NMR (DMSO- d_6) δ 9.22 (br s, 1H, CONH), 4.16 (q, $J = 7.5$ Hz, 2H, CH $_2$ CH $_3$), 3.85 and 3.48 (AB q, $J = 15$ Hz, 2H, NCH $_2$ CO), 2.95–2.05 (m, 4H, CH $_2$ CH $_2$), 1.2 (t, $J = 7.5$ Hz, 3H, CH $_2$ CH $_3$); MS m/z 183 (M – Et), 139 (M – CO $_2$ Et), 83 (C $_3$ H $_3$ N $_2$ O).

2,5-Dioxohexahydro-1*H*-pyrrolo[1,2-*a*]imidazole-7*a*-carboxamide (27). An ice-cold solution of **26** (2.55 g, 12 mmol) in dry methanol (20 mL) was treated with a saturated solution of ammonia in methanol (40 mL) and stirred for 1 h at 0 °C. The precipitate was collected, washed with acetone, and dried to afford 1.7 g (77%) of **27**: mp 295 °C dec; NMR (DMSO- d_6) δ 9.05 (br s, 1H, CONH), 7.50 (br s, 2H, CONH $_2$), 3.80 and 3.50 (AB q, $J = 15$ Hz, 2H, NCH $_2$ CO), 2.85–1.95 (m, 4H, CH $_2$ CH $_2$); MS m/z 139 (M – CONH $_2$), 83 (C $_3$ H $_3$ N $_2$ O).

Dihydro-1-ethyl-1*H*-pyrrolo[1,2-*a*]imidazole-2,5-(3*H*,6*H*)-dione (28). A mixture of **15** (4 g, 28.5 mmol), tetrabutylammonium bromide (4.56 g, 14.1 mmol), and potassium carbonate (20 g, 144.7 mmol) in dry acetonitrile (50 mL) was stirred at room temperature for 1 h. Ethyl bromide (2.3 mL, 30.8 mmol) was added and the suspension was heated at 60 °C for 2.5 h. The precipitate was filtered off, the filtrate was evaporated under vacuum and the residue was chromatographed over silica gel (dichloromethane-methanol 9:1). The appropriate fractions were evaporated under vacuum, and the residue was triturated with light petroleum to afford 2.75 g (57.3%) of **28**: mp 53–56 °C; NMR (CDCl $_3$) δ 5.25 (t, $J = 5$ Hz, 1H, NCHN), 4.20 and 3.55 (AB q, $J = 16$ Hz, 2H, NCH $_2$ CO), 3.40 (m, 2H, CH $_2$ CH $_3$), 2.80–1.75 (m, 4H, CH $_2$ CH $_2$), 1.15 (t, $J = 7.5$ Hz, 3H, CH $_2$ CH $_3$); MS m/z 168 (M), 139 (M – C $_2$ H $_5$), 112 (M – C $_3$ H $_4$ O).

Ethyl 2,5-Dioxohexahydro-1*H*-pyrrolo[1,2-*a*]imidazole-1-acetate (29). A mixture of **15** (0.5 g, 3.57 mmol), tetrabutylammonium bromide (0.57 g, 1.77 mmol), and potassium carbonate (2.5 g, 18.1 mmol) in dry acetonitrile (6 mL) was stirred at room temperature for 1 h. Ethyl bromoacetate (0.5 mL, 4.5 mmol) was added and the suspension was heated at 60 °C for 2.5 h. The precipitate was filtered off, the filtrate was evaporated under vacuum, and the residue was chromatographed over silica gel (ethyl acetate-acetone-methanol 6:3:1) to afford 0.7 g (92%) of **29**: mp 75–80 °C; NMR (CDCl $_3$) δ 5.4 (m, 1H, NCHN), 4.21 (q, $J = 7$ Hz, 2H, CH $_2$ CH $_3$), 4.32 and 3.68 (AB q, $J = 16$ Hz, 2H, NCH $_2$ CO), 4.30 and 3.80 (AB q, $J = 18$ Hz, 2H, NCH $_2$ CO $_2$), 2.8–1.7 (m, 4H, CH $_2$ CH $_2$), 1.28 (t, $J = 7$ Hz, 3H, CH $_2$ CH $_3$); MS m/z 226 (M), 153 (M – CO $_2$ Et), 139 (M – CH $_2$ CO $_2$ Et).

2,5-Dioxohexahydro-1*H*-pyrrolo[1,2-*a*]imidazole-1-acetamide (30). A solution of **29** (1.4 g, 6.18 mmol) in methanol (25 mL) was saturated with ammonia at 0 °C. After stirring at room temperature for 16 h the precipitate was collected, washed with methanol, and dried to yield 0.9 g (75%) of **30**: mp 182–185 °C; NMR (DMSO- d_6) δ 7.50 and 7.10 (br s, 2H, CONH $_2$), 5.25 (m, 1H, NCHN), 3.94 and 3.55 (AB q, $J = 16$ Hz, 2H, NCH $_2$ CO); 3.85 and 3.70 (AB q, $J = 16.5$ Hz, 2H, NCH $_2$ CONH $_2$); 2.90–1.90 (m, 4H, CH $_2$ CH $_2$); MS m/z 139 (M – CH $_2$ CONH $_2$).

1-Acetyldihydro-1H-pyrrolo[1,2-a]imidazole-2,5(3H,6H)-dione (31). A solution of 15 (4 g, 28.5 mmol) in acetic anhydride (20 mL) was refluxed for 2 h. After cooling, the mixture was evaporated and the residue chromatographed over silica gel (ethyl acetate–acetone–methanol 6:3:1), and the appropriate fractions were evaporated under vacuum. The residue was triturated with diethyl ether to afford 4 g (78.4%) of 31: mp 68–72 °C; NMR (CDCl₃) δ 5.67 (t, J = 5 Hz, 1H, NCHN), 4.45 and 3.76 (AB q, J = 17.5 Hz, 2H, NCH₂CO), 3.20–1.75 (m, 4H, CH₂CH₂), 2.50 (s, 3H, CH₃); MS m/z 182 (M), 154 (M – CO), 139 (M – COCH₃).

1-[(Benzoyloxy)carbonyl]dihydro-1H-pyrrolo[1,2-a]imidazole-2,5(3H,6H)-dione (32). To a suspension of 15 (7 g, 50 mmol) in dry acetonitrile (140 mL) cooled to 0–5 °C and maintained under a nitrogen atmosphere was added 55% sodium hydride in oil (2.4 g, 55 mmol) portionwise during 15 min. After 2.5 h at 0–5 °C, a solution of benzyl chloroformate (7.8 mL, 55 mmol) in dry acetonitrile (24 mL) was added dropwise and the mixture was stirred at room temperature for 2 h and at 40 °C for 30 min. After cooling, the mixture was evaporated and the residue, diluted with ethyl acetate (200 mL), was washed twice with water (100 mL). The organic layer was dried and evaporated under vacuum and the residue was triturated with diethyl ether and crystallized from ethanol to yield 4 g (29%) of 32: mp 120–121 °C; NMR (CDCl₃) δ 7.37 (s, 5H, PhH), 5.57 (t, J = 5 Hz, 1H, NCHN), 5.30 (s, 2H, CH₂Ph), 4.35 and 3.68 (AB q, J = 17 Hz, 2H, NCH₂CO), 3.0–1.9 (m, 4H, CH₂CH₂); MS m/z 274 (M), 168 (M – C₇H₆O).

Methyl 1-Benzyl-4-oxo-2-imidazolidinepropanoate (36). To a solution of 2-(benzylamino)acetamide²⁰ (35, 67.9 g, 0.41 mol) in methanol (500 mL) was added 7b (60.3 g, 0.52 mol). The mixture was refluxed for 3 h and most of the solvent was evaporated under vacuum. On addition of water (435 mL) and stirring at 0–5 °C for 1 h, the precipitate was collected by filtration and dried at 40 °C under vacuum to afford 91.7 g (85.3%) of 36 as a yellow solid: mp 84–85 °C; NMR (CDCl₃) δ 7.80 (br s, 1H, CONH), 7.30 (s, 5H, PhH), 4.38 (ABX, J_{AX+BX} = 3 Hz, 1H, NHCHN), 4.01 and 3.53 (AB q, J = 13 Hz, 2H, CH₂Ph), 3.65 (s, 3H, CH₃), 3.37 and 3.01 (ABX, J_{AB} = 15 Hz, 1H, NCH₂CO), 2.60–2.25 (m, 2H, CH₂CO₂), 2.15–1.75 (m, 2H, CHCH₂); MS m/z 175 (M – C₄H₇O₂), 91 (C₇H₇).

Dihydro-1H-pyrrolo[1,2-a]imidazole-2,5(3H,6H)-dione (15). A mixture of 36 (100 g, 0.38 mol), ammonium formate (40.9 g, 0.65 mol), and 5% palladium on charcoal (10 g) in water (112 mL) and methanol (400 mL) was heated at reflux for 3 h. After cooling, the catalyst was filtered off through a Celite pad and the solution was evaporated under vacuum. The residue was dissolved in water (200 mL) and treated batchwise with the ion exchangers Amberlite IR 120 (15 mL) and Amberlite IRA 68 (9 mL) and charcoal (3 g). After filtration the aqueous solution was diluted with 1-butanol (600 mL), and water was azeotropically removed under vacuum. The residue was crystallized from 2-propanol to afford 43.2 g (81%) of 15 as a white crystalline solid, mp 155–157 °C.

Methyl 4-Oxo-2-imidazolidinepropanoate Hydrochloride (37). A mixture of 36 (25 g, 95.3 mmol), water (3.7 mL), and 10% palladium on charcoal (3.75 g) in cyclohexene (250 mL) and 2-propanol (125 mL) was refluxed for 2 h. After cooling, the catalyst was filtered off and the solvent was evaporated under vacuum. The residue was dissolved in methanol and treated with 4 N HCl/MeOH. The precipitate was collected and crystallized from MeOH to afford 11.3 g (56.8%) of 37: mp 161–162 °C; NMR (DMSO-*d*₆) δ 10.75 (br s, 2H, NH₂⁺), 9.25 (br s, 1H, CONH), 5.00 (t, J = 5.5 Hz, 1H, NHCHNH), 3.65 and 3.55 (AB q, J = 15 Hz, 2H, NHCH₂CO), 3.60 (s, 3H, CO₂CH₃), 2.75–2.35 (m, 2H, CH₂CO₂), 2.30–1.90 (m, 2H, CHCH₂); MS m/z 172 (M), 157 (M – CH₃), 141 (M – OCH₃).

Pharmacology. Passive Avoidance Test. Male Wistar rats (Charles River) weighing 200–220 g were housed three per cage and maintained in the animal house for at least 1 week before the experiment. The experimental procedure and the apparatus were similar to those previously described.^{9,38} The passive avoidance apparatus consisted of a two-compartment plexiglass box with an electrifiable grid floor. The transparent side (25 × 25 × 25 cm, lit by a 60-W lamp) was connected with the dark side (painted black and covered by a removable lid) by a guillotine-type door. During the learning trial, the rat was gently placed

in the illuminated compartment. As soon as the rat entered the dark side of the apparatus, the door was closed and an inescapable foot shock (1 mA for 1 s) was delivered through the grid floor. Immediately afterward the rat was put back into its home cage. Twenty-four hours later the rat was again placed in the lighted side of the box with the door open and if the animal did not step into the dark compartment within 120 s (cutoff time) it was considered to have learned the task. The group's percentage of animals that did not enter the dark compartment was taken as an index of learning (percent of retention).

Animals received scopolamine hydrobromide (0.66 mg/kg sc) or saline 60 min before the learning session and the compounds tested or the vehicle 30 min before scopolamine.

In each experiment 45 rats were randomly assigned to three groups of treatment: (1) a saline control group injected with saline 90 and 30 min before the learning session; (2) a scopolamine group treated with saline and scopolamine to evaluate the degree of amnesia produced by scopolamine in that particular experiment, and (3) a treated group injected with the tested compound and scopolamine.

Typically, 80–90% of saline-treated animals remained in the lighted compartment more than 120 s while only 20–30% of scopolamine-treated animals remembered the task. When the difference between saline and scopolamine control groups was less than 40%, the entire experiment was discarded. Since there were some day-to-day and seasonal variability in the percent of retention of saline and scopolamine groups, in order to make comparisons between the effect of treatments, the results were expressed as percent of amnesia reversal employing the following formula:^{25a}

% amnesia reversal =

$$\frac{\% \text{ retention of treated group} - \% \text{ retention of scopolamine group}}{\% \text{ retention of saline group} - \% \text{ retention of scopolamine group}}$$

× 100

Data were analyzed as percent of retention by means of Fisher's exact probability test³⁹ by comparing each treated group with the scopolamine control group in the same experiment. The results obtained are reported in Table I.

Irwin Test. The action of new compounds on gross behavior was tested in mice according to the method of Irwin.³³ Briefly, the compounds were administered at the doses of 100, 300, and 1000 mg/kg ip (three animals per dose were used). The mice were observed continuously for 3 h after treatment and once a day for the following 3 days during which 30 behavioral, neurological, and autonomic signs were assessed and scored as described in the literature. Neither lethality nor pharmacological effects were observed for any of the compounds up to the highest dose tested.

Eight-Armed Radial Maze. Twenty-one male Wistar rats, 2-months-old and weighing 250 g at the beginning of experiments, were used. The rats were gradually reduced to 80–85% of their body weight and trained once a day for 5 days a week in a eight-armed radial maze until they solved the task in no more than nine choices for at least 3 consecutive days (criterion). Training was carried out in a testing room lit by two 70-W halogen lamps. Several visually distinct cues (e.g. door, shelf, posters) were present in the room and remained in the same position with respect to the maze. A television camera was located 200 cm above the maze and transmitted the image to both a closed-circuit television and to an interfaced IBM AT computer located in an adjacent room. A rat's position in the maze was then determined by means of a computerized motion analyzer program (Biomedica Mangoni, Pisa, Italy) and was automatically elaborated and stored on a floppy disk. The following measures of the rat's performance in the maze were taken into account for each trial: (i) efficiency of responding, defined as 8/total number of choices × 100 and (ii) mean running time (in s) obtained by dividing the total running time (the total time spent by the rat to complete the task) by the total number of choices.³⁴

Drug treatments (50 µg/kg sc scopolamine and 1 mg/kg sc compound 15 plus 50 µg/kg sc scopolamine) were begun after the performance had been established. Drugs were dissolved in isotonic saline so that test compound was administered in a volume of 2 mL/kg. Scopolamine was injected 60 min before

testing; 15 was administered 90 min before the test. The tests on the drugs were separated by at least 3 days, during which the rats were trained again to criterion. Seventeen rats received each of the two treatments in a random sequence: the four remaining animals received only scopolamine treatment.

Performance in the days immediately preceding and following a pharmacological treatment was used for comparison. The values for efficiency for each given treatment were compared with each other and with values obtained in the days preceding and following the treatment by means of the one-way ANOVA test. Paired comparisons were subsequently made using Duncan's test for multiple comparison. Values for running time were analyzed in a similar manner.

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