Synthesis and Cognition Activating Properties of Some Mono- and Bicyclic Lactam Derivatives†

Cosimo Altomare,‡ Angelo Carotti,*,‡ Giovanni Casini,*,‡ Saverio Cellamare,‡ Marcello Ferappi,*,‡ Enrico Gavuzzo,§ Fernando Mazza,§ Giancarlo Pantaleoni,∥ and Raffaele Giorgi∥

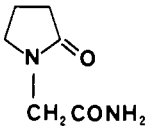
Dipartimento Farmaco-Chimico, University of Bari, Bari, Italy, Istituto di Strutturistica Chimica, CNR, Monterotondo Stazione, Roma, Italy, and Cattedra di Farmacologia, University of L'Aquila, L'Aquila, Italy. *Received February 8, 1988*

Upon reductive cyclization cyano esters **2**, **3**, and **9** yielded piperidones and perhydropyrrolo[3,4-*c*]pyridine lactams, generally as a mixture of diasteromeric cis–trans forms. X-ray crystallographic analyses were carried out on bicyclic dilactam derivatives **6** and **10**, and a cis configuration at the ring junction was determined in both cases. A series of neuropsychopharmacological tests performed on the title compounds indicated that they are generally nontoxic even at high doses (up to 1000 mg/kg ip). The cognition activating properties of lactams **4**, **5**, **6**, and **10** were evaluated in enhancing retention for passive avoidance learning in rats without and after electroconvulsive shock (ECS); compounds **5** and **10** were found to be more potent than piracetam in the amnesia-reversal testing.

It has been recently established that the age- and disease-related short-term memory and learning impairments may be casually connected to loss and/or degeneration of neurotransmitter-specific neurons in such anatomical memory circuits as the base forebrain and the septo–hippocampal–entorhinal areas of the brain.[1] Loss of short-term, or recent, memory, is now recognized as an inevitable manifestation of aging (benign senescent forgetfulness), senile dementia, and primary degenerative dementia (Alzheimer's disease).[2-4]

The development of effective drugs for treatment of the cognitive disorders is thus a real and urgent problem today due to the continuous rise in the average longevity and to the consequent increase of the number of elderly persons who need appropriate medical care.[5] Many comprehensive and useful reviews have been written on the subject and the effects of several drugs for the treatment of cognitive impairments have been reported.[3,4,6] Objective and real benefits however have been rarely proved yet, and for this reason the therapeutic efficiency of most drugs in the market has been questioned.[3,7,8]

Thus search for new drugs that do improve the cognition functions has been undertaken and a relatively new class of compounds, namely nootropics, has been developed on the basis of the significant improvement in learning and memory models observed in a number of behavioral paradigms for piracetam (**1**), the prototype of this class of



1

drugs.[9] The main features defining a nootropic drug are (1) the enhancement of learning acquisitions as well as the resistance of learned behaviors to agents that tend to impair them; (2) the partial enhancement of the general resistance of the brain and particularly its resistance to physical injuries; (3) the increase in the efficacy of the tonic corticosubcortical control mechanism.[10]

The mechanism of action at the molecular level of the nootropic agents is not completely delineated yet; however, recently it was suggested that the pharmacological effects of these drugs could involve functional activation of the cholinergic network.[3,11] It has been established, in fact, that acetylcholine deficiency plays an important role in the etiology of Alzheimer's disease.[12] Piracetam, moreover, was also proved to increase the ratio ATP/ADP, suggesting a direct metabolic effect in the brain,[13] whereas oxiracetam, the cyclic GABOB analogue, and to a lesser extent piracetam itself, have been claimed to stimulate both phospholipids and brain proteins synthesis.[14]

Most compounds described as nootropic agents are 2-pyrrolidone derivatives[15] whereas, more recently, 3-substituted 2-piperazinones,[16] substituted pyridine 1-oxides,[17] and cyclic imides[18] have been described as new chemical

† This paper has been presented in part at the French–Italian Joint Meeting on Medicinal Chemistry, Pisa, Italy, Sept 22–26, 1987, Abstr p 63.

‡ University of Bari.

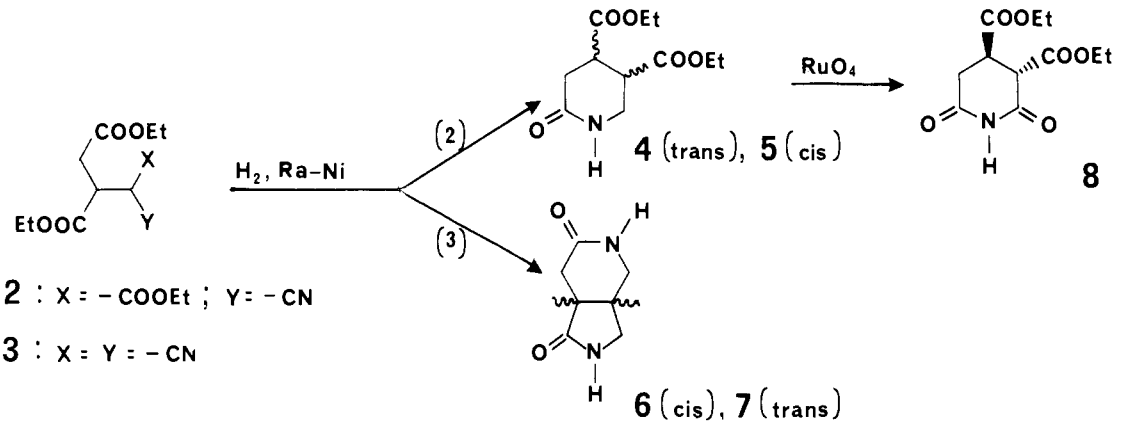
§ Istituto di Strutturistica Chimica.

∥ University of L'Aquila.

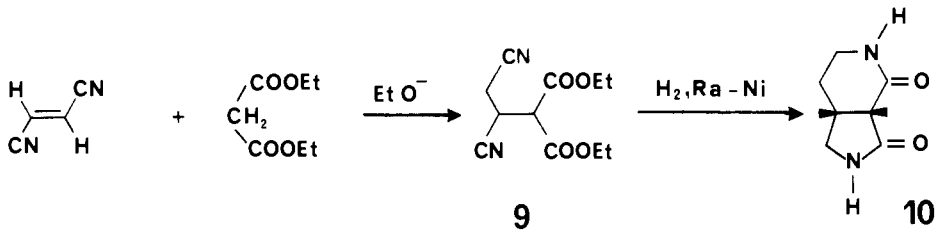
(1) Ordy, J. M. Proceedings of the 191st National Meeting of the American Chemical Society, New York, 1986, Abstr. 17.
(2) Fisher, C. M. *Lancet* **1985**, *i*, 173.
(3) Hershenson, F. M.; Moos, W. H. *J. Med. Chem.* **1986**, *29*, 1125.
(4) Schindler, V.; Rush, D. K.; Fieldings, S. *Drug Dev. Res.* **1984**, *4*, 567.
(5) Busby, J.; Bonelli, A.; Vargas, L.; Stirna, J.; Caranasos, G. *J. Am. Geriatr. Soc.* **1985**, *33*, 366.
(6) See for example: Olton, D. S.; Gamzu, E.; Corkin, S., Eds. *Ann. N.Y. Acad. Sci.* **1985**, 444. Roth, M.; Iversen, L. L. Eds. *Br. Med. Bull.* **1986**, 42 (1). Hershenson, F. M.; Marriott, J. G.; Moos, W. H. *Annu. Rep. Med. Chem.* **1986**, *21*, 31; *Drugs Future* **1985**, *10*, 972, 988.
(7) *Med. Lett.* **1976**, *18*(9), 38.
(8) *Med. Lett.* **1977**, *19*(5), 61.
(9) Di Janni, N.; Wilsher, G. R.; Blank, M. S.; Conners, C. K.; Chase, C. H.; Funkestein, H. H.; Helfgott, E.; Holmes, J. M.; Lougee, L.; Maletta, G. J.; Milewski, J.; Pirozzolo, F. J.; Rudel, R. G.; Tallal, P. *J. Clin. Psychopharmacol.* **1985**, *5*, 272. Skodia, V.; Kabes, J. *J. Int. Med. Res.* **1985**, *13*, 185. Sato, M.; Heiss, W. D. *Arzneim. Forsch.* **1985**, *35*, 790.
(10) Giurgea, C.; Salama, M. *Prog. Neuro-Psychopharmacol.* **1977**, *1*, 235.
(11) Pinza, M.; Farina, C.; Pfeiffer, U.; Banfi, S.; Dorigotti, L. Proceedings of the IX International Symposium on Medicinal Chemistry, Berlin, 1986, p 96.
(12) Gohfries, C. G. *Psychopharmacology* **1985**, *86*, 245.
(13) Pede, J. P.; Shimpfessel, L; Crokoert, R. *Arch. Int. Physiol. Biochem.* **1971**, *79*, 1036.
(14) Pellegata, R.; Pinza, M.; Pifferi, G.; Gaiti, A.; Mozzi, R.; Tirillini, B.; Porcellati, G. *Farmaco, Ed. Sci.* **1981**, *36*, 845.
(15) See, for example: Butler, D. E.; Nordin, I. C.; L'Italien, Y. J.; Eweisler, L.; Poschel, B. P. H.; Marriott, J. G. *J. Med. Chem.* **1984**, *27*, 684. Poschel, B. P. H.; Marriott, J. G.; Gluckman, M. I. *Drugs Exp. Clin. Res.* **1983**, *9*, 853. Popova, R. Y.; Gudasheva, T. A.; Trofimov, S. S.; Ostrovkaya, R. V.; Skoldinov, A. P. *Khim. Farm. Zh.* **1983**, *17*, 1439. Ferrero, E. *Curr. Ther. Res.* **1984**, *36*, 298.
(16) Schindler, V.; Beverle, R.; Nitz, R. E. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1985**, 330.
(17) Greve, W.; Elben, V.; Rudolphi, K.; Schindler, U. Ger. Offen. DE 3514073, 1985.
(18) Butler, D. E.; Leonard, J. D.; Caprathe, B. W.; L'Italien, Y. J.; Pavia, M. R.; Hershenson, F. M.; Poschel, B. P. H.; Marriott, J. G. *J. Med. Chem.* **1987**, *30*, 498.

0022-2623/88/1831-2153$01.50/0

Scheme I



Scheme II



entities possessing potent cognition-activating properties.

These interesting findings prompted us to investigate the amnesia-reversal activity of some mono- and bicyclic lactam compounds that could be easily prepared by using as starting material the same versatile and simple cyano esters **2**, **3**, and **9** easily convertible also into cyclic imides as previously reported by us.[19]

Chemistry

Lactams **4–7** and **10** have been prepared through the reductive cyclization reaction of cyano esters **2**, **3**, and **9** by H_2 with Ra Ni as catalyst (Schemes I and II).[20]

Compound **2** gave a mixture of two isomeric monolactams **4** and **5** (molar ratio 2:1), which could be, in principle, diastereoisomers, as well as ring isomers (5-membered vs 6-membered lactams) as has previously been observed in a patent preparation of some lactam dicarboxylic acids.[21,22]

Since spectroscopic data (IR and ^1H NMR) did not allow a clear discrimination between these two possibilities, both products were oxidized with RuO_4, a reagent that easily and selectively transforms methylene groups, adjacent to nitrogen atom, to carbonyl groups.[23-25] In both cases the same and already known *trans*-4,5-dicarbethoxyglutarimide (**8**)[19] has been obtained, and this result clearly proved that compounds **4** and **5** were diastereomeric 2-piperidones and that, apparently, the racemization of one chiral center, most likely the malonic one generated upon oxidation, took place.

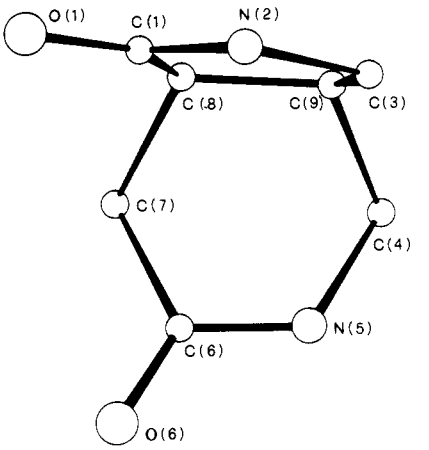


Figure 1. A stereochemical view of *cis*-perhydropyrrolo[3,4-*c*]-pyridine-1,6-dione (**6**).

The configurational assignment to both 2-piperidones has been achieved by careful analysis of their ^1H and ^{13}C NMR spectra, which suggested a trans configuration for the predominant product (**4**) and a cis configuration for the minor product (**5**) (see reference 31).

The reductive cyclization reaction carried out on the dicyano ester **3** produced again two isomeric products, in about 10:1 molar ratio; their analytical and spectroscopic data were clearly indicative of two bicyclic dilactam structures **6** and **7** having different stereochemistry at the ring fusion.

We performed an X-ray crystallographic analysis on the product obtained in the greater amount (**6**) to get an unequivocal structural assignment and with the further scope of correctly evaluating the eventual influence of the different stereochemistry of compounds **6** and **7** on the biological activity. The crystallographic analysis clearly pointed out a cis ring junction for compound **6**. The geometry of the crystalline molecule resulting from this investigation and reported in Figure 1 clearly shows a bent shape very close to that previously determined in a cor-

(19) Ferappi, M.; Carotti, A.; Gasini, G.; De Laurentis, N.; Giardinà, D.; Cingolani, G. M.; Gavuzzo, E.; Mazza, F. *J. Heterocycl. Chem.* **1983**, *20*, 439.
(20) Casini, G.; Carotti, A.; De Laurentis, N.; Ferappi, M.; Ottolino, S. *Chim. Ind. (Rome)* **1983**, *65*, 123.
(21) Kobayashi, T.; Morita, K.; Kitagawa, H.; Yokoyama, T.; Makida, S. Ger. Offen 1 910 344, 1970.
(22) Kobayashi, T.; Naruse, N.; Okuda, S.; Taguchi, R.; Mogami, M.; Komuro, H. Jpn. Kokai Tokkyo Koho 78 109 940, 1978.
(23) Bettoni, G.; Carbonara, G.; Franchini, C.; Tortorella, V. *Tetrahedron* **1981**, *24*, 4159.
(24) Yoshifuji, S.; Arakawa, Y.; Nitta, Y. *Chem. Pharm. Bull.* **1987**, *35*, 357.
(25) Tanaka, K.; Yoshifuji, S.; Nitta, Y. *Chem. Pharm. Bull.* **1987**, *35*, 364.

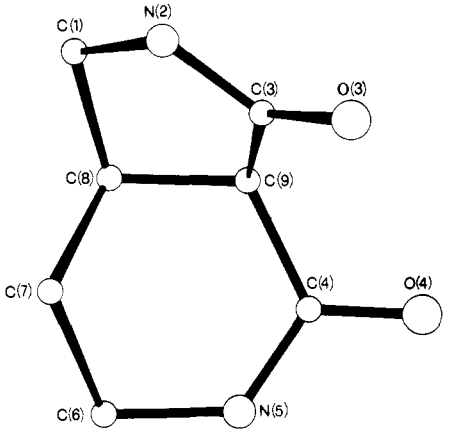


Figure 2. A stereochemical view of *cis*-perhydropyrrolo[3,4-*c*]-pyridine-3,4-dione (**10**).

responding bicyclic diimide.[19]

Finally, with the aim to prepare other bicyclic lactams isomeric with **6** and **7**, we first synthesized another dicyano ester, **9**, through a Michael addition of diethyl malonate to fumaronitrile, and then we obtained compound **10** as the sole reaction product by applying the same reductive cyclization reaction.

The X-ray crystallographic analysis again unequivocally indicated a cis configuration at the ring fusion as showed in Figure 2. A rapid comparison between Figures 1 and 2 easily pointed out a similar structure but with a substantial difference residing in the angle between the mean planes through the two rings. This angle is greater in **10**, most likely because of a repulsive interaction between the two malonic carbonyl groups.

Pharmacology

All the lactam derivatives described in this paper, with the exception of compound **7**, which was almost insoluble in the employed vehicle, were tested to investigate their effect on learning and memory processes in rats. The primary test employed to identify cognition-enhancing properties was facilitation of single trial passive avoidance response in rats. The method used was similar to that described by Giurgea and Salama.[10]

Piperidones **4** and **5** and bicyclic dilactams **6** and **10** also have been evaluated for their ability to antagonize ECS-induced amnesia, by a procedure similar to that describe by Butler et al.,[18] on the basis of the assumption that ECS can induce cognitive and memory impairments in animals.

Bicyclic dilactams **6** and **10**, obtained in higher amounts, have moreover been studied by the water-maze procedure[28] for evaluating their activity on long-term memory.

Finally, the reported lactams were tested in high doses (up to 1000 mg/kg ip) in a battery of standard neuropsychopharmacological tests in mice[29] aimed mainly at detecting eventual sedative, anticonvulsant, or other activities on the CNS as well as neurotoxic effects.

Additionally, the results of the above pharmacological screening could contribute to a better definition and characterization of the neuropsychopharmacological profile of the tested compounds, and consequently the identification of the main chemical features responsible for the neuropharmacological effects and for the effects on memory and learning could be possible.

(26) Nakata, H. *Tetrahedron* **1963**, *19*, 1959.
(27) Nowogrocki, G.; Triclot, G. *Bull. Soc. Chim. Fr.* **1965**, 684.
(28) Batting, K. *Psychopharmacology* **1969**, *15*, 19.
(29) De Martino, G.; Massa, S.; Corelli, F.; Pantaleoni, G.; Fanini, D.; Palumbo, G. *Eur. J. Med. Chem.-Chim. Ther.* **1983**, *18*, 347.

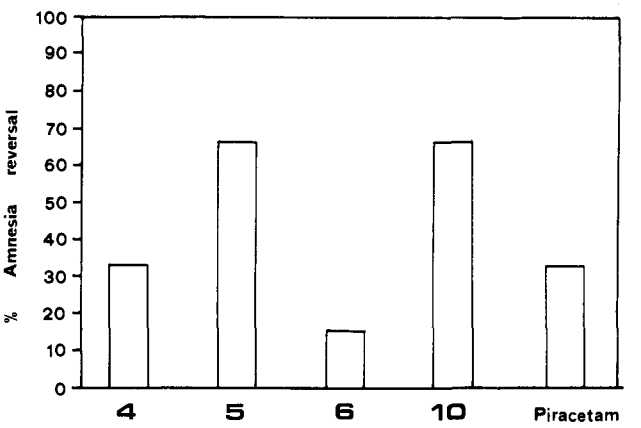


Figure 3. Amnesia reversal activity (percent) of lactam derivatives at equimolar doses to 100 mg/kg ip of piracetam.

Table I. Effects of Lactam Derivatives on Retention in One-Trial Passive Avoidance Test in Rats[a]

compound[b]	% retention	retention time,[c] s (mean ± SD)
vehicle	37.5	70.6 ± 26.0
4	100	44.2 ± 25.9
5	100	47.2 ± 28.7
6	25	37.5 ± 24.7**
10	50	14.5 ± 4.4***
piracetam	100	43.2 ± 38.0

[a] Retention time and percent retention are defined in the Experimental Section. [b] All compounds were tested at equimolar doses to 100 mg/kg ip of piracetam. [c] Statistical significance (*t* test): (**) $p = 0.1/0.05$, (***) $p < 0.01$ vs controls (vehicle).

Results and Discussion

ECS-induced amnesia reversal activity of lactams **4–6** and **10** in equimolar doses of piracetam up to 100 mg/kg ip is reported in Figure 3; piperidones **4** and **5** and bicyclic dilactam **10** showed a good activity in terms of ability to reverse ECS-induced amnesia in rats, at doses producing no other significant CNS signs or symptoms. Compounds **10** and **5** were more potent than piracetam, whereas compound **4** was almost equipotent.

These results confirmed the first observations made with the same compounds in the trial passive avoidance learning procedure (Table I). Piperidones **4** and **5**, in fact, facilitated the single trial passive avoidance in rats, showing an effectiveness, measured as percent of retention, equal to that of piracetam. Among the bicyclic dilactams, compound **10** was slightly more active than the control group whereas compound **6** was found to be inactive.

With the purpose of evaluating the activity on long-term memory, bicyclic compounds **6** and **10**, available in larger amount, were subjected to the water-maze test. Compound **10** and very surprisingly compound **6** (which was the less active in the short-term memory tests) displayed good activity in terms of speed of performance whereas the analysis of the numbers of errors led to some ambiguous results (Table II).

Both compounds were, after ECS, consistently more active than piracetam, with compound **6** slightly more active than compound **10**. Finally, a series of neuropsychopharmacological tests have indicated that all investigated compounds generally showed no effect on spontaneous motor activity in the open field and on explorative behavior; moreover, they were nontoxic even at high doses (up to 1000 mg/kg ip) in mice, with the exception of compound **5**, which showed sedative, myorelaxant, and anticonvulsant effects at 1000 mg/kg ip. None of these

Table II. Effects of Lactam Derivatives on Retention in the Water-Maze Test

compound[a]	day 1[b]		day 2[b]		day 3[b]		day 4[b] 15 min[c]		day 4[b] 30 min[c]		day 4[b] 60 min[c]	
	n	*t*	*n*	*t*	*n*	*t*	*n*	*t*	*n*	*t*	*n*	*t*
vechicle	10.5 ± 3.5 a.m.	300.0 ± 0 a.m.	7.2 ± 2.4 a.m.	259.4 ± 82.3 a.m.	7.2 ± 3.2 a.m.$^{\Delta}$	224.0 ± 112.4 a.m.						
	11.6 ± 1.9 p.m.	292.5 ± 21.2 p.m.	6.2 ± 2.5 p.m.	259.1 ± 77.3 p.m.	9.4 ± 5.8 p.m.	219.1 ± 111.9 p.m.	2.6 ± 0.9	195.3 ± 105.9	4.5 ± 2.1$^{\Delta}$	164.0 ± 121.5	3.5 ± 2.7	86.6 ± 93.2
6	9.7 ± 2.0 a.m.	300.0 ± 0 a.m.	6.0 ± 3.4 a.m.	253.0 ± 88.7 a.m.	8.5 ± 4.3 a.m.$^{\Delta}$	183.0 ± 92.9 a.m.						
	7.2 ± 5.4 p.m.$^{\circ}$	280.0 ± 37.4 p.m.	5.1 ± 3.1 p.m.	243.1 ± 108.3 p.m.	3.0 ± 2.5 p.m.$^{\circ}$	139.4 ± 116.3 p.m.	6.5 ± 4.6°	91.5 ± 77.8$^{\circ\Delta\Delta}$	3.1 ± 1.4	25.3 ± 14.9$^{\circ\Delta\Delta\Delta}$	6.5 ± 5.8	45.8 ± 56.3$^{\Delta\Delta}$
10	12.3 ± 1.8 a.m.$^{\Delta\Delta}$	287.5 ± 35.3 a.m.	6.9 ± 4.6 a.m.	203.1 ± 134.1 a.m.	3.9 ± 1.8 a.m.$^{\circ}$	107.1 ± 120.4 a.m.$^{\Delta}$						
	8.2 ± 3.9 p.m.$^{\circ}$	193.5 ± 115.0 p.m.$^{\circ}$	7.9 ± 2.2 p.m.	173.2 ± 112.4 p.m.$^{\Delta}$	4.5 ± 3.4 p.m.	127.9 ± 114.5 p.m.$^{\Delta}$	3.4 ± 5.1	125.5 ± 144.9	3.2 ± 1.0°	67.1 ± 94.7$^{\Delta}$	5.1 ± 4.1	78.6 ± 50.0$^{\Delta\Delta}$
piracetam	7.6 ± 3.2 a.m.	272.2 ± 78.5 a.m.	7.2 ± 4.5 a.m.	282.7 ± 48.8 a.m.	3.5 ± 3.2 a.m.$^{\circ}$	249.0 ± 100.0 a.m.						
	9.1 ± 2.8 p.m.	265.0 ± 67.4 p.m.	8.1 ± 2.8 p.m.	291.5 ± 24.0 p.m.	5.0 ± 2.7 p.m.	250.1 ± 99.4 p.m.	3.2 ± 2.1	251.0 ± 80.8	2.1 ± 1.7	235.7 ± 110.1	3.6 ± 3.2	197.6 ± 114.6

[a] Compounds 6 and 10 were tested at equimolar doses to 100 mg/kg ip of piracetam. [b] *n* = number of errors. *t* = speed of performance (seconds). Values are reported as mean ± SD (10 rats per group). Statistical significance (*t* test): (Δ) $p < 0.05$, ($\Delta\Delta$) $p < 0.01$, ($\Delta\Delta\Delta$) $p < 0.001$ vs piracetam; ($\circ$) $p < 0.05$, ($\circ\circ$) $p < 0.01$, ($\circ\circ\circ$) $p < 0.001$ vs controls (vehicle). [c] Time after ECS.

effects however were observed at 500 mg/kg ip (see Table III).

Taking into account the good combination of high activity and low toxicity, the tested compounds, especially **5** and **10**, could be considered promising candidates for a further pharmacological evaluation aiming to their possible application on the treatment of symptoms of cerebral insufficiency and mental impairments in the elderly.[30] In this respect, however, the synthesis of new dilactam structures isomeric with those reported herein as well as a suitable functionalization of the lactam group(s) have been planned with the aim to gain further insights on the structure–activity relationships of nootropic agents.

Experimental Section

Chemistry. Melting points were determined by the capillary method on a Electrothermal apparatus (Gallenkamp MFB 595) and are uncorrected. IR spectra (not reported) were recorded as KBr pellets on a Perkin-Elmer 283 spectrophotometer and were fully consistent with the assigned structure. ^{13}C and ^{1}H NMR spectra were taken on a Varian XL 220-MHz FT spectrometer; chemical shifts were expressed in ppm and 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 in the ^{1}H NMR spectra. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; td, triple doublet; m, multiplet(s). Complete proton assignments for compounds **6**, **7**, and **10** have been tentatively made taking into account the X-ray crystallographic results. Elemental analyses were performed by the Analytical Laboratory Service of the Dipartimento Farmaco-Chimico of the University of Bari and agreed with theoretical values to within ±0.40%. Chromatographic separations were carried out on a column using silica gel (35–55 μm, from Merck) as the stationary phase. No efforts were made to optimize the reported yields.

Reductive Cyclization Reactions: General Procedure. Eight milliliters of an EtOH suspension of activated Ra-Ni (commercial sample from Aldrich) was added to an anhydrous EtOH solution (250 mL) of 50 mmol of cyano ester (**2**, **3**, **9**), and the resulting mixture was vigorously stirred in an autoclave under hydrogen at 100 °C and 100 atm pressure for 24 h (**3**) or 48 h (**2**, **9**). The catalyst was removed by filtration on Celite and the solution was evaporated to produce the following compounds.

From **2**: an oily mixture which after chromatographic separation on silica gel (ethyl acetate/MeOH 9:1 as the eluent) gave *trans*-4,5-bis(ethoxycarbonyl)-2-piperidone (**4**)[31] in 36% yield: R_f 0.30; mp 105–106 °C from $CHCl_3$/*n*-heptane; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 6 H, CH_3, J = 7.1), 2.45 (dd, 1 H, H(3ax), J = 17.6, J = 8.9), 2.63 (dd, 1 H, H(3eq), J = 17.6, J = 6.1), 3.05 (td, 1 H, H(5), J = 8.9, J = 8.1, J = 5.1), 3.15 (td, 1 H, H(4), J = 8.9, J = 8.9, J = 6.1), 3.40 (dd, 1 H, H (6ax), J = 12.5, J = 8.1), 3.52 (dd, 1 H, H(6eq), J = 12.5, J = 5.1), 4.12 (q, 4 H, CH_2O, J = 7.1), 7.36 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$) 172.4 and 170.2 [CO(ester)], 171.2 [CO(lactam)], 61.5 and 61.4 [$C(CH_2O)$], 42.1 [C(6)], 40.8 and 40.0 [C(4)/C(5) or C(5)/C(4)], 32.1 [C(3)], 14.1 ppm [$C(CH_3)$]. Anal. ($C_{11}H_{17}NO_5$) C, H, N.

From **2**: *cis*-4,5-bis(ethoxycarbonyl)-2-piperidone (**5**)[31] in 18% yield R_f 0.25; mp 71–72 °C from $CHCl_3$–*n*-heptane; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 6 H, CH_3, J = 7.1), 2.50 (dd, 1 H, H(3ax or 3eq), J = 18.1, J = 7.1), 2.72 (dd, 1 H, H(3eq or 3ax), J = 18.1, J = 7.1), 3.02–3.13 (m, 2 H, H(4) and H(5)), 3.41 (dd, 1 H, H(6eq), J = 12.7, J = 4.8), 3.60 (dd, 1 H, H(6ax), J = 12.7, J = 6.1), 4.06 (q, 4 H, CH_2O, J = 7.1), 7.49 (s, 1 H, NH); ^{13}C NMR ($CDCl_3$) 171.3 [CO(lactam)], 170.3 [CO(ester)], 61.4 and 61.3 [$C(CH_2O)$], 41.6 [C(6)], 39.5 and 39.1 [C(4)/C(5) or C(5)/C(4)], 31.4 [C(3)], 14.0 ppm [$C(CH_3)$]. Anal. ($C_{11}H_{17}NO_5$) C, H, N.

From **3**: a solid mixture which after treatment with hot MeOH left a solid residue which was recrystallized from H_2O to give *trans*-perhydropyrrolo[3,4-*c*]pyridine-1,6-dione (**7**) in 5% yield: mp 324–325 °C dec; ^{1}H NMR (D_2O) δ 2.27 (dd, 1 H, H(7eq or 7ax), J = 12.0, J = 5.5), 2.35–2.45 (m, 1 H, H(3a), $J_{3a\text{-}7a}$ = 8.9 determined by decoupling on irradiation at the center of 3- and 4-proton multiplets), 2.50–2.70 (m, 2 H, H(7a) and H(7ax or 7eq)),

(30) For compounds **4**, **5**, **6**, and **10**, an Italian patent is pending.

(31) The coupling constants $J_{4,5}$ measured upon irradiation of H-3 and H-6 proton signals are 8.9 and 4.8 ± 0.8 Hz in compounds **4** and **5**, respectively, thus indicating that the protons in the junctions were in a prevalent diaxial conformation in the former and in a prevalent cis axial–equatorial conformation in the latter. In the ^{13}C spectra the ester carbonyl groups present two signals (170.2 and 172.4 ppm) in **4** and only one signal in **5** (170.3 ppm), and this result is fully compatible with the previous stereochemical assignment, being the evident nonequivalence of the two ester carbonyl groups possible only in the trans configuration in which the two substituents most likely are in a trans diequatorial conformation. Actually this conformation should be relatively fixed whereas in the cis isomer the probable existence of a rapid equilibrium between axial–equatorial and equatorial–axial conformers should rend the two ethoxycarbonyl groups equivalent.

Table III. Neurobehavioral Effects of High Doses of Lactam Derivatives on Mice

compound	doses, mg/kg ip	test[a] A (mean ± SD)	B (mean ± SD)	C (%)	D (mean ± SD)	E (mean ± SD)
vehicle		3951.7 ± 956.2	22.6 ± 10.6	0	1.4 ± 1.1	120
4	500	3410.5 ± 689.3	20.4 ± 9.8	0	1.0 ± 0.9	75.8 ± 39.5*
	1000	2522.0 ± 1025.8	20.0 ± 4.5	0	3.3 ± 1.0	112.8 ± 34.4
5	500	3025.2 ± 482.2	22.3 ± 12.6	0	0.8 ± 0.8	65.1 ± 40.1*
	1000	782.4 ± 247.0***	8.9 ± 2.3***	40.0	16.4 ± 7.7***	<7***
6	1000	3749.1 ± 872.8	25.1 ± 9.8	12.5	1.0 ± 1.0	68.1 ± 50.1*
10	1000	3429.6 ± 673.2	21.5 ± 13.9	0	0.7 ± 0.03	111.5 ± 24.0
piracetam	1000	3764.3 ± 523.1	19.6 ± 16.2	0	1.7 ± 1.7	79.9 ± 46.3*

[a] A = spontaneous motor activity in open field (total number of movements in 5 min); B = exploratory activity in hole board (total number of holes in 5 min); C = maximal electroshock seizure (percent of animals without seizures); D = motor coordination in rota-rod (total number of falls in 3 min); E = traction test (time in seconds on the horizontal wire with forelegs). statistical significance (*t* test): (*) $p < 0.05$, (***) $p < 0.001$ vs controls (vehicle).

3.13 (apparent t, 1 H, H(4ax), J = 9.8), 3.23 (apparent t, 1 H, H(3ax), J = 11.6), 3.42 (dd, 1 H, H(3eq), J = 11.6, J = 5.5), 3.44 (dd, 1 H, H(4eq), J = 9.8, J = 6.8). Anal. ($C_7H_{10}N_2O_2$) C, H, N. Cooling the MeOH solution gave *cis*-perhydropyrrolo[3,4-*c*]-pyridine-1,6-dione (**6**) in 50% yield: mp 278–280 °C dec; ^{1}H NMR (D_2O) δ 2.40 (dd, 1 H, H (7eq), J = 16.0, J = 4.2), 2.57 (dd, 1 H, H(7ax), J = 16.0, J = 8.2), 2.82–2.96 (m, 1 H, H(3a)), 2.98–3.10 (m, 1 H, H(7a), partially masked), 3.04 (dd, 1 H, H(4eq), J = 10.8, J = 3.5), 3.10 (dd, 1 H, H(3eq or 3ax), J = 13.5, J = 4.7), 3.32 (dd, 1 H, H(3ax or 3eq), J = 13.5, J = 4.7), 3.56 (dd, 1 H, H(4ax), J = 10.8, J = 8.5). Anal. ($C_7H_{10}N_2O_2$) C, H, N.

From **9**: a solid residue which was recrystallized from anhydrous EtOH to give pure *cis*-perhydropyrrolo[3,4-*c*]pyridine-3,4-dione (**10**) in 60% yield: mp 223–225 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.47 (2 dd, 1 H, H(7ax), J = 14.5, J = 12.8, J = 6.8), 1.74 (2 dd, 1 H, H(7eq), J = 14.5, J = 9.6, J = 4.6), 2.66–2.77 (m, 1 H, H(7a)), 2.89 (dd, 1 H, H(1eq), J = 9.8, J = 1.0), 3.00 (d, 1H, H(3a), J = 8.1), 3.05–3.15 (m, 2 H, H(6ax) and H(6eq)), 3.31 (dd, 1 H, H(1ax), J = 9.8, J = 7.0), 7.56 (s, 1 H, NH), 7.70 (s, 1 H, NH). Anal. ($C_7H_{10}N_2O_2$) C, H, N.

1,2-Dicyano-3,3-propanedicarboxylic Acid Bis(ethyl ester) (9). Ethyl malonate (14.15 mL, 0.14 mol) in EtOH (15 mL) was added dropwise to an ice-cooled solution of EtONa (8.17 g, 0.12 mol) in EtOH (50 mL) with mechanical stirring. The mixture was allowed to warm to room temperature and fumaronitrile (10.15 g, 0.13 mol) was then added dropwise over a period of about 15 min. The reaction mixture was neutralized with acetic acid and the solvent was removed under vacuum. The residue was partitioned in water and CCl_4 and the organic layer was dried on Na_2SO_4. After solvent removal the oily residue was distilled under reduced pressure to give 19.5 g (63% yield) of **9**: bp 163–65 °C (1 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3 H, CH_3, J = 7.0), 1.32 (t, 3 H, CH_3, J = 7.0), 2.98 (d, 2 H, CH_2CN, J = 7.0), 3.62 (q, 1 H, CHCN, J = 7.0), 3.81 (d, 1 H, CHCO, J = 7.0), 4.30 (q, 2 H, CH_2O, J = 7.0), 4.32 (q, 2 H, CH_2O, J = 7.0). Anal. ($C_{11}H_{14}N_2O_4$) C, H, N.

***trans*-4,5-Bis(ethoxycarbonyl)glutarimide (8).** An alcohol-free CCl_4 solution of RuO_4 (stochiometric amount), prepared according to literature methods,[26,27] was added dropwise to a solution of 0.01 mol of 2-piperidone derivative (**4** or **5**) in the same solvent (100 mL). The reaction was monitored by TLC, and when the initial product had completely disappeared, 5 mL of 2-propanol was added to the reaction mixture and the precipitate (RuO_2) was filtered off. The remaining solution was evaporated under reduced pressure to give compound **8** in 95% yield; its analytical and spectroscopic data were in full agreement with those already reported.[19]

X-ray Crystallography. Crystal structure analyses were carried out on single crystals of compounds **6** and **10** obtained from MeOH and EtOH solutions, respectively, by slow evaporation. Intensity data were measured on a Syntex $P2_1$ automatic diffractometer. Both structures were solved by direct methods with the program MULTAN,[32] and all the hydrogen atoms were found in the Fourier difference maps.

Crystal data for compound **6** are as follows: $C_7H_{10}N_2O_2$, FW = 154.2, monoclinic space group $P2_1/c$, a = 11.918 (8) Å, b = 6.453 (14) Å, c = 9.609 (12) Å, β = 95.51 (8)°, V = 736 (2) Å^3, Z = 4, D_c = 1.39 g cm^{-3}, λ(Mo Kα) = 0.17069 Å, μ(Mo Kα) = 0.11 mm^{-1}, R = 0.065 for 1405 independent reflections with $I > 2.0\sigma(I)$.

Crystal data for compound **10** are as follows: $C_7H_{10}N_2O_2 \cdot H_2O$, FW = 172.2, monoclinic space group $P2_1/c$, a = 10.693 (6) Å, b = 6.159 (3) Å, c = 12.805 (9) Å, β = 91.32 (5)°, V = 843 (1) Å^3, Z = 4, D_c = 1.36 g cm^{-3}, λ(Mo Kα) = 0.71069 Å, μ(Mo Kα) = 0.12 mm^{-1}, R = 0.056 for 1794 independent reflections with $I > 2.0\sigma(I)$.

As anticipated in the discussion, the fusion between the two rings is cis in both compounds, and therefore the overall shape of the molecules is bent as can be seen from Figures 1 and 2. The angles between the mean planes through the non-hydrogen atoms of the two rings are 99.5° and 123.5° in compounds **6** and **10**, respectively. The torsion angles around the fused bond, H-C-(8)-C(9)-H, are 0.1° and 36.4° in compounds **6** and **10**, respectively. Full details of the molecular and crystal structures of compounds **6** and **10** will be given elsewhere.

Pharmacology. (A) One-Trial Passive Avoidance Learning Procedure.[10] A total of 48 male Sprague–Dawley rats (Charles River Italia), weighing about 250–300 g, was used; they were housed in cages with free access to laboratory chow and tap water and in standardized environmental conditions: temperature 22 ± 1 °C, humidity 52 ± 2%, light period 5 a.m.–9 p.m. The animals were divided into six groups of eight animals each. Control group received vehicle only whereas the other groups were treated twice for 2 days by ip injection of products **4–6** and **10** at equimolar doses up to 100 mg/kg ip of piracetam (reference compound). The animals were trained to avoid a dark compartment with footshock of 720 μA for 15 s as punishment and on the following day they were retested. During the retest, the grid floor in the dark compartment was not electrified. The animals that avoided the dark compartment from 0.2 to 3 min were counted as remembering the painful footshock received 24 h earlier. Retention time (T), calculated for remembering animals, was defined as reported: $T = T' - T_o$, where T_o represents the cumulative time spent by rats in the lightened compartment during acquisition phase and T' represents the cumulative time spent by rats in the same compartment during the test for retention 24 h after acquisition trial.

(B) Amnesia-Reversal Testing. The test was similar to that described by others[18] and was conducted as follows. Male rats (Charles River, CD strain Italia 250–300 g) were divided into groups of 10 rats each. By use of footshock (1.5 mA, 10-s duration) as punishment, the animals were trained to avoid the darkened interior of a box smaller than the lightened chamber (30 × 16 cm) in which the animals were initially placed. Two hours after training, the rats received either ECS (60 mA for 1 s) or sham ECS treatment (no-ECS control) through spring clips attached to the ears. Two hours after ECS, the groups of rats were injected ip with chemicals in equimolar doses up to 100 mg/kg of piracetam or vehicle alone. Each time the compound was tested along with base-line (ECS plus vehicle) and ceiling (no-ECS plus vehicle) control groups. One hour after treatment with the test compounds, the rats were individually returned to the large lightened chamber attached to the test chamber in order to measure retention of the inhibitory avoidance response. Animals entering the box 60 s

(32) Main, P.; Fiske, S. J.; Hull, S E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. MULTAN 80: A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data, Universities of York, England and Louvain, Belgium, 1980.

within the test period were counted as having amnesia or having forgotten the training. The equation used to calculate the percent of amnesia reversal activity reported in Figure 3 was that employed by Butler et al.;[18] that is, percent amnesia reversal = [(drug group - base-line control group)/(ceiling control group - base-line control group)] × 100; data were analyzed according to the screening criteria and statistical constraints used by the same authors.

(C) Water-Maze Procedure. Four groups of 10 male Sprague–Dawley rats weighing about 250–300 g were used. The animals were housed in the same conditions reported for test A. The experiments were carried out in 5 consecutive days in an apparatus similar to that described by Batting et al.[28] In the first day, rats unable to find the exit of the water maze within 5 min were selected. On the second, third, and fourth day the selected rats were treated twice a day with the same dose (equimolar to 100 mg/kg ip of piracetam) of the compounds under study. On the fifth day the same two doses were administered in the morning in 30-min intervals and then the animals were subjected to transtemporal ECS (60 mA/0.2 s) that produced the classical tonic and clonic convulsions. After the shock the animals were observed in three subsequent sessions in the water maze after 15, 60, and 240 min. The time spent to go out of the water maze (speed of performance) and the number of errors, calculated as the sum of reversals and wrong entrances, were recorded.

(D) Neuropsychopharmacological Effects at High Doses.[29] Male and female Swiss inbred mice (Charles River) weighing 22 ± 2 g were used. Animals were housed in the same standardized environmental conditions described in test A. The compounds were injected intraperitoneally after solubilization in 1% Tween 80. Different doses up to 1000 mg/kg were used. Every single dose was administered to a group of 10 mice (five males and five females). Control groups of mice were treated only with vehicle.

(1) **Behavior in Free Conditions.** (a) Activity in the open field. This activity was determined by recording the total number of movements performed in 5 min by every animal.[33,34]

(b) Exploration activity in the hole board. The test was performed by using the Boissier and Simon technique.[35] The number of explorations was recorded automatically by an infrared device. During the experiment the total number of holes explored by each animal during 5 min was recorded.

(2) **Anticonvulsant Activity.** This was tested with use of the maximal electroshock which induced death in 50% of the control animals. The Model U. Basile ECT-Unit 7800 used for the test was adjusted as follows: 200-mA frequency pulses/s; 60-mA current adjustment; 0.4-s shock duration; 0.6-ms pulse width. We considered the animals protected when they did not show seizures.

(3) **Action on Motor Coordination.** This was examined by using the rota-rod test.[36] The number of animal falls, during a 100-s observation period, was recorded.

(4) **Myorelaxant Action.** This was analyzed by using the Boissier and Simon traction test.[37] We recorded how long the animals stayed on an horizontal wire with their forelegs. All tests were performed on each animal as in the following succession (time point, minutes): 0, ip injection; 15, open field activity; 20, exploration activity; 25, traction test; 30, rota-rod test; 60, effect of electroshock. Significance levels were obtained by the Student's *t* test.

Acknowledgment. A partial financial support to this research from NCNS (Nuovo Consorzio Sanitario Nazionale) is gratefully acknowledged.

Registry No. **2**, 116079-14-2; **3**, 116102-36-4; **4**, 116079-15-3; **5**, 116079-16-4; **6**, 116079-17-5; **7**, 116079-18-6; **8**, 116079-19-7; **9**, 116079-20-0; **10**, 116079-21-1; fumaronitrile, 764-42-1; ethyl malonate, 105-53-3.

(33) Minck, K.; Danneberg, P.; Knappen, F. *Psychopharmacologia* **1970**, *19*, 245.
(34) Silvermann, P. *Animal Behaviour in the Laboratory*; Chapman and Hall: London, 1978.
(35) Boissier, J. R.; Simon, P. *Arch. Int. Pharmacodyn.* **1964**, *147*, 372.
(36) Dunham, N. W.; Miya, T. S. *J. Am. Pharm. Assoc.* **1957**, *46*, 208.
(37) Boissier, J. R.; Simon, P. *Therapie* **1960**, *15*, 1170.

Synthesis and Structure–Activity Relationships of a New Series of Antiarrhythmic Agents: Monobasic Derivatives of Disobutamide[1]

Bipin N. Desai,* Kerry W. Fowler, Robert J. Chorvat, Leo G. Frederick, Frida R. Hatley, Kurt J. Rorig, and Susan M. Garthwaite

Cardiovascular Diseases Research Department, G.D. Searle and Company, 4901 Searle Parkway, Skokie, Illinois 60077.
Received April 13, 1988

Analogues of the dibasic antiarrhythmic agent disobutamide (**2**) were prepared and evaluated for antiarrhythmic efficacy, myocardial depression, and anticholinergic activity. The replacement of an isopropyl group in disobutamide by an acetyl group led to the monobasic analogue SC-40230, **7a**, which demonstrated good antiarrhythmic activity accompanied by less myocardial depressant and anticholinergic activities. In addition, it did not induce clear cytoplasmic vacuoles as did the parent compound. SC-40230 was chosen from among other analogues as a candidate for clinical evaluation. Other compounds prepared and evaluated included indolizidinones and a secondary amine isomer of disobutamide.

We have aimed recent efforts of our antiarrhythmic program at the identification of effective class I antiarrhythmic agents[2] with an absence or diminution of the side effects most frequently associated with drugs used to treat chronic ventricular arrhythmias.[3] Emerging from this investigation was the bis[(dialkylamino)alkyl]phenylacetamide series **1**, which included compounds possessing good antiarrhythmic potency and diminished undesirable properties.[4] A representative from this series, disobutamide (**2**), was identified as a candidate for clinical evaluation.[5]

Disobutamide was withdrawn early in the course of clinical testing when clear cytoplasmic vacuoles were found

(1) Presented in part at the 17th Northeast Regional American Chemical Society Meeting, November, 1987, Rochester, NY.
(2) Vaughan Williams, E. M. *Prog. Pharmacol.* **1979**, *2*, 13 and references therein.
(3) Schwarz, J. B.; Keefe, D.; Harrison, D. C. *Drugs* **1981**, *21*, 23.
(4) Yonan, P. K.; Novotney, R. L.; Woo, C. M.; Prodan, K. A.; Hershenson, F. M. *J. Med. Chem.* **1980**, *23*, 1102.
(5) Dohrman, M. L.; Harrell, F. E., Jr.; Strauss, H. C. *J. Pharm. Exp. Ther.* **1981**, *217*, 549.

0022-2623/88/1831-2158$01.50/0