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Monocyclic Analogues of the μ-Opioid Agonist 3,8-Diazabicyclo-[3.2.1]octanes: Synthesis, Modeling, and Activity

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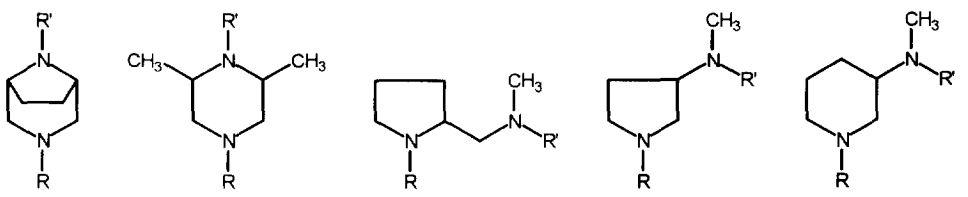
Abstract: Several monocyclic derivatives structurally related to the μ-opioid agonist 3-cinnamyl-8-propionyl-3,8-diazabicyclo[3.2.1]octane have been synthesized and tested in binding studies using the μ-selective ^{3}H-DAMGO as ligand. Modeling studies have been performed on the same compounds in order to explain the observed lack of affinity towards μ-opioid receptors.

Following the discovery that 3-cinnamyl-8-propionyl-3,8-diazabicyclo[3.2.1]octane **1a** is an analgesic about ten times more potent than morphine in the Randall and Selitto test (rat),[1] modifications of its structure have been attempted to obtain analogues with similar or even higher activity. The isomer of **1a** in which the N3 and N8 substituents are exchanged, the so called "reverted" isomer **1b**, was found only slightly less active than **1a**.[2,3] The monocyclic analogue of **1a** deriving from a formal breaking of the endoethylenic bridge, **2a**, maintains a good activity while the same does not occur for **2b**, analogue of **1b**, which was shown to be inactive.[2,4] These results have been rationalized on the basis of the preferred conformation of **2a**, similar to that of **1a**, as the two methyl groups are axially oriented and occupy about the same spatial position as the endoethylenic bridge in **1a**; on the contrary, in **2b** the equatorially oriented methyl groups make its preferred conformation quite different from that of **1b**.[4]



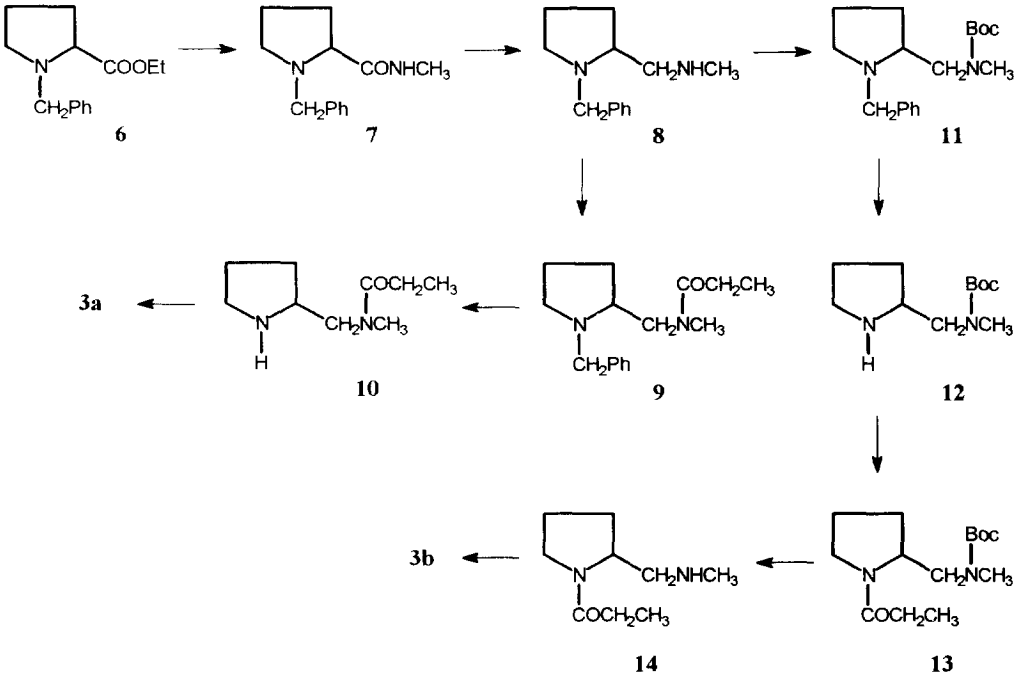
1 **2** **3** **4** **5**

a: R = $CH_2CH{=}CHC_6H_5$, R' = $COCH_2CH_3$ **b**: R = $COCH_2CH_3$, R' = $CH_2CH{=}CHC_6H_5$

More recently a more precise pharmacodynamical model has been proposed for this class of compounds.[5] Besides the well known structural features shared by most opiates, namely an aromatic system placed at an appropriate distance from a tertiary amine function, this model suggests the existence of a small hydrophobic pocket able to accept the endoethylenic bridge of the diazabicyclo-octane moiety. To verify the importance of this requisite, we designed the derivatives **3-5a** and their reverted isomers **3-5b,** structurally related to **1** but lacking the endoethylenic bridge, which, as does the analogue **2**, maintain only one of the two rings present in **1** and show the same through bond distance between the two nitrogen atoms. This paper describes the synthesis of these compounds together with their modeling and the evaluation of their affinity for μ-receptors.

RESULTS AND DISCUSSION

The synthesis of the 2-substituted pyrrolidine derivatives **3a** and **3b**, which were obtained in both enantiomeric forms, is shown in Scheme 1. Accordingly, N-benzyl-L-proline ethyl ester **6**, derived from L-proline, was converted into the corresponding methylamino derivative **8** by condensation with aqueous methylamine and reduction of the amide **7** with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al®). Treatment of **8** with propionic anhydride, debenzylation and final condensation with cinnamyl chloride gave (S)-(-)-**3a**.

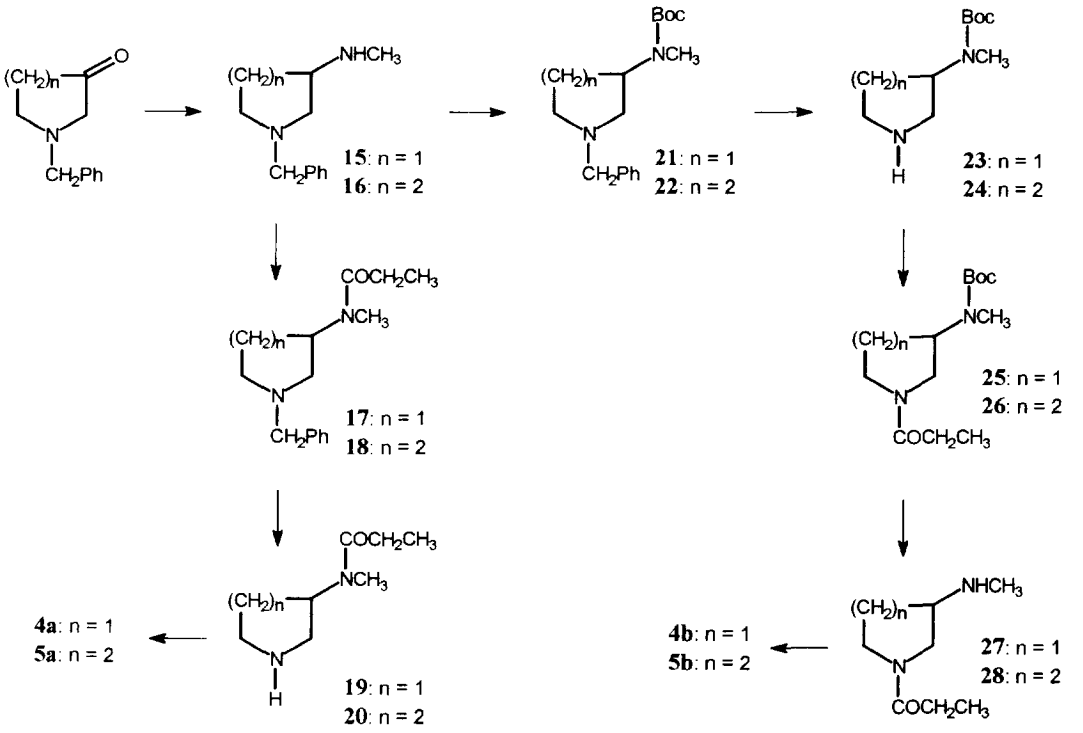


Scheme 1

The reverted isomer (S)-(-)-**3b** was obtained by treatment of **8** with di-*tert*-butyl dicarbonate to give the N-Boc derivative **11**, which was debenzylated by catalytic hydrogenation to **12**, in turn transformed into **13** by reaction with propionic anhydride. Removal of the Boc protecting group by treatment with acidic diethyl ether gave **14**, which was eventually condensed with cinnamyl chloride to the desired (S)-(-)-**3b**. Analogously, (R)-(+)-**3a** and (R)-(+)-**3b** were obtained from N-benzyl-D-proline ethyl ester.

The 3-substituted pyrrolidine derivatives **4** were synthesized (Scheme 2) as racemic mixtures from 1-benzyl-3-pyrrolidinone, which was transformed into the corresponding methylamino derivative **15** by reductive amination. The latter compound was then submitted to the same sequence of reactions above described for the 2-substituted isomers, to give the desired **4a** and **4b**. The higher 3-substituted piperidyl homologues **5a** and **5b** were synthesized as racemic mixtures in a similar way starting from 1-benzyl-3-piperidinone.

Compounds **3-5**, together with **1a** and morphine as reference compounds, were submitted to binding studies on mouse brain homogenates in the presence of ^{3}H-DAMGO as μ-selective ligand. The data reported in Table 1 clearly indicate that none of the new compounds had any significant affinity for the μ-receptors. It should be noted that the two couples of enantiomers **3a** and **3b** do not significantly differ in their affinity, thus indicating that chirality, often a very important parameter in opioids, does not seem to affect their behaviour.



Scheme 2

Table1. Inhibition Constants of Morphine and Compounds **1a** and **3-5** towards μ-Opioid Receptors.

Compd	^{3}H-DAMGO[a] Ki (nM)	Compd	^{3}H-DAMGO[a] Ki (nM)
Morphine	2.8	**1a**	55
(S)-(-)-**3a**	9,500	(R)-(+)-**3a**	> 10,000
(S)-(-)-**3b**	> 10,000	(R)-(+)-**3b**	6,800
(±)-**4a**	> 10,000	(±)-**4b**	> 10,000
(±)-**5a**	8,500	(±)-**5b**	3,900

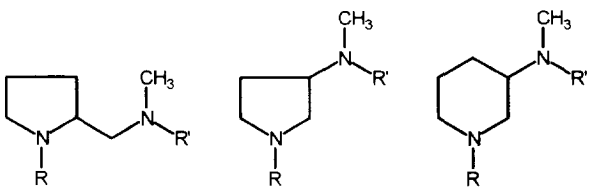
[a] Ki values were calculated with the LIGAND program, based on a Kd value of 1 nM for ^{3}H-DAMGO. Values are the mean from two experiments.

The conformational properties of compounds **3-5** were studied on the simplified models **29-31** in which the cinnamyl moiety is replaced by a methyl group as it was previously shown[4] that the high conformational mobility of the cinnamyl group does not influence the conformational behaviour of the remaining part of the molecule.

The conformational space of these models was fully explored using the MM^+ force field of the HyperChem™ package.[6] The pseudorotational path of the five-membered ring of compounds **29-30** was evaluated while in the case of **31** the chair and the twist-boat conformations were submitted to analysis. The orientation of the ring substituents was studied by considering the rotation around all the exocyclic double bonds.

It could be inferred that the pentacyclic compounds **29a** and **29b** have a high conformational mobility: several conformers contribute for at least 1% to the overall population. In compound **29a** this mobility derives mainly from rotation around the exocyclic single bonds while the five-membered ring is always in a conformation close to ^{1}E. On the contrary, in compound **29b** the ring can assume two different conformations: $^3T_4$ or $^4T_3$. The Z orientation of the amide group is predicted more populated than the E, in particular in the case of **29b**.

A quite different result was obtained for compounds **30a** and **30b**. In these cases the five-membered ring can adopt only one conformation, the envelope ^{1}E in the case of **30a** and the twist $^4T_3$ in the case of **30b** and the side group at C-3 assumes only one preferred orientation; the main geometrical mobility is due



29 **30** **31**

a: R = CH_3, R' = $COCH_2CH_3$ **b**: R = $COCH_2CH_3$, R' = CH_3

to the amide group for which the E and Z orientations are expected almost equipopulated. Thus, for **30a** and **30b** only two significant conformations are predicted by the calculations.

Both compounds **31a** and **31b** show a chair conformation of the six membered ring with a preferred equatorial orientation of the substituents. Compound **31a** has practically only two populated conformations which show an almost perpendicular orientation of the plane of the amide function and of the mean plane of the ring; *vice versa* for all the conformations of **31b** the amide and the ring are almost coplanar. The two conformers of **31a** differ in the orientation of the amide function. **31b** shows several conformers which differ not only in the amide function but also in the orientation of the dimethylamino function which is almost completely free to rotate. In figure 1 are reported the most populated conformers of each compound with the indication of their percentage contribution to the overall population.

^{1}H NMR analysis of the compounds **3-5** confirmed the results of the modeling. The six spectra showed two sets of signals deriving from Z/E interconversion at the amide bond. The ratios between the two isomers (slowly equilibrating in the NMR time scale) were 50:50 for **3a**, 68:32 for **3b**, 67:33 for **4a**, 52:48 for **4b**, 43:57 for **5a**, 50:50 for **5b**. The spectroscopic parameters were in agreement with the results of the calculations, e.g. the large (10-11 Hz) axial-axial coupling constants found for the compounds with a six membered ring confirmed its rigid chair conformations.

In conclusion, our results indicate that, though in the presence of a large variety of 3D structures (*e.g.* different degree of flexibility and different orientations of the substituents), none of the conformers of compounds **3-5** has a hydrophobic group oriented in the same direction as the endoethylenic bridge of the bicyclic compound **1a** and capable to interact correctly with the hydrophobic pocket of the receptor defined in the model previously reported.[5] So, the low affinity of this class of monocyclic derivatives towards μ-opioid receptors could give further support to this model, though several other factors cannot be excluded as responsible for the observed inactivity.

EXPERIMENTAL

Elemental analyses of all new compounds were within ± 0.4 % of the theoretical values. ^{1}H NMR spectra were recorded on a Bruker AC200 or on a Bruker AM500 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as internal standard; $CDCl_3$ was used as the solvent, unless otherwise noted. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck, 70-230 mesh) was used for column chromatography.

(S)-1-Benzyl-pyrrolidine-2-carboxylic acid methylamide **7**. A mixture of N-benzyl-L-proline ethyl ester[7] **6** (7 g; 30.0 mmol) and 40% aqueous methylamine (15 mL) was heated overnight at 100 °C. After cooling, the residue was extracted with dichloromethane (3 x 25 mL), dried (Na_2SO_4) and the solvent evaporated to give 5.8 g of **7** (26.6 mmol, 89%), which was used as such for the next step. ^{1}H NMR: 1.7-1.8 (m, 3H); 2.2-2.4 (m, 2H); 2.8 (s, 3H); 3.0 (m, 1H); 3.2 (m, 1H); 3.5 (d, 1H); 3.9 (d, 1H); 7.3 (m, 5H); 8.5 (s, 1H, exch. with D_2O).

(S)-1-Benzyl-2-methylaminomethyl-pyrrolidine **8**. To an ice-cooled solution of **7** (1.7 g; 7.79 mmol) in toluene (25 mL) a 3.5M solution of sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al®) in toluene

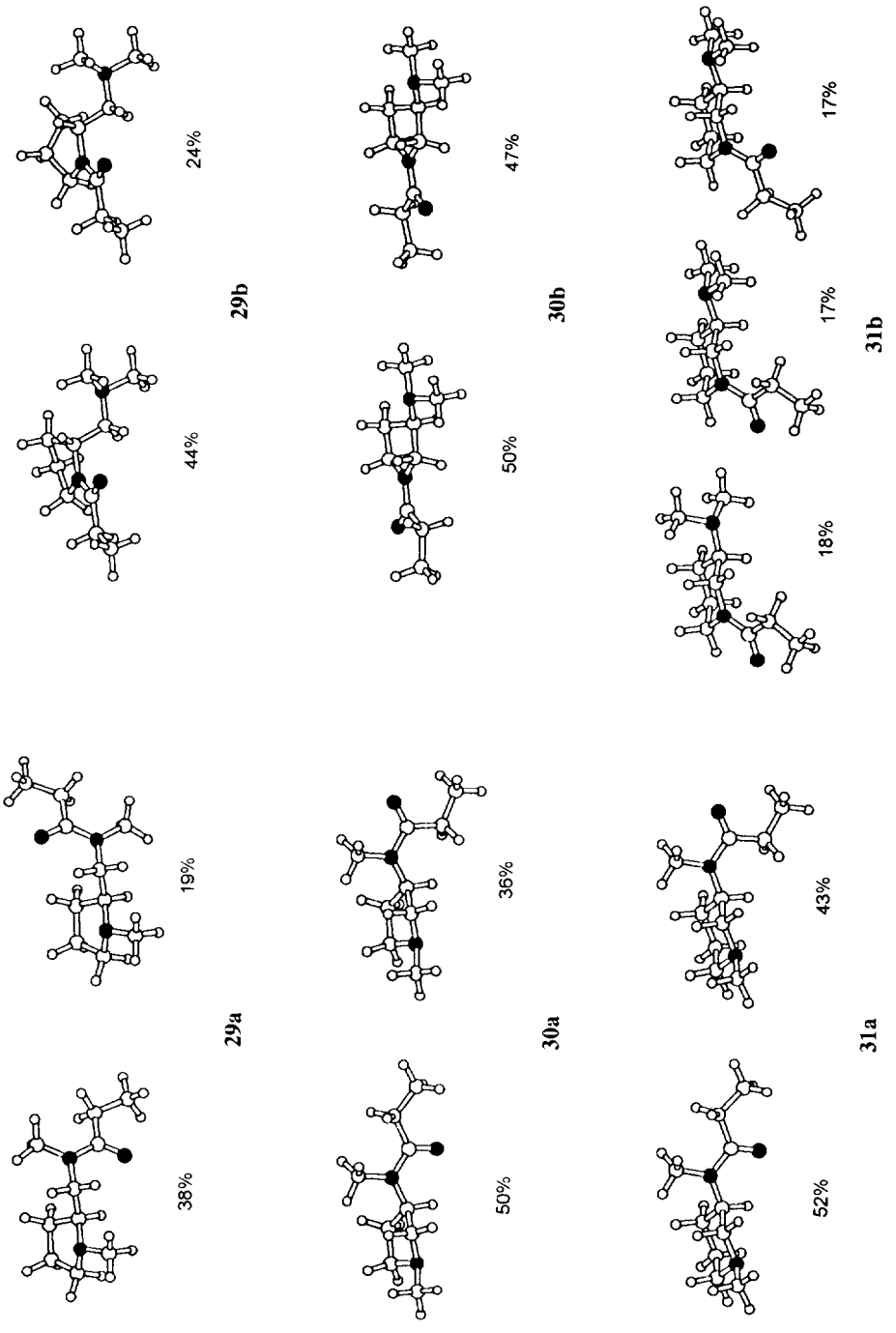


Fig. 1. Three-dimensional plots of the most populated conformers of compounds **29-31**.

(37 mL) was added dropwise and the mixture was then heated at 50 °C for 2 h. After cooling, aqueous sodium carbonate was added, the salts were filtered off and the aqueous layer were extracted with toluene (3 x 15 mL). After drying (Na_2SO_4) and evaporation of the solvent, the residue was distilled to give 0.98 g of **8** (4.80 mmol, 62%). Bp = 100 °C/0.3 mmHg. ^{1}H NMR 1.8-2.8 (m, 7H); 2.0 (bs, 1H, exch. with D_2O); 2.4 (s, 2H); 2.9 (m, 1H); 3.4 (d, 2H); 3.9 (d, 2H); 7.3 (m, 5H).

(S)-1-Benzyl-2-methylpropionylaminomethyl-pyrrolidine **9**. To ice-cooled propionic anhydride (5 mL), **8** (0.80 g; 3.92 mmol) was added dropwise and the mixture stirred at 100 °C for 1 h. After cooling, 10% sodium hydroxide (15 mL) was added, the mixture stirred overnight, extracted with diethyl ether and dried (Na_2SO_4). After evaporation of the solvent, the residue was purified by silica gel chromatography eluting with dichloromethane/methanol 95/5 to yield 0.86 g of 9 (3.30 mmol, 84%). ^{1}H NMR: 1.1 (t, 3H); 1.5-2.3 (m, 8H); 2.8 (s, 3H); 2.9 (s, 2H); 3.2-3.8 (m, 2H); 3.9-4.1 (m, 1H); 7.3 (m, 5H).

(S)-2-Methylpropionylaminomethyl-pyrrolidine **10**. A mixture of **9** (3.5 g; 13.5 mmol) and 10% Pd-C (0.32 g) in ethanol (35 mL) was hydrogenated at room temperature. The catalyst was filtered off and the solvent evaporated to give 2.24 g of **10** (13.2 mmol, 98%). ^{1}H NMR: 1.1 (t, 3H); 1.8-2.3 (m, 6H); 2.8 (bs, 1H; exch. with D_2O); 2.9 (s, 2H); 3.0 (s, 3H); 3.4 (m, 2H); 3.8 (m, 1H).

(S)-(-)-2-Methylpropionylaminomethyl-1-(1-phenylpropen-3-yl)-pyrrolidine **3a**. A mixture of **10** (0.51 g; 3.00 mmol), cinnamyl chloride (0.46 g; 3.00 mmol) and K_2CO_3 (0.41 g; 3.00mol) in acetone (12 mL) was refluxed overnight. After cooling, the salts were filtered off, the solvent evaporated and the residue chromatographed on silica gel eluting with dichloromethane/methanol 98/2 to give 0.65 g of (S)-(-)-**3a** (2.27 mmol, 76%). ^{1}H NMR: 1.08, 1.09 (2t, 3H); 1.5-2.8 (m, 8H); 2.90, 2.99 (2s, 3H); 3.0-3.6 (m, 5H); 6.14-6.30 (m, 1H); 6.44-6.52 (m, 1H); 7.15-7.35 (m, 5H). $[\alpha]_D^{20}$ = - 41.9° (c 1.0, CH_2Cl_2).

(R)-(+)-2-Methylpropionylaminomethyl-1-(1-phenylpropen-3-yl)-pyrrolidine **3a**. Compound (R)-(+)-**3a** was obtained starting from N-benzyl-D-proline ethyl ester with the same procedure above described for (S)-(-)-**3a**. $[\alpha]_D^{20}$ = + 42.0° (c 1.0, CH_2Cl_2).

(S)-1-Benzyl-2-[(t-butyloxycarbonyl)methylaminomethyl]-pyrrolidine **11**. To a solution of **8** (2.8 g; 13.7 mmol) in anhydrous chloroform (20 mL) di-*t*-butyldicarbonate (3 g; 13.7 mmol) in anhydrous chloroform (20 mL) was added dropwise under nitrogen and the mixture was refluxed overnight. After evaporation of the solvent, the obtained residue was used as such for the next step (4.09 g, 13.4 mmol, 98%). ^{1}H NMR: 1.4 (s, 9H); 1.7-2.2 (m, 6H); 2.7 (m, 1H); 2.8 (s, 3H); 3.2 (s, 2H); 4.0 (d, 2H); 7.3 (m, 5H).

(S)-2-[(t-Butyloxycarbonyl)methylaminomethyl]-pyrrolidine **12**. Compound **12** was prepared by hydrogenolysis starting from **11** as above reported for **10** (55%). ^{1}H NMR: 1.4 (s, 9H); 1.7-2.1 (m, 4H); 2.9 (s, 3H); 3.3-3.6 (m, 2H); 3.4 (s, 2H); 4.0 (m, 1H); 5.0 (bs, 1H, exch. with D_2O).

(S)-2-[(t-Butyloxycarbonyl)methylaminomethyl]-1-propionyl-pyrrolidine **13**. Compound **13** was prepared starting from **12** as above reported for **9** (55%). ^{1}H NMR: 1.2 (t, 3H); 1.4 (s, 9H); 1.7-1.9 (m, 5H); 2.3 (q, 2H); 2.9 (s, 3H); 3.2-3.4 (m, 3H); 4.2 (m, 1H).

(S)-2-Methylaminomethyl-1-propionyl-pyrrolidine **14**. A solution of **13** (1.6 g; 5.92 mol) in diethyl ether (20 mL) was treated with hydrochloric diethyl ether to pH 1 and stirred overnight at room temperature. Aqueous 20% NaOH was added and the aqueous layer was extracted with diethyl ether (2 x 25 mL), the solvent dried (Na_2SO_4) and evaporated to give 0.99 g of **14** (5.81 mmol, 98%). ^{1}H NMR: 1.1 (t, 3H); 1.8-2.4 (m, 7H); 2.6 (s, 3H); 3.0 (d, 2H); 3.3-3.4 (m, 2H); 4.4 (bs, 1H, exch. with D_2O).

(S)-(-)-2-[Methyl-(1-phenylpropen-3-yl)aminomethyl]-1-propionyl-pyrrolidine **3b**. Compound (S)-(-)-**3b** was prepared starting from **14** as above reported for **3a** (78%). ^{1}H NMR: 1.09, 1.10 (2t, 3H); 1.8-2.4 (m, 7H); 2.29, 2.33 (2s, 3H); 3.0-3.4 (m, 5H); 3.89, (m, 0.32H); 4.22 (m, 0.68H); 6.14-6.25 (m, 1H); 6.44-6.54 (m, 1H); 7.15-7.35 (m, 5H). $[\alpha]_D^{20}$ = - 93.2° (c 1.0, CH_2Cl_2).

(R)-(+)-2-[Methyl-(1-phenylpropen-3-yl)aminomethyl]-1-propionyl-pyrrolidine **3b**. Compound (R)-(+)-**3b** was obtained with the same procedure above described for (S)-(-)-**3b**. $[\alpha]_D^{20}$ = + 94.6° (c 1.0, CH_2Cl_2).

1-Benzyl-3-methylamino-pyrrolidine **15**. To an ice-cooled solution of 1-benzyl-3-pyrrolidinone (2.0 g; 11.4 mmol) in methanol (16 mL), 40% aqueous methylamine was added (2.6 mL) followed by sodium borohydride (0.22 g; 5.8 mmol) and the mixture was stirred for 1 h at room temperature. The solvent was evaporated, the residue treated with water and extracted with dichloromethane. After drying (Na_2SO_4) and evaporation of the solvent, the residue was distilled to give 1.33 g of **15** (7.0 mmol, 61%). Bp = 75 °C/0.4 mmHg. ^{1}H NMR: 1.6-1.7 (m, 2H); 2.0-2.1 (m, 1H); 2.3 (s, 3H); 2.4-2.7 (m, 4H); 3.2 (m, 1H); 3.6 (s, 2H); 7.3 (s, 5H).

1-Benzyl-3-methylamino-piperidine **16**. Compound **16** was prepared starting from 1-benzyl-3-piperidinone as above reported for **15** (71%). Bp = 100 °C/0.3 mmHg. ^{1}H NMR: 1.1-1.2 (m, 2H); 1.5-2.0 (m, 5H); 2.4 (s, 3H); 2.6 (m, 2H); 2.8 (m, 1H); 3.6 (s, 2H); 7.3 (s, 5H).

1-Benzyl-3-methylpropionylamino-pyrrolidine **17** *and 1-benzyl-3-methylpropionylamino-piperidine* **18**. Compounds **17** and **18** were prepared starting from **15** and **16**, respectively, as above reported for **9**. **17**: (85%) ^{1}H NMR: 1.1 (t, 3H); 1.8-2.4 (m, 8H); 2.8-3.0 (m, 4H); 3.6-3.8 (m, 2H); 7.3 (m, 5H). **18**: (90%) ^{1}H NMR: 1.1 (t, 3H); 1.4-2.0 (m, 6H); 2.4-2.8 (m, 8H); 3.6 (m, 2H); 7.3 (m, 5H).

3-Methylpropionylamino-pyrrolidine **19** *and 3-methylpropionylamino-piperidine* **20**. Compounds **19** and **20** were prepared starting from **17** and **18**, respectively, as above reported for **10**. **19**: (90%) ^{1}H NMR: 1.0 (t, 3H); 1.6-2.3 (m, 9H); 2.8-2.9 (m, 2H); 3.7-4.0 (m, 2H). **20**: (85%) ^{1}H NMR: 1.1 (t, 3H); 1.5-1.9 (m, 4H); 2.2-3.0 (m, 9H); 3.8 (m, 1H); 4.4 (m, 1H).

3-Methylpropionylamino-1-(1-phenylpropen-3-yl)-pyrrolidine **4a** *and 3-methylpropionylamino-1-(1-phenylpropen-3-yl)-piperidine* **5a**. Compounds **4a** and **5a** were prepared starting from **19** and **20**, respectively, as above reported for **3a**. **4a**: (48%) ^{1}H NMR: 1.11, 1.12 (2t, 3H); 1.6-2.8 (m, 8H); 2.88, 2.96 (2s, 3H); 3.1-3.3 (m, 2H); 4.45 (m, 0.33H); 5.24 (m, 0.67H); 6.12-6.32 (m, 1H); 6.48-6.54 (m, 1H); 7.18-7.37 (m, 5H). **5a**: (50%) ^{1}H NMR: 1.09, 1.11 (2t, 3H); 1.4-2.4 (m, 9H); 2.80, 2.82 (2s, 3H); 2.84-2.96 (m, 1H); 3.07-3.20 (m, 2H); 3.80 (m, 0.57H); 4.59 (m, 0.43H); 6.19-6.27 (m, 1H); 6.44-6.51 (m, 1H); 7.17-7.35 (m, 5H).

1-Benzyl-3-[(t-butyloxycarbonyl)methylamino]-pyrrolidine **21**. To a solution of **15** (1.33 g; 7.0 mmol) in dichloromethane (10 mL) a solution of di-*t*-butyldicarbonate (1.53 g; 7.0 mmol) in dichloromethane (10 mL) was added under nitrogen and the mixture stirred overnight. After evaporation of the solvent, the residue was purified by silica gel chromatography eluting with dichloromethane/methanol 98/2 to give 1.50 g of **21** (5.2 mmol, 74%). ^{1}H NMR: 1.4 (s, 9H); 1.6-2.4 (m, 6H); 2.8 (s, 3H); 3.6 (m, 2H); 4.8 (m, 1H); 7.3 (s, 5H).

1-Benzyl-3-[(t-butyloxycarbonyl)methylamino]-piperidine **22**. Compound **22** was prepared starting from **16** as above reported for **21** (85%). ^{1}H NMR: 1.4 (s, 9H); 1.6-2.0 (m, 7H); 2.7 (s, 3H); 2.8-3.0 (m, 2H); 3.4-3.5 (m, 2H); 4.0 (m, 1H); 7.3 (s, 5H).

3-[(t-Butyloxycarbonyl)methylamino]-pyrrolidine **23** *and 3-[(t-butyloxycarbonyl)methylamino]-piperidine* **24**. Compounds **23** and **24** were prepared starting from **21** and **22**, respectively, as above reported for **10**. **23**: (92%) ^{1}H NMR: 1.3 (s, 9H); 1.8-2.2 (m, 3H); 2.8 (s, 3H); 2.9-3.0 (m, 4H); 3.8 (bs, 1H; exch. with D_2O). **24**: (90%) ^{1}H-NMR: 1.4 (s, 9H); 1.6-1.8 (m, 4H); 2.3 (bs, 1H; exch. with D_2O); 2.4-2.8 (m, 2H); 2.9 (s, 3H); 3.0 (m, 2H); 3.8 (s, 1H).

3-[(t-Butyloxycarbonyl)methylamino]-1-propionyl-pyrrolidine **25** *and 3-[(t-butyloxycarbonyl)-methylamino]-1-propionyl-piperidine* **26**. Compounds **25** and **26** were prepared starting from **23** and **24**, respectively, as above reported for **9**. **25**: (76%) ^{1}H NMR: 1.0 (t, 3H); 1.4 (s, 9H); 1.8-2.2 (m, 4H); 2.8 (s, 3H); 3.2-3.8 (m, 4H); 4.7 (m, 1H). **26**: (80%) ^{1}H NMR: 1.0 (t, 3H); 1.4 (s, 9H); 1.8-2.0 (m, 4H); 2.3-2.4 (m, 2H); 2.8 (s, 3H); 3.8 (m, 4H); 4.8 (m, 1H).

3-Methylamino-1-propionyl-pyrrolidine **27** *and 3-methylamino-1-propionyl-piperidine* **28**. Compounds **27** and **28** were prepared starting from **25** and **26**, respectively, as above reported for **14**. **27**: (83%) ^{1}H NMR: 1.0 (t, 3H); 1.2-2.2 (m, 6H); 2.5 (s, 3H); 3.0-3.8 (m, 4H). **28**: (87%) ^{1}H NMR: 1.0 (s, 3H); 1.3-1.4 (m, 3H); 1.8-2.0 (m, 2H); 2.2-2.3 (m, 3H); 2.4 (s, 3H); 2.8-3.0 (m, 2H); 3.8-4.2 (m, 2H).

3-[Methyl-(1-phenylpropen-3-yl)amino]-1-propionyl-pyrrolidine **4b** *and 3-[methyl-(1-phenylpropen-3-yl)amino]-1-propionyl-piperidine* **5b**. Compounds **4b** and **5b** were prepared starting from **27** and **28**, respectively, as above reported for **3b**. **4b**: (54%) ^{1}H NMR: 1.13 (t, 3H); 1.7-2.3 (m, 4H); 2.28 (s, 3H); 2.9-3.9 (m, 7H); 6.20-6.28 (m, 1H); 6.48-6.54 (m, 1H); 7.19-7.35 (m, 5H). **5b**: (59%) ^{1}H NMR: 1.09, 1.10 (2t, 3H); 1.18-1.52 (m, 3H); 1.70-1.80 (m, 1H); 2.30 (s, 3H); 2.25-2.52 (m, 4H); 2.84-2.91 (m,

1H); 3.25-3.36 (m, 2H); 3.70 (m, 0.52H); 3.90 (m, 0.48H); 4.54 (m, 0.48H); 4.73 (m, 0.52H); 6.17-6.24 (m, 1H); 6.49 (d, 1H); 7.15-7.36 (m, 5H).

Binding studies. Male Sprague-Dawley rats (Charles River, Italy) weighing 180-200 g were used. Rat brain membrane binding studies were carried out as described by Gillan and Kosterlitz[8] with slight modifications. Whole brain minus cerebellum was homogenized with Polytron in 50 volumes (w/v) of 50 mM Tris HCl pH 7.4, centrifuged at 48,000 x g for 20 min at 4 °C, resuspended in 50 volumes of the same buffer and incubated at 37 °C for 45 min. After centrifugation at 48,000 x g for 20 min at 4 °C, the final pellet was resuspended in the same buffer to final concentration of 0.8 to 1.0 mg prot/mL. ^{3}H-DAMGO (2 nM) (New England Nuclear, F.R.G.) was used to label μ-receptors. Membrane suspensions were incubated with the ligand at 25 °C for 60 min in the presence or the absence of 10-5 M naloxone. Final protein concentrations were determined by the method of Lowry *et al.*[9] K_i values were calculated with the LIGAND program,[10] from displacement curves of each compound at concentrations ranging between 10^{-10} M and 10^{-4} M. Values are the mean from at least two assays.

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