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2,2,6- and 2,3,5-Trimethylpiperazines as Monocyclic Analogues of the μ-Opioid Agonist 3,8-Diazabicyclo[3.2.1]octanes: Synthesis, Modeling, and Activity

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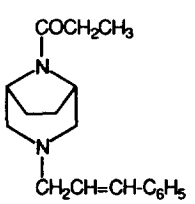
Abstract: A series of trimethylpiperazine derivatives, structurally related to the μ-agonist 3-cinnamyl-8-propionyl-3,8-diazabicyclo[3.2.1]octane, have been synthesized and tested in binding studies, using ^{3}H-DAMGO as μ-selective ligand. Their different affinity towards μ-opioid receptors has been interpreted on the basis of a molecular modeling study performed through molecular mechanics calculations and high field ^{1}H MNR spectroscopy.

3-Cinnamyl-8-propionyl-3,8-diazabicyclo[3.2.1]octane, **1**, and its isomer **2** have been shown to be potent μ-selective opioid agents.[1-3] Their affinity was shown to be comparable with that of morphine in binding experiments towards μ-receptors (^{3}H-DHM, ^{3}H-DAMGO as radioligands). Several monocyclic analogues of **1** have been synthesized and tested;[4-5] among them, the one deriving from a formal breaking of the endoethylenic bridge, *i.e.* the *cis*-2,6-dimethylpiperazine **3**, maintained a good activity whereas its isomer **4**, formally deriving from **2**, was shown to be inactive.[2-4] The activity of **3** was rationalized[4] on the basis of its preferred conformation in which the two methyl groups are forced by steric constraints to assume an axial orientation and hence to occupy the same spatial position as the endoethylenic bridge of **1**; thus, the two axial methyl groups can correctly interact with a small hydrophobic pocket of the receptor, whose existence has been proposed in a pharmacodynamical model.[6] On the contrary, the two methyl groups of **4** are equatorially oriented,[4] thus making its preferred conformation quite different from that of **2**.



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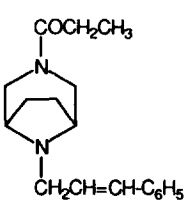

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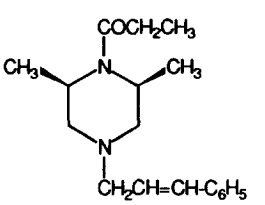

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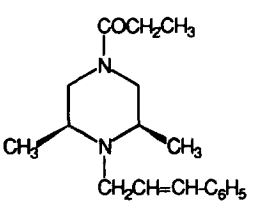

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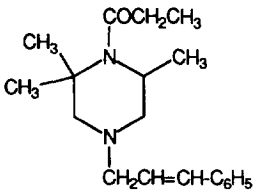

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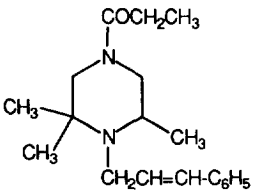

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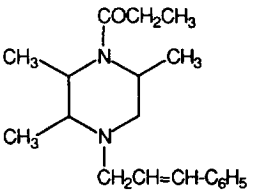

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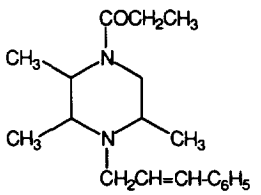

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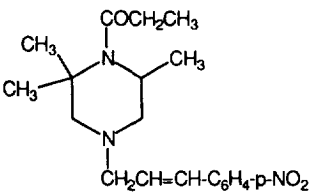

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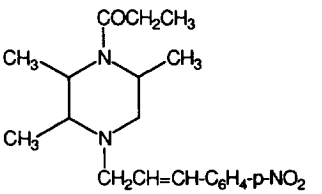

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In the course of previous studies on diazabicyclooctane analogues,[2] we also synthesized a 2,2,6-trimethylpiperazine derivative, **5**, which proved to possess interesting analgesic properties in Randall and Selitto test together with low toxicity. In order to verify whether this further substitution with a methyl group could improve the affinity and to check different spatial arrangements of the methyl groups, we have again focused our attention on compound **5** and on its unknown analogues **6-8**, all presenting an additional methyl group with respect to **3** and **4**. We report here their synthesis[7] and their modeling together with the results of binding tests for μ-opioid receptors that are discussed in comparison with the results of the conformational study. Two related compounds, **9** and **10**, were also studied to evaluate the effects of phenyl substitution with a nitro group.

RESULTS AND DISCUSSION

The synthesis of compounds **5-8** started from the known 2,2,6- and 2,3,5-trimethylpiperazines **11** and **14**. While the former was commercially available as a racemic mixture, its 2,3,5-isomer was synthesized according to a modification of a previously reported method[8] and, due to the presence of three stereogenic carbon atoms, resulted in a mixture containing about 85% of the major stereoisomer accompanied by small amounts of the other stereoisomers. This mixture was used as such for the following steps. Treatment of the trimethylpiperazines with cinnamyl chloride followed by condensation with propionic anhydride afforded the desired **5** and **7**. By inverting the sequence of the reactions, compounds **6** and **8** were easily prepared from the same precursors. Compounds **9** and **10** were obtained through similar sequences by using p-nitro-cinnamyl chloride instead of cinnamyl chloride. The 2,3,5-trimethylpiperazines **7**, **8**, and **10** resulted 90% pure by NMR analysis. The configuration of the major stereoisomer was determined as 2R,3S,5R/2S,3R,5S through molecular mechanics and ^{1}H NMR data (see below) while the configuration of the minor components was not determined.

Compounds **5-10**, together with **1** and morphine as reference compounds, were submitted to binding studies on mouse brain homogenates in the presence of ^{3}H-DAMGO as selective ligand for μ-receptors. The

Table 1. Inhibition Constants of Morphine and Compounds **1** and **5-10** towards μ-Opioid Receptors.

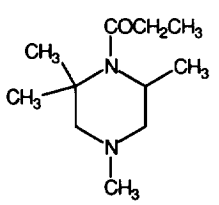
Compd	^{3}H-DAMGO[a] Ki (nM)	Compd	^{3}H-DAMGO[a] Ki (nM)
Morphine	2.8	**7**	56
1	55	**8**	188
5	40	**9**	2200
6	1540	**10**	286

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

data reported in Table 1 show that, while **5** and **7** still retain an affinity comparable with that of the models, the inversion of the substituents (**6** and **8**) caused a certain decrease of affinity more pronounced in the case of **6**. Affinity was also lowered by introduction of the nitro group on the cinnamyl moiety (see **9** vs. **5** and **10** vs. **7**).

The conformational properties of compounds **5-10** were studied on the simplified models **17-20** in which the cinnamyl or p-nitro-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 different orientations of the three methyl groups of the 2,3,5-trimethylpiperazines **7**, **8**, and **10** (and **19**, **20**) can produce eight different stereoisomers for each compound, *i.e.* four stereoisomeric racemic mixtures; each of these four cases was separately modeled. On the contrary for the 2,2,6-trimethylpiperazines **5**, **6**, and **9** (and **17**, **18**) only one racemic mixture is possible. The conformational space of the four models **17-20** was fully explored through the MM$^+$ force field of the HyperChem™ package.[9]

The following degrees of conformational freedom were considered for compounds **17-18**: chair inversion of the piperazine ring, nitrogen inversion at the tertiary amine center, E/Z isomerism at the amide function. Thus, eight conformers can be envisaged for each of the 2,2,6-trimethylpiperazines whose geometry was optimized and energy minimized. In Table 2 are reported the data of all the conformers which contribute for at least 0.5% to the overall population while in figure 1 are reported their 3D plots. It can be inferred that, while compound **17** can be described by a single conformer (**17A**) which contribute for more than 99% to the overall population, compound **18** can be described as a mixture of the two conformers **18A** and **18B**, almost equally populated, with only minor amounts of conformers **18C** and **18D** (about 1.5% each).



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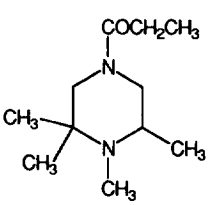

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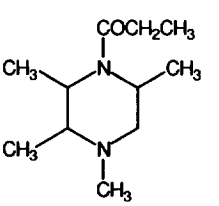

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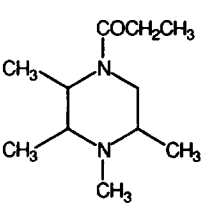

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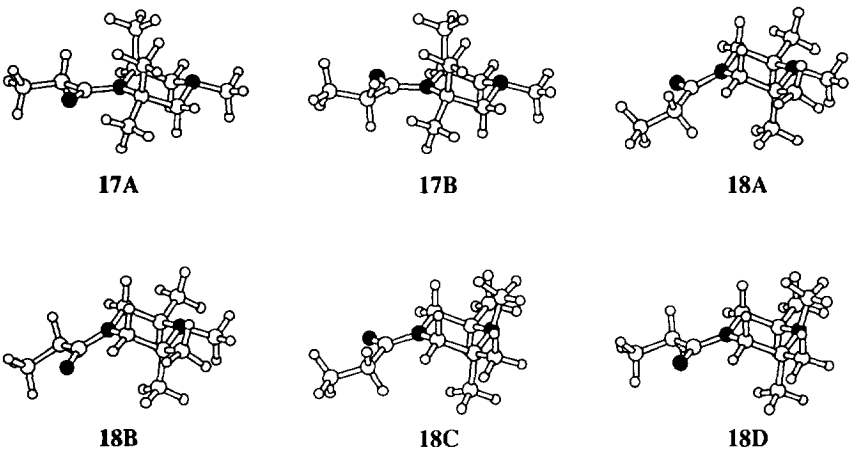

Fig. 1. Three dimensional plots of the most populated conformers of compounds **17-18**.

Modeling of compounds **19** and **20** was performed similarly, but it required the analysis of four stereoisomers for each compound (**19a-d** and **20a-d**). Table 2 and figure 2 report the results of the modeling, and show that only two conformers are usually significantly populated for each stereoisomer and in some cases (**19b** and **19d**) a single conformer is largely prevailing.

Finally, in order to confirm the calculated conformations of the model compounds **17-20**, the corresponding N-cinnamyl compounds **5-10** were submitted to high field ^{1}H NMR analysis to determine the

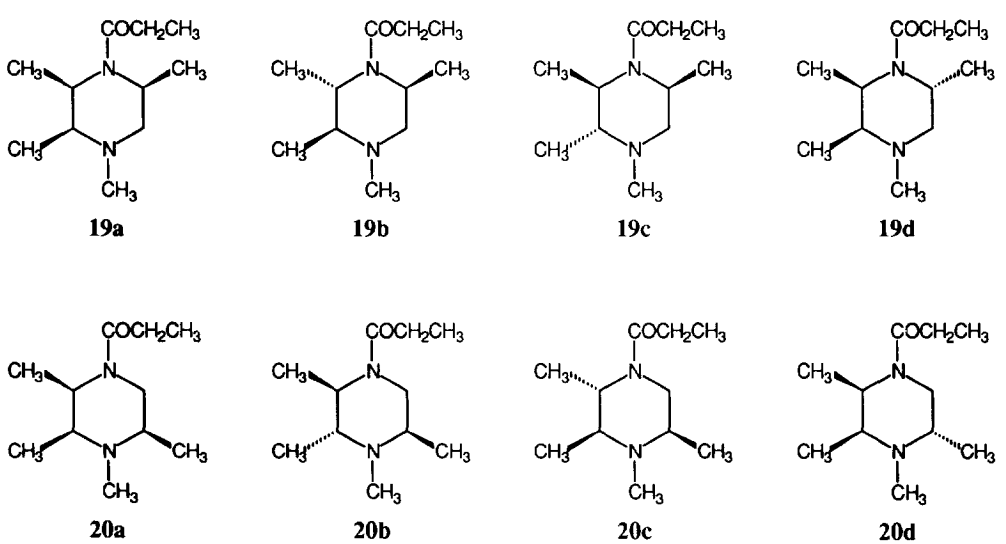

Table 2. Relative Energies (kcal/mol) and Equilibrium Percentages for the Conformers of Compounds **17-20** which Contribute for at least 0.5% to the Overall Population.

Conf.	Erel	%	Conf.	Erel	%
17A	0.00	99.5	**18A**	0.00	53.8
17B	3.14	0.5	**18B**	0.13	43.1
			18C	2.04	1.6
			18D	2.15	1.3
19aA	0.00	51.3	**20aA**	0.00	53.3
19aB	0.03	48.7	**20aB**	0.08	46.6
19bA	0.00	96.1	**20bA**	0.00	57.6
19bB	1.88	3.8	**20bB**	0.18	42.3
19cA	0.00	54.2	**20cA**	0.00	40.2
19cB	0.10	45.8	**20cB**	0.04	37.5
			20cC	0.40	20.2
19dA	0.00	88.9	**20cD**	1.84	1.7
19dB	1.22	10.9			
			20dA	0.00	51.8
			20dB	0.05	47.6
			20dC	2.60	0.6

coupling constants of vicinal protons in the six membered ring. In four cases (**6-8**, **10**) two distinct series of signals were obtained deriving from the E/Z isomerism at the C-N amide bond while in the cases of **5** and **9** the spectrum was simpler as it presented only one series of signals. However, in all the cases the coupling constants between the vicinal hydrogen atoms in the piperazine ring could be determined (Table 3). Application of the Altona equation[10] to the allowed conformations of **17-20** yielded, as weighed averages, the calculated coupling constants also reported in Table 3. The agreement between calculated and experimental values ensures that the calculated conformations of **17** and **18** exactly represent the solution conformations of **5/9** and **6**, respectively. Moreover, the experimental coupling constants of **7/10** and **8** are in close agreement with those of the stereoisomers **19a** and **20a** of compounds **19** and **20**, respectively, thus allowing to assign the relative configuration of the stereogenic centers. It is worthy of note that they have the same all cis configuration (2R,3S,5R/2S,3R,5S) as expected for products deriving from the same synthetic precursor.

The conformational results above illustrated indicate that compounds **5** and **7** have a geometry very similar to that of compound **3**. Two methyl groups are axially oriented and the third methyl group is equatorial;

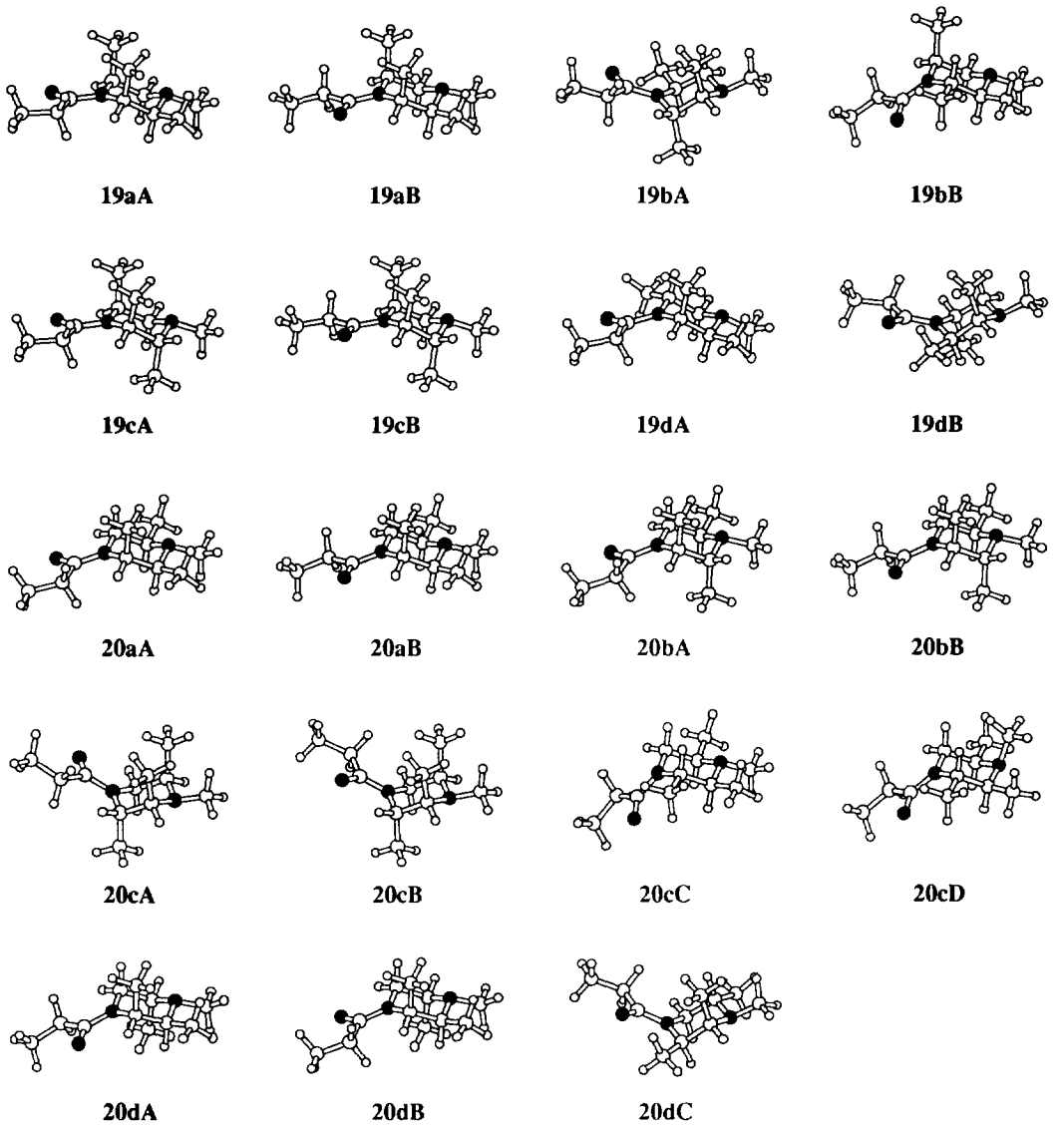

Fig. 2. Three dimensional plots of the most populated conformers of compounds **19-20**.

in the case of **5** it is in geminal position with respect to an axial methyl group while, in the case of **7**, it is in vicinal position. This additional methyl group does not disturb the interaction of these compounds with the receptor as they have an affinity quite similar to that of **3**. On the contrary compounds **6** and **8** have a geometry very similar to that of **4** as they have two methyl groups adjacent to the tertiary amine function equatorially oriented. The third methyl group is axially oriented in these cases, in position either geminal (**6**) or vicinal (**8**).

Table 3. ^1H NMR Coupling Constants (Hz) of the Vicinal Hydrogen Atoms of the Piperazine Ring: Calculated Values for Compounds **17-20** and Experimental Values for Compounds **5-10** (two values are indicated when the spectrum showed two series of signals).

Compd	$J_{5,6}$	$J_{5',6}$	Compd	$J_{5,6}$	$J_{5',6}$
17	1.5	4.8	**5**	1.0	4.0
			9	1.0	4.0
18	11.3	3.7	**6**	11.0; 10.0	3.0; 3.0

	$J_{2,3}$	$J_{5,6}$	$J_{5,6'}$		$J_{2,3}$	$J_{5,6}$	$J_{5,6'}$
19a	3.1	1.5	4.9	**7**	3.5; 3.5	1.0; 1.0	4.5; 4.5
19b	1.6	3.5	11.1	**10**	3.5; 4.0	1.0; 1.0	4.5; 4.5
19c	1.0	1.3	5.4				
19d	2.6	3.1	10.6				
20a	2.7	11.4	2.8	**8**	3.5; 3.5	11.0; 11.0	3.5; 2.0
20b	1.2	11.3	4.1				
20c	2.8	6.0	1.9				
20d	3.0	4.0	1.9				

Compounds **6** and **8** show, as **4**, a low affinity towards μ receptors, though, in the case of **8**, the loss of affinity is less pronounced, probably due to the positive influence of the axial methyl group. Compounds **9** and **10**, though presenting the correct geometry at the piperazine moiety, suffer a decrease of affinity due to the nitro group which negatively influences the interaction with the receptor.

In conclusion, our results indicate that the region below the piperazine ring should be occupied by two axial methyl substituents in order to favour a correct interaction with the μ-receptor. The side regions, on the contrary, permit only a single substitution, as two equatorial methyl groups on the two sides of the molecule make less active, or even inactive, these compounds.

EXPERIMENTAL

Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. Analyses of all new compounds were within ± 0.4 % of the theoretical values. ^1H NMR spectra were recorded in $CDCl_3$ on a Bruker AM500 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane as internal standard. TLC on silica gel plates was used to check product purity. Silica gel 60 (Merck, 70-230 mesh) was used for column chromatography.

2,3,5-Trimethylpiperazine ***14***. A solution of 2,3,5-trimethylpyrazine (5 g, 40.9 mmol) in CH_3COOH (50 mL) was hydrogenated in the presence of 10% Pd-C (0.5 g) at 70-80 °C under a pressure of 3 atm. After 18 h the hydrogen absorption was complete, the mixture was cooled, the catalyst filtered off and thourougly washed with CH_3COOH. After evaporation of the solvent, the residue was suspended in MeOH (30 mL), added of a 5 fold excess Na_2CO_3 and refluxed for 2 h. The still warm mixture was filtered and the solvent evaporated under vacuum to give **14** (2.62 g, 50%,). Oil; ^1H NMR spectrum (298 K) δ 1.02 (3H,d, J 6 Hz, 3-CH_3), 1.08 (3H,d, J 6 Hz, 5-CH_3), 1.26 (3H,d, J 5.5 Hz, 2-CH_3), 2.00 (2H, bs, NH), 2.62 (1H, t, J 11 Hz, 6-Hax), 2.96 (1H, bd, J 11 Hz, 6-Heq), 3.13 (1H, m, 5-Hax), 3.26-3.37 (2H, m, 2-H and 3-H).

General method for the synthesis of 1,4-substituted-trimethylpiperazines ***5***, ***7***, ***9***, ***10***. A mixture of **11** or **14** (2 g, 15.6 mmol), the required cinnamyl chloride (15.6 mmol) and K_2CO_3 (2.16 g, 15.6 mmol) in acetone (40 mL) was refluxed for 6 h. The residue was dissolved in 2N HCl (10 mL) and extracted with diethyl ether (2 x 10 mL) to eliminate the unreacted cinnamyl chloride. The aqueous layer was then made alkaline by 10 % Na_2CO_3 and extracted with dichloromethane (3 x 20 mL). After drying (Na_2SO_4) and evaporation of the solvent, the crude residue (**12** or **15**) was suspended in an excess of propionic anhydride and stirred at 100 °C for 1 h. After cooling, NaOH was added until basic pH, the mixture was stirred overnight at room temperature and then extracted with dichloromethane (3 x 20 mL). After drying and evaporation of the solvent, the residue was purified by silica gel chromatography eluting with dichloromethane-ethyl acetate 1:1. Compound **5** (30 %): oil; m.p. as hydrochloride 165-166° C (lit.[10] 165-167 °C); ^1H NMR (298 K) δ 1.13 (3H, t, J 7.5 Hz, $COCH_2CH_3$), 1.41 (3H, d, J 7 Hz, 6-CH_3), 1.50 (6H, s, 2-CH_3), 1.97 (1H, d, J 11.5 Hz, 3-Hax), 2.27 (1H, dd, J 11, 4 Hz, 5-Hax), 2.33 (1H, dq, J 15, 7.5 Hz, CO*CH*a), 2.45 (1H, dq, J 15, 7.5 Hz, CO*CH*b), 2.57 (1H, dd, J 11.5, 2.5, 3-Heq), 2.73 (1H, dd, J 2.5, 1 Hz, 5-Heq), 3.18 (2H, m, N-CH_2), 4.02 (1H, ddq, J 4, 1, 7 Hz, 6-Heq), 6.25 (1H, ddd, J 16, 6, 6 Hz, CH_2-*CH*=), 6.57 (1H, d, J 16 Hz, =*CH*-Ph), 7.20-7.40 (5H, m, Ph). Compound **7** (25 %): oil; ^1H NMR (273 K) δ 1.13-1.18 (6H, m, $COCH_2CH_3$ and 2-CH_3), 1.20 (1.5H, d, J 7 Hz, 3-CH_3 II isomer), 1.28 (1.5H, d, J 7 Hz, 5-CH_3 I isomer), 1.29 (1.5H, d, J 7 Hz, 3-CH_3 I isomer), 1.38 (1.5H, d, J 7 Hz, 5-CH_3 II isomer), 2.22-2.52 (4H, m, $COCH_2$, 2-Hax and 6-Hax), 2.83 (1H, dd, J 12, 1 Hz, 6-Heq), 2.98, 3.66 (2H, 2m, N-CH_2), 3.71 (0.5H, dq, J 3.5, 7 Hz, 3-Heq I isomer), 3.93 (0.5 Hz, ddq, J 4.5, 1, 7 Hz, 5-Heq II isomer), 4.45 (0.5H, dq, J 3.5, 7 Hz, 3-Heq II isomer), 4.60 (0.5H, ddq, J 4.5, 1, 7 Hz, 5-Heq I isomer), 6.27 (1H, ddd, J 16, 8, 5 Hz, CH_2-*CH*=), 6.56 (1H, d, J 16 Hz, =*CH*-Ph), 7.23-7.42 (5H, m, Ph). Compound **9** (36 %): m.p. 74-75 °C; ^1H NMR (298 K) δ 1.11 (3H, t, J 7.5 Hz, $COCH_2CH_3$), 1.39 (3H, d, J 7 Hz, 6-CH_3), 1.48 (6H, s, 2-CH_3), 1.96 (1H, d, J 11.5 Hz, 3-Hax), 2.26 (1H, dd, J 11, 4 Hz, 5-Hax), 2.32 (1H, dq, J 15, 7.5 Hz, CO*CH*a), 2.43 (1H, dq, J 15, 7.5 Hz, CO*CH*b), 2.55 (1H, dd, J 11.5, 2.5, 3-Heq), 2.72 (1H, dd, J 2.5, 1 Hz, 5-Heq), 3.17 (2H, m, N-CH_2), 4.00 (1H, ddq, J 4, 1, 7 Hz, 6-Heq), 6.44 (1H, ddd, J 16, 6, 6 Hz, CH_2-*CH*=), 6.66 (1H, d, J 16 Hz, =*CH*-Ph), 7.49 (2H, d, J 8 Hz, Ph), 8.17 (2H, d, J 8 Hz, Ph). Compound **10** (30 %): m.p. 102-103 °C; ^1H NMR (273 K) δ 1.10-1.14 (6H, m, $COCH_2CH_3$ and 2-CH_3), 1.18 (1.5H, d, J 7 Hz, 3-CH_3 II isomer), 1.25 (1.5H, d, J 7 Hz, 5-CH_3 I isomer), 1.26 (1.5H, d, J 7 Hz, 3-CH_3 I isomer), 1.35 (1.5H, d, J 7 Hz, 5-CH_3 II isomer), 2.20-2.45 (3H, m, $COCH_2$, 2-Hax), 2.29 (1H, dd J 11.5, 4.5 Hz, 6-Hax), 2.73 (1H, dd, J 11.5, 1 Hz, 6-Heq), 2.93 (1H, dd, J 14.5, 7.5 Hz, N-*CH*a), 3.65 (1H, dd, J 14.5, 5.5 Hz, N-*CH*b), 3.68 (0.5H, dq, J 3.5, 7 Hz, 3-Heq I isomer), 3.90 (0.5 Hz, ddq, J 4.5, 1, 7 Hz, 5-Heq II isomer), 4.41

(0.5H, dq, J 4, 7 Hz, 3-Heq II isomer), 4.57 (0.5H, ddq, J 4.5, 1, 7 Hz, 5-Heq I isomer), 6.45 (1H, ddd, J 16, 7.5, 5.5 Hz, CH_2-*CH*=), 6.60 (1H, d, J 16 Hz, =*CH*-Ph), 7.47 (2H, d, J 8 Hz, Ph), 8.17 (2H, d, J 8 Hz, Ph).

General method for the synthesis of 1,4-substituted-trimethylpiperazines ***6, 8***. A solution of **11** or **14** (2 g, 15.6 mmol) in dichloromethane (20 mL) was heated at 50 °C and an equimolar amount of propionic anhydride in dichloromethane (10 mL) was added dropwise. If necessary, during the addition the internal temperature was kept below 60 °C by external cooling. The mixture was then stirred for further 30 min. After cooling, the solvent was evaporated, the residue was treated with 2N HCl and extracted with dichloromethane (3 x 20 mL). The aqueous layer was then made alkaline by 4N NaOH and extracted with dichloromethane (3 x 20 mL). After drying (Na_2SO_4) and evaporation of the solvent, the crude residue (**13** or **16**) was dissolved in toluene (20 mL), equimolar amounts of cinnamyl chloride and triethylamine were added and the mixture was refluxed for a time ranging from 8 to 24 h, depending on the substrate. After cooling, the salts were filtered off, the solvent was evaporated and the residue was purified by silica gel chromatography, eluting with dichloromethane-ethyl acetate 1:1. Compound **6** (28 %): oil; ^{1}H NMR (298 K) δ 0.93, 1.03, 1.15, 1.15 (6H, 4s, 2-CH_3), 1.05 (1.5H, d, J 6.5 Hz, 6-CH_3 I isomer), 1.06 (1.5H, d, J 6 Hz, 6-CH_3 II isomer), 1.15, 1.17 (3H, 2t, J 7 Hz, $COCH_2CH_3$), 2.29-2.42 (2H, m, $COCH_2$), 2.46 (0.5H, dd, J 13, 11 Hz, 5-Hax I isomer), 2.65 (0.5H, d, J 12.5 Hz, 3-Hax II isomer), 2.78 (0.5H, m, 6-Hax I isomer), 2.83 (0.5H, m, 6-Hax II isomer), 2.90 (0.5H, dd, J 12.5, 10 Hz, 5-Hax II isomer), 3.05 (0.5H, d, J 13 Hz, 3-Hax I isomer), 3.04-3.13, 3.43-3.52 (2H, 2m, N-CH_2), 3.38 (0.5H, dd, J 13, 2.5 Hz, 3-Heq I isomer), 3.59 (0.5H, ddd, J 12.5, 3, 2.5 Hz, 5-Heq II isomer), 4.14 (0.5H, dd, J 12.5, 2.5 Hz, 3-Heq II isomer), 4.39 (0.5H, ddd, J 13, 3, 2.5 Hz, 5-Heq I isomer), 6.25 (1H, ddd, J 16, 6, 6 Hz, CH_2-*CH*=), 6.54 (1H, d, J 16 Hz, =*CH*-Ph), 7.18-7.38 (5H, m, Ph). Compound **8** (26%): oil; ^{1}H NMR (298 K) δ 1.10-1.18 (9H, m, $COCH_2CH_3$, 3-CH_3 and 5-CH_3), 1.11 (1.5H, d, J 6.5 Hz, 2-CH_3 I isomer), 1.21 (1.5H, d, J 6.5 Hz, 2-CH_3 II isomer), 2.22-2.40 (2H, m, $COCH_2$), 2.58 (0.5H, dd, J 11, 11 Hz, 6-Hax II isomer), 2.59-2.64 (1H, m, 5-Hax), 2.77 (0.5H, dq, J 3.5, 6.5 Hz, 3-Hax I isomer), 2.80 (0.5H, dq, J 3.5, 6.5 Hz, 3-Hax II isomer), 3.03 (0.5H, dd, J 11, 13.5 Hz, 6-Hax I isomer), 3.42 (0.5H, ddd, J 13.5, 3.5, 1.5 Hz, 6-Heq I isomer), 3.48-3.66 (2H, m, N-CH_2), 3.70 (0.5H, ddq, J 3.5, 1.5, 6.5 Hz, H-2eq II isomer), 4.31 (0.5H, ddd, J 11, 2.0, 1.5 Hz, 6-Heq II isomer), 4.55 (0.5H, ddq, J 3.5, 1.5, 6.5 Hz, 2-Heq I isomer), 6.32 (1H, ddd, J 16, 6.5, 6.5 Hz, CH_2-*CH*=), 6.56 (1H, d, 16 Hz, =*CH*-Ph), 7.20-7.40 (5H, m, Ph).

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[11] 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, Germany) 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 μmolar naloxone. Final protein concentrations were determined by the method of Lowry et al.[12] K_i values were calculated with the ligand program,[13] from displacement curves of each compound at a concentration range between 10^{-10} and 10^{-4} M. Values are the mean from two assays.

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