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Benzocondensed derivatives as rigid analogues of the μ-opioid agonist 3(8)-cinnamyl-8(3)-propionyl-3,8-diazabicyclo[3.2.1]octanes: synthesis, modeling, and affinity☆

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Abstract

A new series of rigid analogues (**1a–g**, **2a–g**) of the previously reported analgesic 3-cinnamyl-8-propionyl-3,8-diazabicyclo[3.2.1]octane (**I**) and its reverted isomer 3-propionyl-8-cinnamyl (**II**) were synthesized, in which the cinnamyl substituent is incorporated in benzocondensed bicyclic systems. Binding assays for the affinity towards μ receptors indicated that, while in the reverted series **2** the β-naphthylmethyl (**2d**) and the benzocycloheptenylmethyl derivative (**2g**) favorably compared with **II**, all compounds **1** displayed a μ-affinity lower than that of the parent **I**. Modeling studies suggest that the flexibility of the cinnamyl side chain plays an important role for activity.

Keywords: Opioid receptors; Analgesic activity; Diazabicyclooctanes

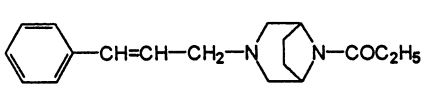
1. Introduction

The class of 3,8-diazabicyclo[3.2.1]octanes (DBO) substituted by a N3 (N8)-arylpropenyl and by a N8 (N3)-propionyl group is provided by central analgesic properties related to a selective affinity towards μ-opiod receptors. It is however to note that, though μ-affinity of the most active term is similar to or one order of magnitude lower than that of morphine, their in vivo potency was found from five to twenty times higher than that of the reference drug [1].

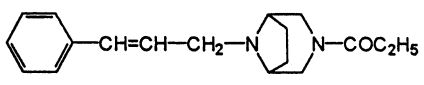
In this paper we wish to report on a structure–activity study carried out taking as models 3-cinnamyl-8-propionyl-DBO (**I**) and its reverted isomer 3-propionyl-8-cinnamyl-DBO (**II**) [1], based on the insertion of the styrene moiety of the cinnamyl substituent into benzocondensed systems, to give compounds **1a–g**, **2a–g**.

This research aimed at the evaluation of the effects on μ-affinity determined by the hampered rotation of the phenyl group out of the plane of the cinnamyl double bond. Previous studies [2] had shown that modifications of the cinnamyl substituent of DBO, like hydrogenation of the double bond or insertion on it of a methyl group left μ-affinity almost unchanged. On the contrary, it was markedly decreased by substitution of the double bond with a cyclopropane ring or inversion of its configuration from *trans* to *cis*.

This paper describes the synthesis of compounds **1a–g** and **2a–g**, together with their modeling and the evaluation of their affinity towards μ-opioid receptors.



I



II

☆ Dedicated to Professor Antonio Maccioni.

* Corresponding author.

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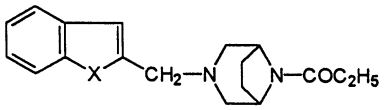
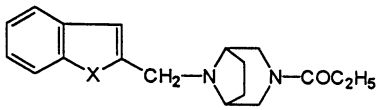


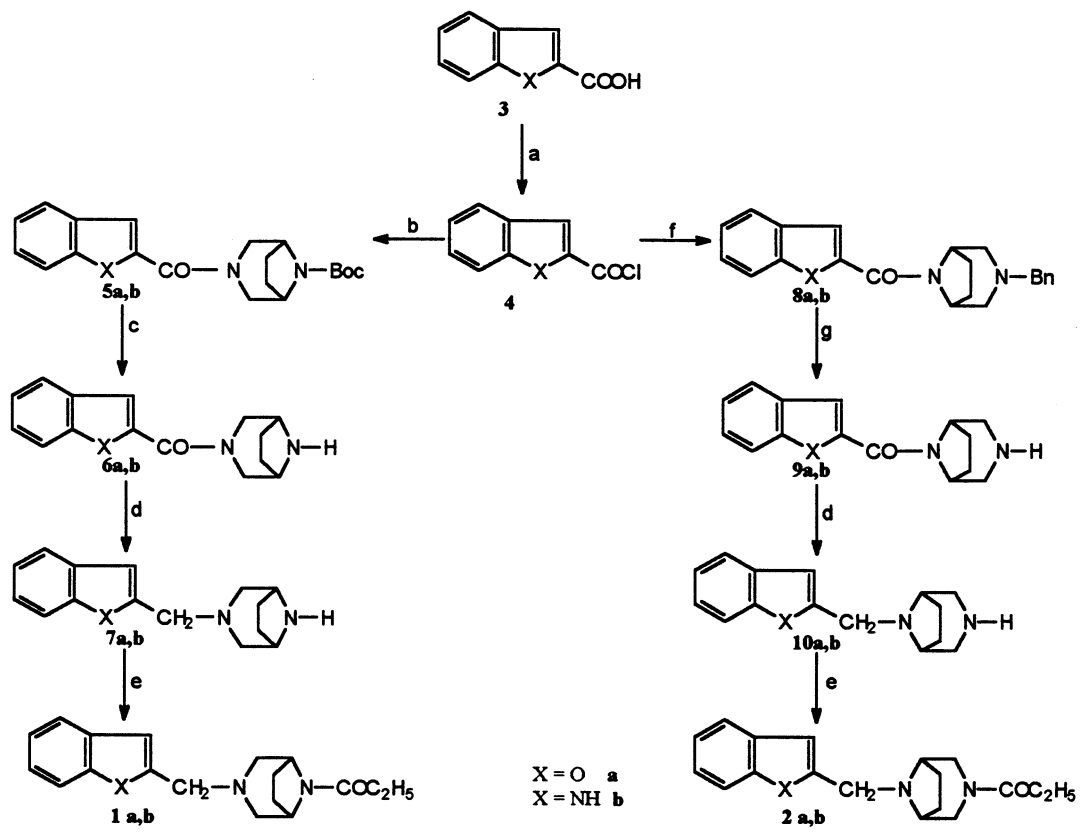
1 a-g



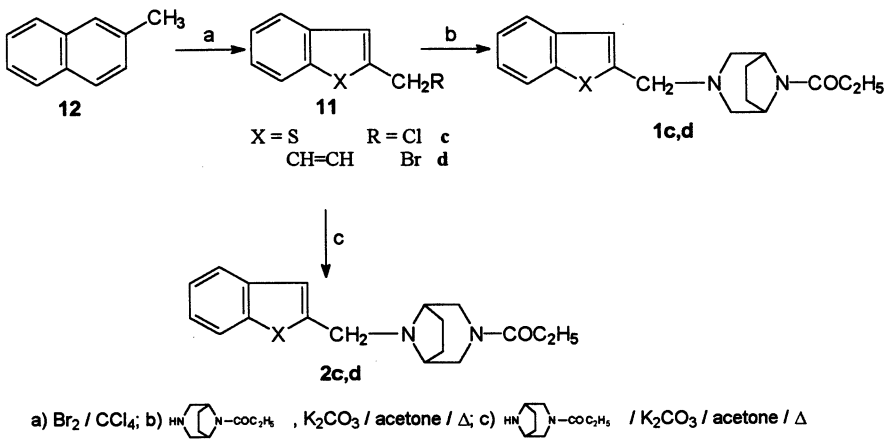
2 a-g

2. Chemistry

Compounds were prepared following three different methods, depending on the available starting material. According to Scheme 1, the appropriate carboxylic acid (**3**) was treated with thionyl chloride to give the corresponding chloride (**4**), which was condensed with 8-Boc-3,8-diazabicyclo[3.2.1]octane [3] in refluxing toluene to give the amide **5**. Deprotection by an ethereal solution of hydrochloric acid and reduction of the so obtained **6** by sodium-bis-(2-methoxyethoxy)-aluminumhydride (Red-Al®) in toluene, gave the intermediate **7**, finally acylated by propionic anhydride to the desired **1a**,**b**. The reverted **2a**,**b** were similarly obtained by condensing the appropriate acyl chloride (**4**) with 3-benzyl-DBO [4] to **8**, which was debenzylated by catalytic hydrogenation (**9**) then reduced (**10**) and finally acylated as reported for the isomers **1a**,**b**. The benzothieno derivatives (**1c**, **2c**) were synthesized as shown in Scheme 2, starting from the known 2-chloromethyl derivative **11c** [5,6], which was condensed with 8-propionyl-DBO [7] or 3-propionyl-DBO [8] to give **1c** and **2c**, respectively. The 2-naphthyl derivatives **1d**, **2d** were analogously prepared from **11d**, easily obtained by bromination of the commercial 2-methylnaphthalene. For the synthesis of **1e–g**, **2e–g** (Scheme 3), the key chloromethyl derivative (**13**) was prepared following literature methods from the appropriate bicyclic ketone [9–11] and finally condensed with 8-propionyl-DBO or 3-propionyl-DBO (see Table 1 for data).



Scheme 1.



Scheme 2.

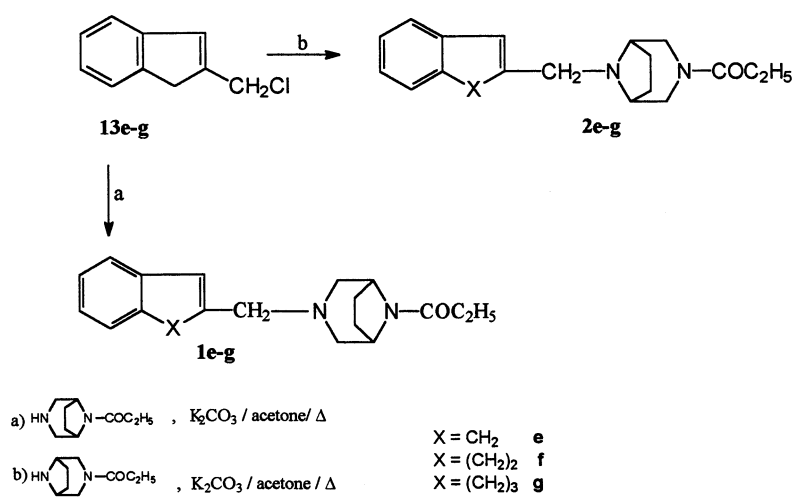
3. Binding

Compounds **1a–g**, **2a–g** together with **I** and **II** were submitted to binding studies on mouse brain homogenates in the presence of ^{3}H-DAMGO [(D-Ala^{2},*N*-Me-Phe^{4},Gly-ol^{5})enkephalin] as μ-selective ligand. Morphine was used as the reference compound. Their inhibition constants are reported in Table 2.

4. Modeling

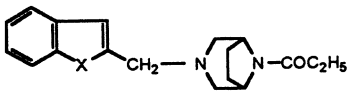
The conformational properties of compounds **1** and of their isomers **2** were investigated with the MM^{+} force field of the HyperChem™ package [12]. The 3,8-diazabicyclo[2.2.1]octane system is in both cases quite rigid. Actually, only a chair conformation can be assumed by the piperazine ring due to the presence of the endoethylenic bridge. Nitrogen inversion at the tertiary amine center and rotation around the two single bonds N3–C9 and C9–C10 represent the only degrees of freedom which could influence the conformational behavior of compounds **1** and **2**.

The exploration of the conformational space of these compounds gave in all the cases similar results. A main difference between **1** and their isomers **2** could be observed: while for the former only an equatorial orientation of the N3-aralkyl substituent is allowed, for the latter both the equatorial and the axial orientations are permitted. However, no significant difference in the conformational behavior is found among the seven compounds belonging to the **1** series as well as among those belonging to the **2** series: the freedom of rotation around the two N3–C9 and C9–C10 single bonds is high and quite similar for all the compounds. A comparison of **1** and **2** with the parent compounds **I** and **II** shows that the insertion of the styrene moiety of the cinnamyl substituent into a benzocondensed system does not significantly influence the behavior of the two above cited single bonds.

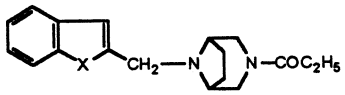


Scheme 3.

Table 1
Physicochemical properties of compounds **1** and **2**



1a–g



2a–g

Compound	X	Yield (%)	M.p. (°C)	Formula	^{1}H NMR δ
1a	O	40	88–90	$C_{18}H_{22}N_2O_2$	1.2 (t, 3H); 1.7–2.1 (m, 4H); 2.2–2.5 (m, 4H); 2.6–2.9 (m, 2H); 3.5–3.8 (m, 2H); 4.0–4.2 (bs, 1H); 4.6–4.7 (bs, 1H); 6.6 (s, 1H); 7.1–7.4 (m, 2H); 7.4–7.6 (m, 2H)
1b	NH	40	63–64	$C_{18}H_{23}N_3O$	1.2 (t, 3H); 1.8–2.1 (m, 4H); 2.1–2.4 (m, 4H); 2.6–2.8 (m, 2H); 3.6 (dd, 2H); 4.0–4.2 (bs, 1H); 4.6–4.7 (bs, 1H); 6.4 (s, 1H); 7.0–7.2 (m, 2H); 7.4 (d, 1H); 7.6 (d, 1H); 8.3 (bs, 1H exch. with D_2O)
1c	S	60	208–209[a]	$C_{18}H_{22}N_2OS{\cdot}HCl$	1.2 (t, 3H); 1.7–2.1 (m, 4H); 2.2–2.4 (m, 4H); 2.7–2.9 (m, 2H); 3.6–3.8 (m, 2H); 4.0–4.1 (bs, 1H); 4.6–4.7 (bs, 1H); 7.1 (s, 1H); 7.2–7.4 (m, 2H); 7.6–7.8 (m, 2H)
1d	CH=CH	27	205–207[a]	$C_{20}H_{24}N_2O{\cdot}HCl$	1.1 (t, 3H); 1.8–2.2 (m, 4H); 2.3–2.5 (m, 4H); 3.1–3.3 (m, 4H); 4.3–4.4 (bs, 1H); 4.5–4.7 (bs, 1H); 7.6–7.7 (m, 2H); 7.9– 8.1 (m, 4H); 8.2 (s, 1H); 10.9–11.0 (s, 1H exch. with D_2O)
1e	CH_2	35	193–195[a]	$C_{19}H_{24}N_2O{\cdot}HCl$	1.17 (t, 3H); 1.7–2.5 (m, 8H); 2.72 (q, 2H); 3.35 (s, 2H); 3.38 (s, 2H); 4.08 (bs, 1H); 4.65 (bs, 1H); 6.66 (s, 1H); 7.10–7.50 (m, 4H)
1f	$(CH_2)_2$	31	217–218[a]	$C_{20}H_{26}N_2O{\cdot}HCl$	1.2 (t, 3H); 1.7–1.9 (m, 4H); 2.1–2.4 (m, 6H); 2.6–2.9 (m, 4H); 3.02 (s, 2H); 4.1 (bs, 1H); 4.7 (bs, 1H); 6.3 (s, 1H) 7.0–7.1 (m, 4H)
1g	$(CH_2)_3$	16	155–156[a]	$C_{21}H_{28}N_2O{\cdot}HCl$	1.2 (m, 3H); 1.8–2.1 (m, 6H); 2.2–2.4 (m, 6H); 2.8–2.9 (m, 4H); 3.0 (d, 1H); 4.1 (bs, 1H); 4.4 (d, 1H); 4.7 (bs, 1H); 6.4 (s, 1H); 7.1–7.4 (m, 4H)
2a	O	50	156–157[a]	$C_{18}H_{22}N_2O_2{\cdot}HCl$	1.1 (t, 3H); 1.6–2.1 (m, 2H); 2.2–2.5 (m, 2H); 3.2–3.7 (m, 3H); 3.6–4.1 (m, 4H); 4.2–4.3 (m, 1H); 4.5 (d, 2H); 7.2–7.5 (m, 3H); 7.6 (d, 1H); 7.8 (d, 1H): 11.5 (bs, 1H exch. with D_2O)
2b	NH	45	119–121[a]	$C_{18}H_{23}N_3O{\cdot}HCl$	1.1 (t, 3H); 1.6–2.0 (m, 2H); 2.2–2.4 (m, 2H); 3.2–3.3 (m, 1H); 3.4 (q, 2H); 3.6–3.8 (m, 4H); 4.2–4.4 (m, 1H); 7.0–7.3 (m, 2H); 7.4 (d, 1H); 7.6 (d, 1H); 11.4 (s, 1H exch. with D_2O)
2c	S	60	108–109[a]	$C_{18}H_{22}N_2OS{\cdot}HCl$	1.1 (t, 3H); 1.5–1.7 (m, 2H); 1.8–2.1 (m, 2H); 2.1–2.4 (m, 2H); 2.9–3.0 (m, 1H); 3.2–3.4 (m, 4H); 3.8 (s, 2H); 4.1–4.3 (m, 1H); 7.1 (s, 1H); 7.2–7.4 (m, 2H); 7.7 (d, 1H); 7.8 (d, 1H)
2d	CH=CH	40	195–200[a]	$C_{20}H_{24}N_2O{\cdot}HCl$	1.1 (t, 3H); 1.8–2.0 (m, 2H); 2.2–2.4 (m, 2H); 3.1–3.2 (m, 1H); 3.2–3.4 (m, 2H); 3.6–3.8 (m, 4H); 4.2–4.3 (m, 1H); 4.5 (s, 2H); 7.6–7.7 (m, 2H); 7.8–8.1 (m, 4H); 8.2 (s, 1H); 10.8 (bs, 1H exch. with D_2O)
2e	CH_2	20	182–184[a]	$C_{19}H_{24}N_2O{\cdot}HCl$	1.2 (t, 3H); 1.5–2.1 (m, 6H); 2.3 (q, 2H); 2.9 (d, 1H); 3.2 (s, 2H); 3.4–3.5 (m, 4H); 4.2 (d, 1H); 6.7 (s, 1H); 7.2–7.4 (m, 4H)
2f	$(CH_2)_2$	58	183–185[a]	$C_{20}H_{26}N_2O{\cdot}HCl$	1.1 (t, 3H); 1.5–1.6 (m, 2H); 1.9–2.0 (m, 2H); 2.3–2.4 (m, 4H); 2.8–2.9 (m, 2H); 3.1 (s, 2H); 3.2–3.4 (m, 5H); 4.2 (d, 1H); 6.4 (s, 1H) 7.0–7.1 (m, 4H)
2g	$(CH_2)_3$	40	192–193[a]	$C_{21}H_{28}N_2O{\cdot}HCl$	1.2 (t, 3H); 1.6–1.7 (m, 2H); 1.9–2.1 (m, 4H); 2.2–2.5 (m, 4H); 2.7–3.0 (m, 4H); 3.2–3.5 (m, 5H); 4.2 (d, 1H); 6.4 (s, 1H); 7.0–7.2 (m, 4H)

[a] As the hydrochloride.

However, this structural modification involves two main differences with respect to the cinnamyl: the hampered rotation of the phenyl group out of the plane of the double bond and the modified orientation of the bicyclic substituent. As examples, in Fig. 1 the superimpositions of the most populated conformers of compounds **1a**, **1b**, **1g** are reported with respect to **I**. Besides a complete correspondence of the diazabicyclooctane moiety and of the N3–C9, C9–C10, C10–C11 bonds, different orientations of the benzo ring of **1** with respect to the phenyl ring of **I** can be observed.

5. Discussion

The data reported in Table 2 clearly show that none of compounds **1** is as active as the model **I**. This lowering of affinity could be due to the fact that the

Table 2
Inhibition constants of morphine, **I**, **II** and compounds **1** and **2** towards μ-opioid receptors

Comp.	^{3}H-DAMGO[a] K_i (nM)	Comp.	^{3}H-DAMGO[a] K_i (nM)
I[b]	55	**II**[b]	160
1a	2500	**2a**	3050
1b	120	**2b**	404
1c	2200	**2c**	2400
1d	328	**2d**	126
1e	303	**2e**	766
1f	408	**2f**	215
1g	213	**2g**	150
Morphine	2.8		

[a] K_i values were calculated with the LIGAND program [15], based on a K_d value of 1 nM for ^{3}H-DAMGO. Values are the mean from two experiments.
[b] See Ref. [1].

aromatic moieties of **1** cannot accommodate into the proper pocket of the active site of the receptor as easily as the phenyl ring of **I**. However, different degrees of potency are shown along the series. In particular, while

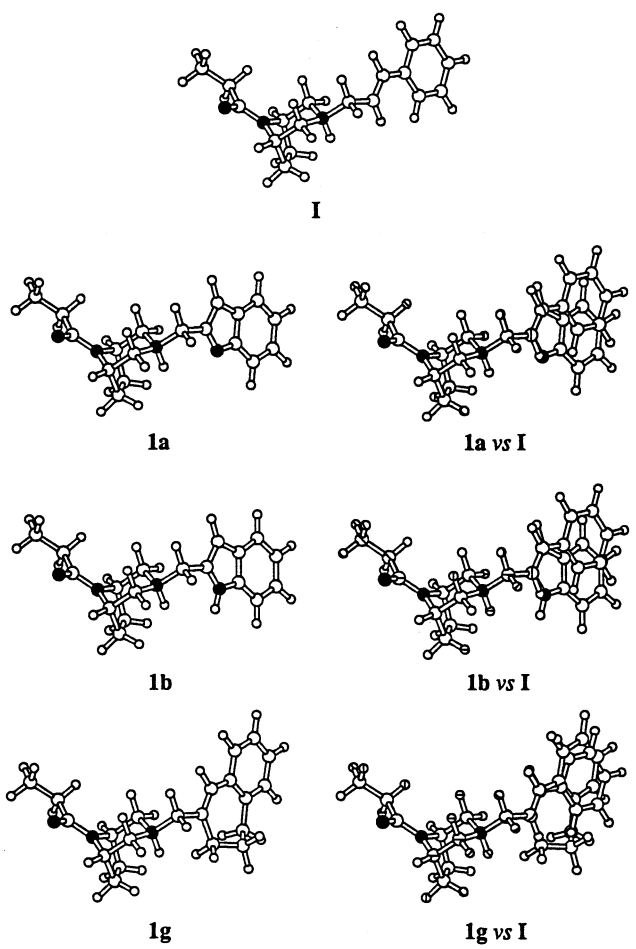


Fig. 1. 3D plots of the most populated conformations of compounds **1a**, **1b** and **1g** in comparison with the corresponding conformation of **I**.

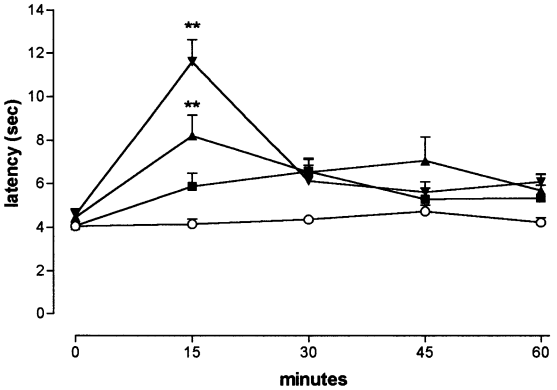


Fig. 2. Dose–response curve of **1b** in the hot plate test. Mice received **1b** sc at the indicated dose and were controlled every 15 min. Each point represents the mean ± SEM of eight mice per group. ** $P <$ 0.01 vs. vehicle. ○, vehicle; ■, **1b** 10 mg kg^{-1} sc; ▲, **1b** 20 mg kg^{-1} sc; ▼, **1b** 40 mg kg^{-1} sc.

the benzofurane derivative (**1a**) as well as the benzothiophene analog (**1c**) have K_is in the micromolar range, the indolyl (**1b**) as well as the indenyl (**1e**) and the benzocycloheptenyl derivative (**1g**) are by one order of magnitude more potent. As far as compounds **2** are concerned, they show a slightly better profile, when compared to their corresponding model **II**. The β-naphthyl (**2d**), the dihydronaphthyl (**2f**), and the benzocycloheptenyl derivative (**2g**) favorably compare to the reference compound **II**. Also in this series, however, a large range of potency was observed, K_is ranging from 150 to 3050 nM.

The most significant derivatives (**1b**, **2d**) were also tested in vivo, in the hot plate method. Though their binding values were fully comparable, when administered subcutaneously **1b** was found by about 7-fold more potent than **2d** (ED_{50} 3.3 and 24.0 mg kg^{-1}, respectively) (see Figs. 2 and 3). However, both **1b** and

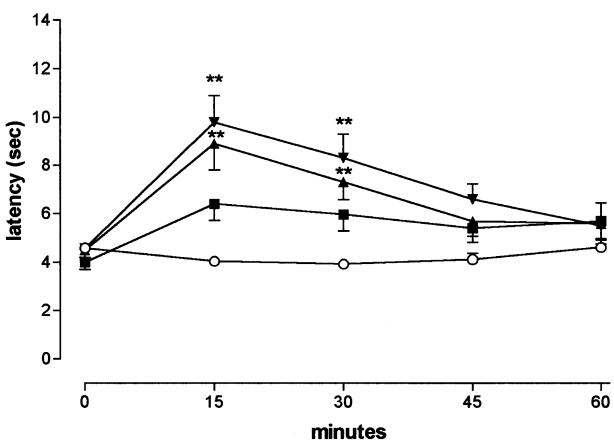


Fig. 3. Dose–response curve of **2d** in the hot plate test. Mice received **2d** sc at the indicated dose and were controlled every 15 min. Each point represents the mean ± SEM of eight mice per group. ** $P <$ 0.01 vs. vehicle. ○, vehicle; ■, **2d** 10 mg kg^{-1} sc; ▲, **2d** 20 mg kg^{-1} sc; ▼, **2d** 40 mg kg^{-1} sc.

Table 3
Elemental analyses

Compound	Mol. wt.	Calc. (found) (%)				
		C	H	N	S	Cl
1a	298.38	72.45 (72.05)	7.43 (7.65)	9.39 (9.06)		
1b	297.38	72.69 (72.30)	7.79 (7.74)	14.13 (13.99)		
1c	350.91	61.61 (61.35)	6.60 (6.43)	7.98 (7.58)	9.13 (8.83)	10.11 (10.27)
1d	344.87	69.65 (70.03)	7.31 (6.99)	8.12 (8.02)		10.28 (10.09)
1e	332.86	68.55 (68.63)	7.57 (7.65)	8.42 (8.41)		10.65 (10.81)
1f	346.90	69.25 (69.54)	7.85 (7.83)	8.08 (8.09)		10.22 (10.15)
1g	360.93	69.88 (69.50)	8.10 (8.07)	7.76 (7.40)		9.82 (9.56)
2a	334.85	64.56 (64.17)	6.92 (7.01)	8.37 (8.43)		10.59 (10.78)
2b	333.87	64.75 (64.68)	7.25 (7.29)	12.59 (12.60)		10.62 (10.24)
2c	350.84	61.61 (61.95)	6.60 (6.28)	7.98 (8.02)	9.13 (9.45)	10.11 (10.35)
2d	344.87	69.65 (70.27)	7.31 (7.27)	8.12 (8.31)		10.28 (10.19)
2e	332.86	68.55 (68.75)	7.57 (7.68)	8.42 (8.57)		10.65 (10.79)
2f	346.90	69.25 (69.43)	7.84 (8.03)	8.07 (7.88)		10.22 (10.34)
2g	360.93	69.88 (70.11)	8.10 (8.23)	7.76 (7.56)		9.82 (9.75)

2d were less active than their corresponding models **I** and **II** (ED_{50} 1.1 and 16.0 mg kg^{-1}) in this test [1].

In conclusion, the present study gives a further contribution to the elucidation of the structural and geometrical requirements of the cinnamyl chain of **I** and **II**. In particular, it indicates that a precise orientation of the phenyl ring is indispensable for a good affinity, as already evidenced by the lowering of activity observed when going from *trans*- to *cis*-cinnamyl [2]. Moreover, it suggests that the phenyl ring and the double bond of the cinnamyl moiety in **I** and **II** should not be coplanar to allow a good interaction with the μ-receptor.

6. Experimental

6.1. Chemistry

Melting points were determined on a Büchi 510 capillary melting points apparatus and are uncorrected. Analyses indicated by the symbols were within ± 0.4 of the theoretical values. ^{1}H NMR spectra were recorded on a Bruker AC200 spectrometer; chemical shifts are reported as δ (ppm) relative to tetramethylsilane (TMS) 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. The structures of all compounds were consistent with their analytical (see Table 3) and spectroscopic data.

6.1.1. 3-Aryloyl-8-Boc-3,8-diazabicyclo[3.2.1]octanes (**5a,b**)

The appropriate acid **3** (0.01 mol) was treated with an excess thionyl chloride in dichloromethane and stirred overnight at room temperature (r.t.). A mixture of the obtained chloride **4** (0.01 mol), 8-Boc-3,8-diazabicyclo[3.2.1]octane [3] (0.01 mol) and triethylamine (0.01 mol) in toluene (20 ml) was stirred overnight at r.t. The inorganic salts were filtered off, the solvent evaporated to give **5a**,**b** which were used without further purification.

For **5a**: yield 65%, m.p. 109°C, ^{1}H NMR δ: 1.5 (s, 9H); 1.7–2.1 (m, 4H); 2.9–3.3 (m, 1H); 3.4.–3.7 (m, 1H); 4.0–4.6 (m, 4H); 7.2–7.8 (m, 5H).

For **5b**: yield 60%, m.p. 227°C, ^{1}H NMR δ: 1.5 (s, 9H); 1.6–2.0 (m, 4H); 3.0–3.8 (m, 2H); 4.0–4.5 (m, 4H); 6.9 (s, 1H); 7.1 (t, 1H); 7.2 (t, 1H); 7.5 (d, 1H); 7.7 (d, 1H); 11.7 (s, 1H, exch. with D_2O).

6.1.2. 3-Aryloyl-3,8-diazabicyclo[3.2.1]octanes (**6a,b**)

To a cooled solution of the required **5a**,**b** (0.003 mol) in diethyl ether (10 ml) a solution of HCl in diethyl ether was added until the pH was about 1 and the mixture stirred overnight at r.t. Sodium hydroxide (6 N) was then added until pH 9, the layers separated and the aqueous phase extracted twice by diethyl ether (2×20 ml). The reunited organic layers where dried over sodium sulfate and the solvent evaporated to give **6a**,**b**, which were used without further purification.

For **6a**: yield 70%, m.p. 125–127°C, ^{1}H NMR δ: 1.8 (m, 4H); 2.7 (s, 1H exch. with D_2O); 3.0–3.2 (m, 1H); 3.4–3.8 (m, 3H); 4.0–4.6 (m, 2H); 7.2–7.4 (m, 3H); 7.5 (d, 1H); 7.6 (d, 1H).

For **6b**: yield 75%, m.p. 186–188°C, ^{1}H NMR δ: 1.6–2.2 (m, 4H); 3.6–3.9 (m, 2H); 4.0–4.2 (bs, 2H); 4.3–4.5 (m, 2H); 6.9 (s, 1H); 7.1 (t, 1H); 7.3 (t, 1H); 7.5 (d, 1H); 7.7 (d, 1H); 9.4–10.0 (bs, 1H, exch. with D_2O); 11.7 (s, 1H exch. with D_2O).

6.1.3. 3-Arylmethyl-3,8-diazabicyclo[3.2.1]octanes (**7a,b**)

To an ice-cooled solution of the required **6a**,**b** (0.02 mol) in anhydrous toluene (40 ml) a 65% (w/w) solution

of bis-(2-methoxyethoxy)aluminum hydride (Red-Al®) (0.04 mol) in toluene was added. The mixture was brought to r.t. and monitored by TLC (CH_2Cl_2/MeOH 7/3) until the starting material disappeared. The mixture was again cooled at 0°C and cautiously added of a 10% aqueous solution of sodium carbonate until pH 10, the inorganic salts were filtered off and the aqueous layer repeatedly extracted with dichloromethane. After drying over sodium sulfate, the solvent was evaporated to give the desired **7a**,**b**, which were used without further purification.

For **7a**: yield 80%, oil, ^{1}H NMR δ: 1.8–2.1 (m, 4H); 2.6 (s, 1H, exch. with D_2O); 2.8 (s, 2H); 2.9–3.2 (m, 1H); 3.5–3.8 (m, 3H); 4.0–4.6 (m, 2H); 7.2–7.35 (m, 3H); 7.5 (d, 1H); 7.6 (d, 1H).

For **7b**: yield 90%, oil, ^{1}H NMR δ: 1.8–2.2 (m, 5H); 2.9 (s, 2H); 3.1–3.4 (m, 2H); 3.5 (s, 1H); 3.5–3.7 (m, 2H); 4.3–4.5 (m, 2H); 6.8 (s, 1H); 7.1 (d, 1H); 7.2–7.4 (m, 1H); 7.45 (d, 1H); 7.6 (d.1H); 9.5 (s, 1H exch. with D_2O).

*6.1.4. 3-Arylmethyl-8-propionyl-3,8-diazabicyclo-[3.2.1]octanes (**1a,b**)*

To an ice cooled solution of the required **7a**,**b** (0.02 mol) in CH_2Cl_2 (20 ml) a solution of propionic anhydride (0.2 mol) in CH_2Cl_2 (10 ml) was added and the mixture refluxed for 1 h. After cooling, the suspension was made alkaline by 20% NaOH and stirred until excess propionic anhydride was destroyed. The aqueous layer was extracted with dichloromethane (2 × 10 ml), the solvent dried (Na_2SO_4) and evaporated to give **1a**,**b**. Compounds were purified by silica gel chromatography, eluting with CH_2Cl_2/MeOH 98/2 (see Table 1 for data).

*6.1.5. 8-Aryloyl-3-benzyl-3,8-diazabicyclo[3.2.1]octanes (**8a,b**)*

A mixture of the appropriate **4** (0.01 mol), 3-benzyl-3,8-diazabicyclo[3.2.1]octane [4] (0.01 mol) and triethylamine (0.01 mol) in diethyl ether (20 ml) was stirred for 2 h at r.t. The suspension was added of water (10 ml) and stirred for further 10 min then extracted with dichloromethane (3 × 10 ml). The organic layer was dried over sodium sulfate and the solvent evaporated to give **8a**,**b** which were purified by silica gel chromatography, eluting with cyclohexane/ethyl acetate 7/3.

For **8a**: yield 75%, m.p. 108–111°C, ^{1}H NMR δ: 2.0–2.3 (m, 5H); 2.8 (s, 2H); 3.0–3.2 (m, 2H); 4.1–4.3 (m, 2H); 4.8 (s, 2H); 4.9–5.0 (m, 2H); 7.3–7.7 (m, 10H).

For **8b**: yield 80%, m.p. 152–154°C, ^{1}H NMR δ: 1.8–2.1 (m, 5H); 2.7–2.9 (m, 2H); 3.0–3.2 (m, 2H); 4.7 (s, 2H); 4.8 (s, 2H); 4.9–5.1 (m, 2H); 6.7 (s, 1H); 7.0–7.6 (m, 8H); 7.7 (d, 1H); 9.7 (bs, 1H exch. with D_2O).

*6.1.6. 8-Aryloyl-3,8-diazabicyclo[3.2.1]octanes (**9a,b**)*

A mixture of the required **8a**,**b** (0.01 mol) and 10% Pd–C (10/1 w/w) in ethanol (35 ml) was hydrogenated at r.t. After the hydrogen absorption ceased, the catalyst was filtered off and the solvent evaporated to give **9a**,**b**, which were purified by silica gel chromatography, eluting with dichloromethane/methanol 95/5.

For **9a**: yield 53%, m.p. 167–168°C, ^{1}H NMR δ: 2.1–2.3 (m, 5H); 3.1–3.3 (m, 2H); 4.1–4.3 (m, 2H); 4.9–5.1 (m, 2H); 7.3–7.5 (m, 4H); 7.6 (d, 1H).

For **9b**: yield 40%, m.p. 162–164°C.

*6.1.7. 8-Arylmethyl-3,8-diazabicyclo[3.2.1]octanes (**10a,b**)*

The compounds were prepared as above reported for the isomers **7**, starting from the appropriate **9a**,**b**.

For **10a**: yield 45%, oil, ^{1}H NMR δ: 2.1–2.4 (m, 5H); 3.2–3.4 (m, 2H); 2.6–2.8 (m, 2H); 4.2–4.3 (m, 2H); 4.8–5.0 (m, 2H); 7.2–7.5 (m, 4H); 7.6 (d, 1H).

For **10b**: yield 40%, oil, ^{1}H NMR δ: 1.8–2.2 (m, 5H); 2.6–2.7 (m, 2H); 2.8–3.0 (m, 2H); 3.0–3.3 (m, 2H); 4.8–5.0 (m, 2H); 6.7 (s, 1H); 7.0–7.1 (m, 1H); 7.2–7.3 (m, 1H); 7.5 (d, 1H); 7.8 (d, 1H); 9.8 (bs, 1H, exch. with D_2O).

*6.1.8. 3-Propionyl-8-arylmethyl-3,8-diazabicyclo[3.2.1]-octanes (**2a,b**)*

The compounds were prepared as above reported for the isomers **1**, starting from the required **10a**,**b** (see Table 1 for data).

*6.1.9. 2-Bromomethyl-naphthalene (**11d**)*

To a solution of **12** (2.5 g, 0.018 mol) in CCl_4 (25 ml), bromine (2.8 g, 0.018 mol) in CCl_4 was added. During addition the mixture was irradiated with a 500 W lamp. The mixture was stirred for 1 h at r.t., the solvent evaporated and the residue purified by silica gel chromatography, eluting with petroleum ether/diethyl ether (9/1 w/w) to give **11d**.

Yield 71%, oil, ^{1}NMR δ: 4.65 (s, 2H); 7.5–7.6 (m, 3H); 7.8–7.9 (m, 4H).

*6.2. 3-Arylmethyl-8-propionyl-3,8-diazabicyclo[3.2.1]-octanes (**1c–g**)—general method*

An equimolar mixture of the required halide **11** [5,6], **13** [9–11] (0.001 mol), 8-propionyl-3,8-diazabicyclo[3.2.1]octane [7] (0.16 g), and K_2CO_3 (0.14 g) in acetone (10 ml) was refluxed overnight. After cooling, the salts were filtered off, the solvent evaporated and the residue purified by flash chromatography, eluting with dichloromethane/methanol (98/2 w/w). If required, the so obtained **1c–g** could be transformed into the corresponding hydrochloride by treatment with a solution of hydrochloric acid in diethyl ether (see Table 1 for data).

6.3. 8-Arylmethyl-3-propionyl-3,8-diazabicyclo[3.2.1]-octanes (**2c–g**)

Compounds **2c–g** were obtained according to the procedure above reported for their isomers **1**, starting from 3-propionyl-3,8-diazabicyclo[3.2.1]octane [8].

6.4. 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 with slight modifications [13]. Whole brain minus cerebellum was homogenized with Polytron in 50 volumes (w/v) of 50 mM Tris–HCl pH 7.7, centrifuged at $48\,000 \times g$ for 20 min at 4°C, resuspended in 50 volumes of the same buffer and incubated for 45 min at 37°C. After centrifugation at $48\,000 \times g$ for 20 min at 4°C, the final pellet was resuspended in the same buffer to final concentration of 0.8–1.0 mg protein ml^{-1}. ^{3}H-DAMGO (2 nM) (New England Nuclear, Germany) was used to label μ-receptors. Membrane suspensions were incubated with the ligand for 60 min at 0°C in the presence or the absence of 10 μM of naloxone. Final protein concentrations were determined by the method of Lowry et al. [14]. K_i values were calculated with the LIGAND program [15], from displacement curves of each compound at a concentration range between 10^{-10} and 10^{-4} M. Values are the mean from two assays.

6.5. Antinociception

Male Albino-Swiss mice weighing 20–25 g (Charles River, Italy) were used. Antinociception was estimated by means of the hot plate method described by Oden and Oden [16]. The effect of compounds on the reaction time of mice placed on a hot plate, thermostatically maintained at 65°C were determined. The time at which mice displayed a nociceptive response, licking the front paws, fanning the hind paws or jumping was recorded and the animal was removed from the hot plate. In each case, post treatment hot plate latencies were determined at the indicated times for every experiment and 14 s was set as the cut off time to avoid damage. To establish the dose–response curve, at least four drug doses were used on eight to ten mice per each dose. The 50% antinociceptive doses (ED_{50}) and their 95% confidence intervals were determined by the method of Litchfield and Wilcoxon [17].

References

[1] G. Cignarella, D. Barlocco, M.E. Tranquillini, A. Volterra, N. Brunello, G. Racagni, Pharm. Res. Commun. 20 (1988) 383.
[2] D. Barlocco, G. Cignarella, S. Villa, XI Convegno Nazionale-Divisione di Chimica Farmaceutica, Bari, Oct. 1994.
[3] D. Barlocco, G. Cignarella, D. Tondi, P. Vianello, S. Villa, A. Bartolini, C. Ghelardini, N. Galeotti, D. Colombo, L. Toma, J. Med. Chem. 41 (1998) 674.
[4] G. Cignarella, G.G. Nathansohn, J. Org. Chem. 26 (1961) 2747.
[5] F.F. Blicke, US Patent 2,533,086, 1950; Chem. Abstr. 45 (1949) 2508e.
[6] F.F. Blicke, US Patent 2,533,087, 1950; Chem. Abstr. 45 (1949) 2508g.
[7] G. Cignarella, E. Occelli, E. Testa, J. Med. Chem. 8 (1965) 326.
[8] G. Cignarella, E. Testa, C.R. Pasqualucci, Tetrahedron 19 (1963) 143.
[9] H.O. House, C.B. Hudson, J. Org. Chem. 35 (1979) 647.
[10] J. Vebrel, R. Carrie, Bull. Soc. Chem. Fr. II 3-4 (1982) 116.
[11] R.B. Miller, J.M. Frincke, J. Org. Chem. 45 (1980) 5312.
[12] HyperChem™ is a package from HyperCube, Inc., Canada.
[13] M.G.G. Gillan, H.W. Kosterlitz, J. Pharmacol. 77 (1982) 461.
[14] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J.J. Randall, Biol. Chem. 193 (1951) 265.
[15] P.J. Munson, D. Rodbard, Anal. Biochem. 107 (1980) 220.
[16] D.L. Oden, K.L Oden, Life Sci. 31 (1982) 1245.
[17] J.T. Litchfield, F. Wilcoxon, J. Pharmacol. Exp. Ther. 96 (1949) 99.