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Il Farmaco 53 (1998) 557–562

IL FARMACO

Synthesis and μ-opioid receptor affinity of a new series of nitro substituted 3,8-diazabicyclo[3.2.1]octane derivatives

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Received 4 July 1998; accepted 1 September 1998

Abstract

A new series of analogues (**1c–j**; **2c–i**) of the previously reported analgesic 3,8-diazabicyclo[3.2.l]octanes (**1a,b**; **2a,b**) was synthesized and tested for their affinity towards μ-opioid receptors. Modifications were introduced either at the cinnamyl or the acyl side chains. The majority of the new compounds, with the exception of **1c,j** and **2c**, showed K_i values better or comparable with those of the models. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Opioid receptors; Analgesic activity; Diazabicyclooctane

1. Introduction

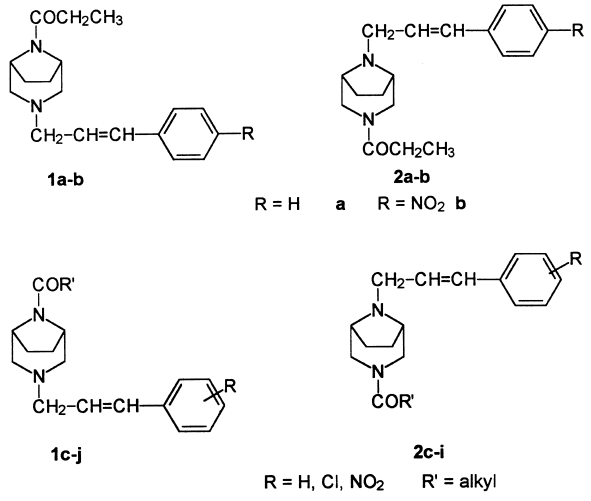
The development of potent opioid agonist and antagonist with high specificity for each of the three major receptor types (μ, δ, κ) continues to be of great concern in opioid pharmacology.

In previous papers [1,2] we described the 3-(cinnamyl)-8-propionyl-3,8-diazabicyclo[3.2.1]octane (**1a**) as a potent, μ-selective opioid agonist ($K_i = 55.2$ nM) showing significant analgesic activity ($ED_{50} = 1.1$ mg/kg). Inversion of the substituent at the N_3 and N_8 gave a compound **2a** still provided with high μ-affinity and selectivity ($K_i = 160$ nM), though with lower analgesic potency ($ED_{50} = 16.0$ mg/kg). Insertion of a *para*-nitro group on the phenyl ring of both **1a** and **2a** led to compounds **1b** and **2b**, respectively, which, besides in vitro higher μ/δ selectivity with respect to morphine, showed in vivo better analgesic properties and minor side-effects [3]. It is interesting to note that contrary to the unsubstituted **1a** and **2a**, inversion of **1b** to give **2b**, increased both μ affinity and analgesic potency. A possible explanation for the remarkable activity of these compounds was based on the existence of an additional H-bond between one of the nitro oxygens and a complementary site on the receptor [4].

To further explore the role of the nitro group, we have now synthesized and tested several analogues (**1c–j**; **2c–i**) of **1a,b** and **2a,b** for their affinity towards μ-opioid receptors. In particular, the following modifications were considered:

1. shift of the nitro group to different positions of the phenyl ring (**1c,d**; **2c,d**);
2. insertion of a chlorine atom adjacent the nitro group (**1e–g**; **2e–g**);
3. elongation of the acyl chain up to 5 atoms (**1h–j**; **2h,i**).

This paper describes the synthesis of compounds **1** and **2** as well as their binding affinity towards μ-receptors.



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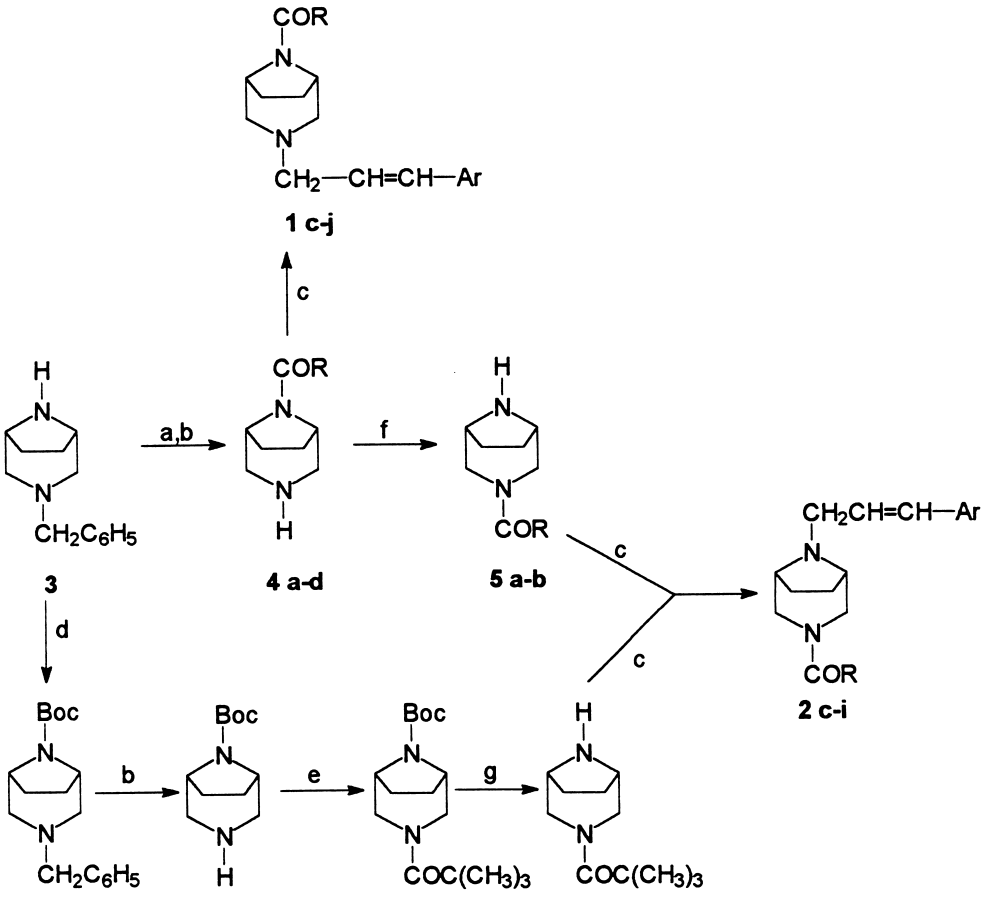


4,5	R
a	Ethyl
b	*n*-propyl
c	*t*-butyl
d	*n*-pentyl

6	Ar
a	C_6H_5
b	4-$NO_2C_6H_4$
c	2-$NO_2C_6H_4$
d	3-$NO_2C_6H_4$
e	2-NO_2,5-ClC_6H_3
f	3-NO_2,4ClC_6H_3
g	2-Cl,5-$NO_2C_6H_3$

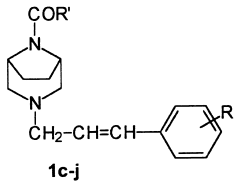
1,2	Ar	R
a	C_6H_5	Ethyl
b	4-$NO_2C_6H_4$	Ethyl
c	2-$NO_2C_6H_4$	Ethyl
d	3-$NO_2C_6H_4$	Ethyl
e	2-NO_2,5-ClC_6H_3	Ethyl
f	3-NO_2,4ClC_6H_3	Ethyl
g	2-Cl,5-$NO_2C_6H_3$	Ethyl
h	4-$NO_2C_6H_4$	*n*-propyl
i	4-$NO_2C_6H_4$	*t*-butyl
j	4-$NO_2C_6H_4$	*n*-pentyl

Scheme 1. (a) RCOCl/toluene/NEt_3/Δ; (b) H_2, 10% Pd–C/EtOH; (c) Ar–CH=$CHCH_2Cl$ (**6**)/K_2CO_3/acetone; (d) $O[COOC(CH_3)_3]_2$, $CHCl_3$; (e) $(CH_3)_3CCOCl$/$CH_2Cl_2/NEt_3/\Delta$; (f) 150°C/3 h; (g) Et_2O/H^+.

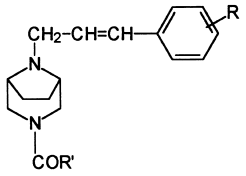
2. Chemistry

The 3-aralkenyl-8-acyl derivatives (**1c–j**; **3-DBO**) were prepared starting from the known 3-benzyl-3,8-diazabicyclo[3.2.1]octane (**3**) [5], following a previously described procedure [6], as shown in Scheme 1. Accordingly, a mixture of **3** and equimolar amounts of the required acyl chloride and triethylamine in toluene was refluxed for 2 h to give the benzyl derivative of the corresponding **4**, easily deprotected by catalytic hydrogenation in the presence of 10% Pd–C. The so obtained **4** were then treated with equimolar amounts of the required aralkenyl chloride (**6**) and potassium carbonate in refluxing acetone for 6 h. The inorganic salts were filtered off, the filtrate evaporated and the residue purified by silica gel chromatography to give the desired **1c–j**. The 3-acyl-8-aralkenyl isomers (**2c–i**; **8-DBO**) were prepared in the same way starting from the appropriate 3-acyl-3,8-diazabicyclo[3.2.1]octane (**5**), which could either directly be obtained from the corresponding **4** by shifting the acyl chain to the 3-position [7] (compounds **5a,b**) or from the intermediate **3**

Table 1
Physicochemical properties of compounds **1c–j**; **2c–i**



1c-j



2c-i

Compound	R	R′	% Yield	M.p. (°C)	Formula	^{1}H NMR δ (ppm)
1c	2-NO_2	Et	51	220–223[a,b]	$C_{18}H_{23}N_3O_3$	1.2 (t, 3H); 1.8–2.1 (m, 4H); 2.2–2.5 (m, 4H); 2.7–2.9 (m, 2H); 3.1–3.3 (m, 2H); 4.1–4.2 (m, 1H); 4.6–4.8 (m, 1H); 6.1–6.3 (m, 1H); 7.0 (d, 1H); 7.3–7.5 (m, 1H); 7.5–7.7 (m, 2H); 7.9 (d, 1H)
1d	3-NO_2	Et	45	168–170[a]	$C_{18}H_{23}N_3O_3$	1.1 (t, 3H); 2.0–2.4 (m, 6H); 3.0–3.1 (m, 2H); 3.9–4.0 (m, 2H); 4.5–4.6 (m, 2H); 6.7–6.8 (m, 1H) 7.0 (d, 1H); 7.8 (t, 1H); 8.0 (d, 1H); 8.2 (dd, 1H); 8.4 (s, 1H)
1e	2-NO_2,5-Cl	Et	20	206–208[a]	$C_{18}H_{22}ClN_3O_3$	1.1 (t, 3H); 1.5–2.0 (m, 2H); 2.9 (d, 1H); 3.8 (s, 2H); 4.2 (d, 1H); 7.1 (s, 1H); 7.3–7.8 (m, 4H)
1f	3-NO_2,4-Cl	Et	45	225–226[a]	$C_{18}H_{22}ClN_3O_3$	1.2 (t, 3H); 1.9–2.6 (m, 8H); 2.7 (t, 3H); 3.1 (d, 1H); 4.1 (s, 1H); 4.6; (s, 1H); 6.5 (d, 1H); 7.4 (d, 2H); 7.9 (s, 1H);
1g	2-Cl,5-NO_2	Et	56	210–212[a]	$C_{18}H_{22}ClN_3O_3$	1.1 (t, 3H); 1.6–2.0 (m, 6H); 2.3–2.4 (m, 2H); 2.9 (d, 1H); 3.2–3.5 (m, 4H); 4.2 (d, 1H); 6.4 (dt, 1H); 6.9 (d, 1H); 7.5 (d, 1H); 8.0 (dd, 1H); 8.4 (d, 1H)
1h	4-NO_2	*n*-prop	50	82–83	$C_{19}H_{25}N_3O_3$	1.0 (t, 3H); 1.1–2.4 (m, 10H); 2.6–2.7 (m, 2H); 3.1 (d, 2H); 4.0–4.1 (m, 1H); 4.5–4.6 (m, 1H); 6.3–6.7 (m, 2H); 7.3–7.4 (m, 2H); 8.1–8.2 (m, 2H)
1i	4-NO_2	*t*-but	45	128–29	$C_{20}H_{27}N_30_3$	1.3 (s, 9H); 1.7–1.8 (m, 4H); 2.3 (d, 2H); 2.8 (d, 2H); 3.2 (d, 2H); 4.6 (s, 2H); 6.3–6.4 (m, 1H); 6.6 (d, 1H); 7.5 (d, 2H); 8.1 (d, 2H)
1j	4-NO_2	*n*-pent	37	76–78	$C_{20}H_{27}N_3O_3$	1.2 (t, 3H); 1.2–1.3 (m, 14H); 2.6–2.7 (m, 2H); 3.1 (d, 2H); 4.1 (s, 1H); 4.6 (s, 1H); 6.5 (d, 1H); 7.4 (d, 2H); 7.9 (s, 1H)
2c	2-NO_2	Et	48	220–221[a]	$C_{18}H_{23}N_3O_3$	1.1 (t, 3H);1.8–2.6 (m, 6H); 3.6–3.8 (m, 4H); 4.0–4.1 (m, 2H); 4.3–4.6 (m, 2H); 6.5–6.7 (m, 1H); 7.1–7.4 (m, 1H); 7.4–7.7 (m, 3H); 8.0 (d, 1H); 13.1 (br s, 1H)
2d	3-NO_2	Et	38	170–175[a]	$C_{18}H_{23}N_3O_3$	1.0 (t, 3H); 1.6–1.9 (m, 2H); 2.2–2.3 (m, 4H); 3.4 (d, 1H); 4.1 (br s, 2H); 4.4 (d, 1H); 6.9–7.0 (m, 1H); 7.1 (d, 1H); 7.8 (t, 1H); 8.0 (d, 1H); 8.2 (dd, 1H); 8.4 (s, 1H)
2e	2-NO_2,5-C1	Et	20	200–204[a]	$C_{18}H_{22}ClN_3O_3$	1.1 (t, 3H); 1.6–1.8 (m, 6H); 2.9 (d, 1H); 3.1–3.5 (m, 4H); 4.2 (d, 1H) 6.3 (dt, 1H); 7.0 (d, 1H); 7.4 (dd, 1H); 7.6 (d, 1H); 7.9 (d, 1H)

(*continued*)

Table 1 (*continued*)

Compound	R	R′	% Yield	M.p. (°C)	Formula	^{1}H NMR δ (ppm)
2f	3-NO_2,4-Cl	Et	35	210–11a	$C_{18}H_{22}ClN_3O_3$	1.1 (t, 3H); 1.6–1.7 (m, 2H); 1.8–2.0 (m, 2H); 2.2–2.4 (m, 2H); 2.8–3.2 (m, 3H); 3.3–3.5 (m, 4H); 4.2 (d, 1H); 6.3–6.4 (m, 1H); 6.5 (d, 1H) 7.5 (s, 2H); 7.8 (s, 1H)
2g	2-C1,5-NO_2	Et	56	180–184[a]	$C_{18}H_{22}ClN_3O_3$	1.5 (t, 3H); 1.6–2.0 (m, 6H); 2.3–2.4 (m, 2H); 2.9 (d, 1H); 3.2–3.5 (m, 4H); 4.2 (d, 1H); 6.4 (dt, 1H); 6.9 (d, 1H); 7.5 (d, 1H); 8.0 (dd, 1H); 8.4 (d, 1H)
2h	4-NO_2	*n*-prop	45	94–96	$C_{19}H_{25}N_3O_3$	1.0 (t, 3H); 1.5–2.4 (m, 8H); 2.9–3.4 (m, 7H); 4.1–4.2 (m, 1H); 6.4–6.7 (m, 2H); 7.5 (d, 2H); 8.2 (d, 2H)
2i	4-NO_2	*t*-But	40	142–43	$C_{20}H_{27}N_3O_3$	1.3 (s, 9H); 1.6–1.9 (m, 4H); 3.1–3.2 (m, 6H); 4.0–4.1 (m, 2H); 6.5–6.6 (m, 2H); 7.3 (d, 2H); 8.2 (d, 2H)

[a] As the hydrochloride.
[b] See Ref. [1].

(compound **5c**), according to standard procedures (see Scheme 1). Finally, the required aralkenyl chlorides **6** were synthesized according to reported methods [8,9].

3. Binding studies

Compounds **1c–j**; **2c–i** together with **1a,b** and **2a,b** 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.

4. Experimental

4.1. 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 Brüker AC200 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. The structures of all compounds were consistent with their analytical and spectroscopic data.

*4.2. 3-Aralkenyl-8-acyl-3-8-diazabicyclo[3.2.1]octanes (**1c–j**): general method*

A mixture of the appropriate 8-acyl derivative (**4**; 0.01 mol), the required halide (**6**; 0.01 mol) and K_2CO_3 (0.01 mol) in acetone (20 ml) was refluxed for 6 h. The inorganic salts were filtered off, the solvent evaporated and the residue purified by silica gel chromatography (eluent CH_2Cl_2/EtAc 1:1) to give the desired **1c–j**. When stable, the corresponding hydrochloride was prepared. See Table 1 for data.

*4.3. 3-Acyl-8-aralkenyl-3,8-diazabicyclo[3.2.1]octanes (**2c–i**): general method*

Compounds were prepared as above reported for **1**, starting from the required **5**. See Table 1 for data.

*4.4. 8-Acyl-3,8-diazabicyclo[3.2.1]octanes (**4a–d**): general method*

(a) A mixture of 3-benzyl-3,8-diazabicyclo[3.2.1]octane (0.01 mol) (**3**) [5], TEA (0.01 mol) and the required acyl chloride (0.01 mol) in toluene (20 ml) was refluxed for 2 h. After cooling, the salts were filtered off, the solvent evaporated and the residue purified by silica gel chromatography, eluting with cyclohexane/ethylacetate 1:1.

(b) A mixture of the appropriate above described 3-benzyl derivative of **4** (0.01 mol), and 10% Pd–C (40 mg) in ethanol (10 ml) was hydrogenated at room temperature. The catalyst was filtered off and the solvent evaporated to give the desired **4**, which were used without further purification.

1. **4a**: see Ref. [6].
2. **4b**: yield 65%, ^{1}H NMR δ: 1.0 (t, 3H); 1.6–1.8 (m, 2H); 1.8–2.1 (m, 4H); 2.2–2.5 (m, 4H); 2.7–2.8 (m, 1H); 2.8–3.0 (m, 1H); 4.0 (s, 1H); 4.7 (s, 1H).
3. **4c**: yield 70%, m.p. = 103–104°C, ^{1}H NMR δ: 1.3 (s, 9H); 1.9–2.2 (m, 7H); 2.8–3.0 (m, 4H).
4. **4d**: yield 65%, oil, ^{1}H NMR δ: 1.0 (t, 3H); 1.3–1.4 (m,

6H); 1.5–1.6 (br s, 1H, exch with D_2O); 1.9–2.0 (m, 4H); 2.2-2.3 (m, 4H); 2.8-2.9 (m, 2H); 4.1 (br s, 1H); 4.7 (br s, 1H).

4.5. 3-Propionyl-3,8-diazabicyclo[3.2.1]octane (**5a**)

The compound was prepared by heating **4a** at about 150°C for 3 h, according to a previously reported method [7]. The homologue **5b** was obtained from **4b** by the same procedure.

4.6. 3-t-Butyl-3,8-diazabicyclo[3.2.1]octane (**5c**)

(a) To a solution of **3** [5] (0.01 mol) in anhydrous chloroform (20 ml) di-tert-butyldicarbonate (3 g; 0.01 mol) in anhydrous chloroform (20 ml) was added dropwise under nitrogen and the mixture stirred at room temperature overnight. After evaporation of the solvent, the residue (**7**) was dissolved in ethanol (40 ml) and hydrogenated at room temperature in the presence of 10% Pd–C (0.04 g). The catalyst was filtered off, the solvent evaporated and the thus obtained 8-Boc-diazabicyclooctane (**8**) dissolved in toluene and treated with trimethylacetyl chloride as reported for the corresponding **4**. Finally, the protecting group was removed by treatment with a solution of hydrochloric acid in diethyl ether. The solution was then made alkaline by 20% NaOH, extracted with diethyl ether (2 × 25 ml), the solvent dried (Na_2SO_4) and evaporated to give **5c**. Yield 60%. ^{1}H NMR δ: 1.3 (s, 9H); 1.5–1.8 (m, 4H); 2.1–2.4 (m, 2H); 2.7 (d, 1H); 3.2 (d, 1H); 3.4–3.6 (m, 2H); 4.2 (d, 1H).

4.7. General method for the synthesis of the nitro, chloro-substituted aralkenyl chloride (**6e–g**)

(a) To a suspension of NaH (1 g; 80% dispersion in mineral oil) in toluene (15 ml), triethyl phosphonoacetate (7.2 g; 0.03 mol) was added dropwise under a nitrogen stream at such a rate to keep the temperature below 40°C. The mixture was stirred for 1 h at room temperature, added of a solution of the required substituted benzaldehyde (0.03 mol) in anhydrous toluene (20 ml) and stirred for further 2 h. Water (20 ml) was added under cooling and the aqueous layer was extracted with CH_2Cl_2. After evaporation of the solvent, the residue was purified by silica gel chromatography, eluting with CH_2Cl_2/MeOH 9:1.

(b) To an ice-cooled solution of the above described ester (0.015 mol) in anhydrous toluene (70 ml) a 1 M solution of diisobutylaluminum hydride (DIBALH) in toluene (3.4 ml) was added dropwise under a nitrogen stream. The mixture was stirred for 1 h at 5°C, cautiously added to a saturated solution of K^+/Na^+ tartrate and then stirred at room temperature for 18 h. The aqueous layer was repeatedly extracted with diethyl ether and dried over sodium sulfate. After evaporation of the solvent, the thus obtained alcohol and freshly distilled thionyl chloride (30 ml) were refluxed for 30 min. After usual work-up, the residue was purified by silica gel chromatography (eluent cyclohexane/ethylacetate 7:3).

1. **6e** yield 74%, ^{1}H NMR δ: 4.2 (d, 2H); 6.3–6.4 (m, 1H); 7.2 (d, 1H); 7.4 (dd, 1H); 7.6 (d, 1H); 8.0 (d, 1H).
2. **6f**: yield 55%, ^{1}H NMR δ: 4.4 (d, 2H); 6.3-6.4 (m, 1H); 6.6 (d, 1H); 7.5 (s, 2H) 7.9 (s, 1H).
3. **6g**: yield 73%, ^{1}H NMR δ: 4.3 (d, 2H); 6.4-6.5 (m, 1H); 7.1 (d, 1H); 7.5 (d, 1H); 8.1 (dd. 1H); 8.41 (d, 1H).

4.8. Binding studies

Male Sprague–Dawley rats (Charles River, Italy) weighting 180–200 g were used. Rat brain membrane binding studies were carried out as described by Gillan and Kosterlitz with slight modifications [10]. Whole brain minus cerebellum was homogenized with Polytron in 50 vols. (w/v) of 50 mM Tris–HCl (pH 7.7), centrifuged at 48 000 × *g* for 20 min at 4°C, resuspended in 50 vols. of the same buffer and incubated at 37°C for 45 min. After centrifugation at 48 000 × *g* for 20 min at 4°C, the final pellet was resuspended in the same buffer to a final concentration of 0.8–1.0 mg protein/ml. [^{3}H]DAMGO (2 nM) (New England Nuclear, Germany) was used to label μ-receptors. Membrane suspensions were incubated with the ligand at 0°C for 60 min in the presence or the absence of 10 μ-molar naloxone. Final protein concentrations were determined by the method of Lowry et al. [11]. K_i values were cal-culated with the LIGAND program [12], from displace-ment curves of each compound at a concentration range between 10^{-10} M and 10^{-4} M. Values are the mean from two assays.

5. Results and discussion

As shown in Table 2, shifting the nitro group from the *para* to *meta* position of the cinnamyl chain led to derivatives still comparable with the models both in the normal **3-DBO** (**1d**, K_i = 10 versus 33 nM for **1b**) and in the reverted **8-DBO** series (**2d**, K_i = 9.5 versus 5.1 nM for **2b**). On the contrary, the presence of an *ortho*-nitro group led to a relevant loss of

Table 2
Inhibition constants of morphine and compounds **1a–j, 2a–i** towards μ-opioid receptors

Compound	^{3}H-DAMGO[a] K_i (nM)	Compound	^{3}H-DAMGO[a] K_i (nM)
1a[b]	55	**2ab**	160
1b[b]	33	**2b**[b]	5.1
1c	1750	**2c**	325
1d	10	**2d**	9.5
1e	30	**2e**	112
1f	48	**2f**	14
1g	55	**2g**	87
1h	21	**2h**	18
1i	22	**2i**	13
1j	360	Morphine	2.8

[a] K_i values were calculated with the LIGAND program [12], based on a k_d value of 1 nM for ^{3}H-DAMGO. Values are the mean from two experiments.
[b] See Ref. [1].

affinity, though by different degree in the two series (**1c**, $K_i = 1750$; **2c**, $K_i = 325$). However, it should be noted that insertion of a chlorine atom *para* to the nitro group of **1c** gave a compound (**1e**) whose potency ($K_i = 30$ nM) was fully comparable to that of **1b**. On the contrary, in the *meta*-nitro derivatives the presence of a chlorine in different positions of the phenyl ring, either left the affinity almost unchanged (compound **2f**, $K_i = 14$ nM) or slightly decreased (compounds **1f,g**; **2g**). Finally, modification of the acyl substituent was well tolerated in both series up to **4** carbon atom chains, either linear or branched (compounds **1h,i**; **2h**). Further elongation brought about a remarkable loss of activity (compound **1j**, $K_i = 360$ nM).

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