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Synthesis, modelling, and μ-opioid receptor affinity of *N*-3(9)-arylpropenyl-*N*-9(3)-propionyl-3,9-diazabicyclo[3.3.1]nonanes

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Dedicated to Professor Pietro Pratesi

Abstract

A series of *N*-3-arylpropenyl-*N*-9-propionyl-3,9-diazabicyclo[3.3.1]nonanes (**1a–g**) and of reverted *N*-3-propionyl-*N*-9-arylpropenyl isomers (**2a–g**), as homologues of the previously reported analgesic 3,8-diazabicyclo[3.2.1]octanes (**I–II**), were synthesized and evaluated for the binding affinity towards opioid receptor subtypes μ, δ and κ. Compounds **1a–g** and **2a–g** exhibited a strong selective μ-affinity with K_i values in the nanomolar range, which favourably compared with those of **I** and **II**. In addition, contrary to the trend observed for DBO-**I**, **II**, the μ-affinity of series **2** is markedly higher than that of the isomeric series **1**. This aspect was discussed on the basis of the conformational studies performed on DBN which allowed hypotheses on the mode of interaction of these compounds with the μ receptor.

Keywords: Diazabicyclononanes; Modelling; μ-Affinity

1. Introduction

The class of *N*-8-propionyl-*N*-3-arylpropenyl-3,8-diazabicyclo[3.2.1]octane (DBO-**I**) as well as of the *N*-3-propionyl-*N*-8-arylpropenyl isomers (DBO-**II**) are central analgesics displaying a potency comparable or greater than morphine [1,2].

Despite the structural dissimilarity with common opioids, their activity is related to an affinity towards μ-opioid receptors in the nanomolar range, similarly to morphine but with higher μ/δ, κ selectivity [2].

Molecular modelling studies suggest that the endoethylenic bridge of DBO-**I** and -**II** plays an essential role in modulating μ-affinity by fitting lipophylic pockets of the receptor. The hypothesis was supported by the significantly lower μ-affinity found in the corresponding piperazine and equatorial *cis*-2,6-dimethyl piperazine derivatives [3].

On the basis of this assumption, we have now planned to evaluate the affinity towards μ-opioid receptors of 3,9-diazabicyclo[3.3.1]nonanes (DBN), higher homologues of DBO-**I** and -**II**, in which the two carbon bridge of DBO is replaced by a three carbon bridge [4].

Accordingly, representative 9-propionyl-3-arylpropenyl DBN (**1a–g**) and their 3-propionyl-9-arylpropenyl isomers (**2a–g**) have been synthesized and tested in vitro towards μ-opioid receptors (μ, δ, κ for **1a**, **2a**) comparing their affinity values with those of the corresponding DBO recently reported [5,6].

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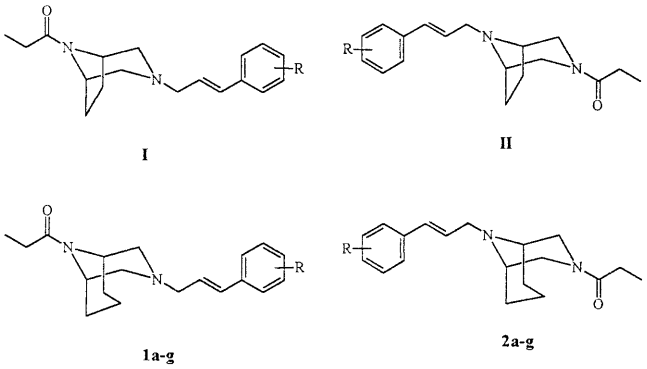


2. Chemistry

The synthesis of **1a–g** and **2a–g** required as intermediate the known 3-benzyl-3,9-diazabicyclo[3.3.1]nonane (**10**) which was obtained by modifying the previously reported procedure[1]. Thus, as outlined in Scheme 1, *meso*-dimethyl-α,α′-dibromopimelate (**6**), was condensed with benzylamine in refluxing benzene to give in 88% yield *N*-benzyl-2,6-dicarbomethoxypiperidine (**7**) as a mixture of the *cis* and the *trans* isomers, which, as such, was allowed to react with benzylamine in refluxing toluene for 18 h, and, after evaporation of the solvent, at 160–170°C for a further 4 h. The oily product was dissolved in ethanol–ether (1/1) and treated with ethereal HCl to separate in 40% yield 3,9-dibenzyl-3,9-diazabicyclo[3.3.1]nonane-2,4-dione (**8**) as the HCl salt. Owing to the comparable yield of **8**, this procedure was preferred to that involving a preliminar separation by flash-chromatography of *cis*-**7** and *trans*-**7**, followed by condensation of *cis*-**7** with benzylamine. Catalytic hydrogenolysis (Pd/C) of **8** · HCl in ethanol afforded in almost quantitative yield 3-benzyl-3,9-diazabicyclo[3.3.1]nonane-2,4-dione (**9**) as the HCl salt. Reduction of **9** with $LiAlH_4$ in ether gave in 80% yield the expected **10** as an oil.

Acylation of **10** with propionic anhydride to the 3-benzyl-9-propionyl-3,9-diazabicyclo[3.3.1]nonane (**11**) followed by debenzylation (H_2, Pd/C) led to 9-propionyl-3,9-diazabicyclo[3.3.1]nonane (**3**) in 82% overall yield from **10**. Finally, condensation of **3** with the appropriate arylpropenyl chlorides (**14**) in refluxing acetone or butanone in the presence of K_2CO_3 gave compounds **1a–g** (Scheme 2).

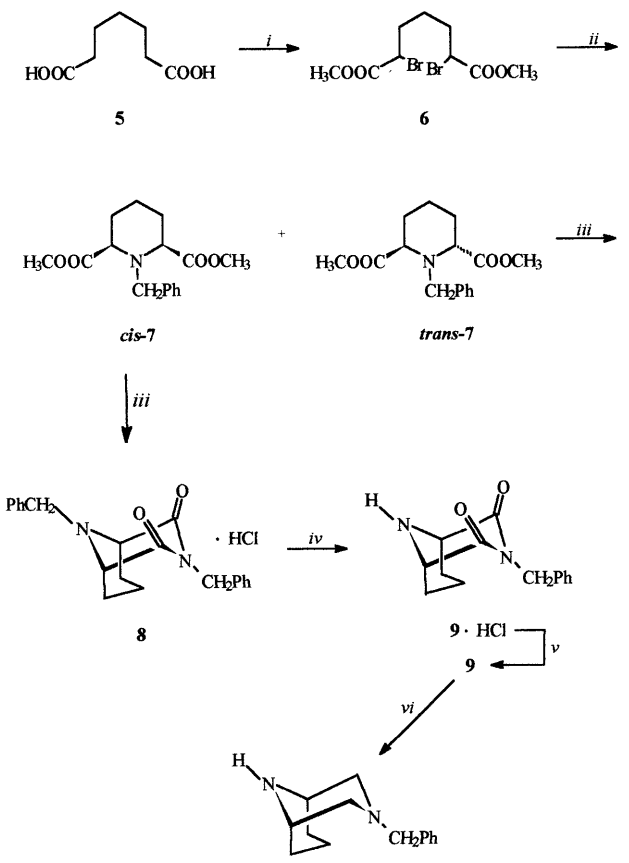
The isomeric series **2a–g** was synthesized by a similar procedure starting from 3-propionyl-3,9-diazabicyclo[3.3.1]nonane (**4**) still unknown in the literature, which was obtained in 88% yield by heating **3** at 150°C for 2 h with consequent N_9–N_3 acyl migration, following a procedure described for the DBO analogues [7].

The needed chlorides (**14**) were prepared from the appropriate aryl acrylic acid (**12b,d**) or ester (**12c,e–g**) by reduction with diisobutylaluminum hydride in toluene to the corresponding arylpropenyl alcohol (**13**) eventually converted to **14** with thionyl chloride or concentrated HCl (Scheme 3).

3. Receptor binding

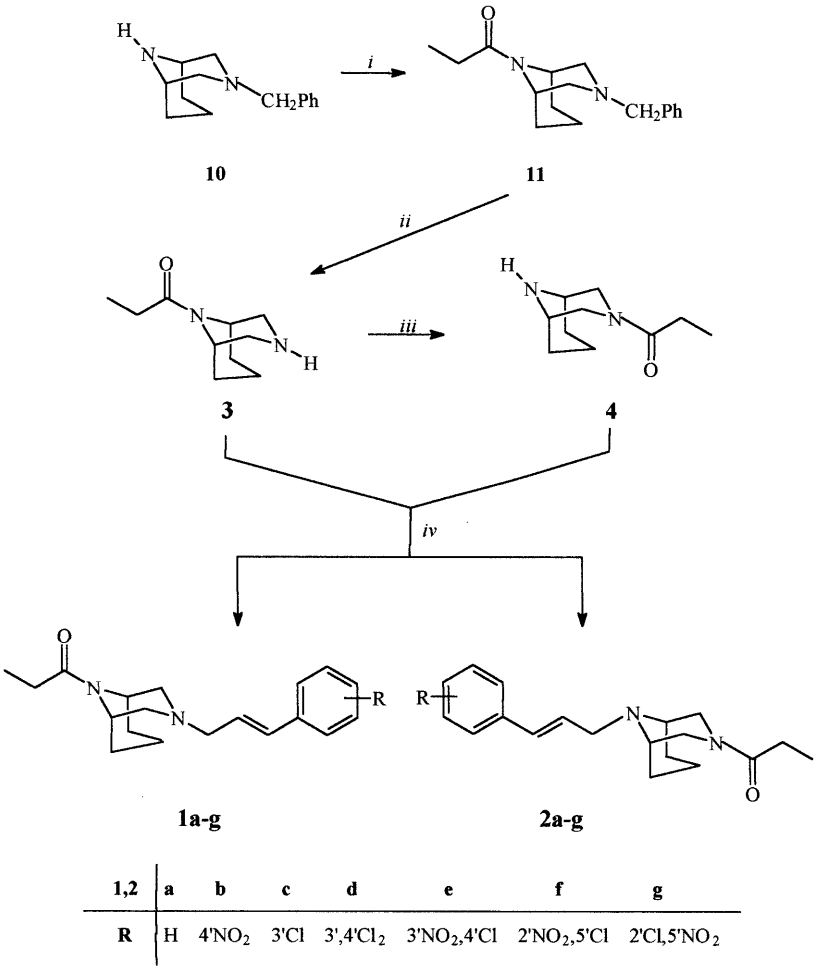
3.1. Opioid receptor binding assays

Binding affinities to opioid receptors were measured on mouse brain homogenates in the presence of [^{3}H]-DAMGO for μ, [^{3}H]-DELTORPHINE II for δ. [^{3}H]-U69 593 was used on guinea pig homogenates to evaluate κ binding. Morphine was used as the reference compound. The inhibition constants of the compounds tested are reported in Tables 1 and 2.

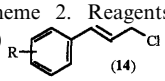


Scheme 1. Reagents and conditions: (i) $SOCl_2$, MeOH, Br_2, Δ; (ii) $PhCH_2NH_2$, C_6H_6, Δ; (iii) $PhCH_2NH_2$, toluene, Δ, Et_2O/HCl; (iv) EtOH, Pd/C 10%, H_2; (v) H_2O, $NaCO_3$ 10%; (vi) Et_2O, $LiAlH_4$, Δ.

[1] Actually, at the very beginning of our research on DBOs, one example was reported of homologation of the mild analgesic 3-methyl-8-propionyl DBO to 3-methyl-9-propionyl DBN. The resulting decrease of the activity by about 50% (Randall and Selitto test, rat) discouraged this approach, see [4].



1,2	a	b	c	d	e	f	g
R	H	4'NO_2	3'Cl	3',4'Cl_2	3'NO_2,4'Cl	2'NO_2,5'Cl	2'Cl,5'NO_2

Scheme 2. Reagents and conditions: (i) CH_2Cl_2, $(CH_3CH_2CO)_2O$, Δ, NaOH 20%; (ii) EtOH, Pd/C 10%, H_2, (iii) Δ; 150°C, 2 h; (iv)  (14), K_2CO_3, acetone or butanone, Δ.

4. Modelling

The conformational properties of compounds **1a** and **2a**, ground terms of the two series of compounds **1** and **2**, were investigated with the MM^+ force field of the HYPER CHEM™ package [9] considering the protonated form of each compound. First, attention was focused on the bicyclic moiety by considering the simplified models **1h** and **2h** (Chart 1) in which a methyl group replaces the phenylpropenyl group, as it was previously shown [10] on the DBO analogues that the conformational mobility of this group does not influence the conformational mobility of this group does not influence the conformational behaviour of the remaining part of the molecule.

In principle, the DBN system could present a conformational flexibility higher with respect to the DBO system. In fact, the two six-membered rings that constitute the bicycle could assume four combinations of conformations: chair–chair, chair–boat, boat–chair, boat–boat exemplified by the four structures **A**–**D** (Chart 2). Actually, exploration of the conformational space of compounds **1h** and **2h** showed a large preference for the chair–chair conformations (type **A**) in both cases; in fact, all other conformations were more than 4 kcal/mol higher in energy.

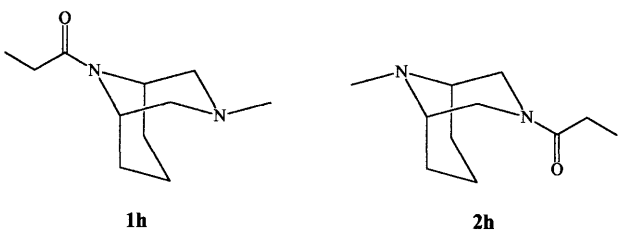


Chart 1.

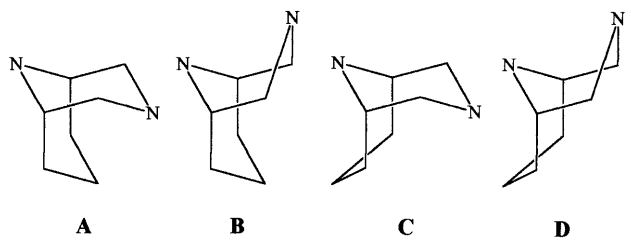
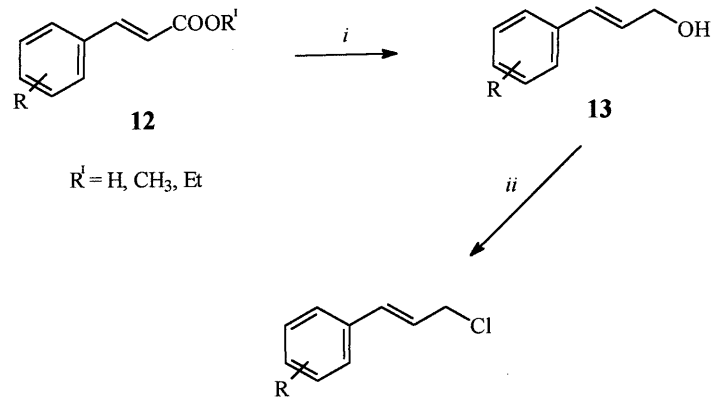


Chart 2.



12-14	a	b	c	d	e	f	g
R	H	4'NO_2	3'Cl	3',4'Cl_2	3'NO_2, 4'Cl	2'NO_2, 5'Cl	2'Cl, 5'NO_2

Scheme 3. Reagents and conditions: (i) DIBALH, Et_2O or toluene, 0–5°C; (ii) $SOCl_2$, dry pyridine, CH_2Cl_2 or concentrated HCl, Δ.

Fig. 1 reports the 3D plots of the only populated conformation of **1h** (**1hA**) and of the two populated conformations of **2h** (**2hA** and **2hA′**); these last two conformations differ only for the orientation of the methyl group at N9 and are practically isoenergetic.

The modelling study was then extended to compounds **1a** and **2a**, exploring the conformations deriving from rotation of the single bonds of the phenylpropenyl group. As expected, this group is very flexible as several conformations were found in a range of 1 kcal/mol above the global minima; however, the geometry of the bicyclic system strictly corresponded to those shown in Fig. 1 for **1h** and **2h**.

In order to confirm the calculated conformation of **1a** and **2a**, they were submitted to high field ^{1}H NMR spectroscopy; the chemical shifts of all the atoms were assigned as well as almost all the coupling constants of the vicinal protons in the diazabicyclo[3.3.1]nonane system (Table 3). Application of the Altona equation [11] to the calculated conformations of **1a** and **2a** 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 **1a** and **2a** represent their solution conformations thus confirming the chair–chair geometry of the bicyclic system. As the other compounds in the **1** and in the **2** series differ from **1a** and **2a** only for the presence of substituents on the phenyl ring, it can be safely affirmed that the conformations calculated for the latter compounds hold also for all compounds in each series.

5. Discussion

The ground terms **1a** and the isomer **2a** were evaluated for their affinity towards μ, δ and κ receptors (Table 1). Having found for both compounds a μ-affinity in the nanomolar range ($K_i = 29$ and 13 nM, respectively) with a μ/δ and μ/κ selectivity from two to three orders of magnitude, the compounds **1b–g** and **2b–g** were only tested on μ receptors. The K_i values of DBN series **1** and **2** are shown in Table 3 in comparison with the K_i values of corresponding DBO-**I** and DBO-**II** recently reported [6].

It is worth noting the μ-affinity of DBN-**2** favourably compared with that of the lower homologues DBO-**II**, as indicated by the K_i values of **2c**,**d**,**f**,**g** which are significantly lower than those of the corresponding **II**.

On the contrary, DBN-**1** are definitely less potent than the corresponding DBO-**I** with the only exception being **1a** ($K_i = 29$ nM) versus **Ia** ($K_i = 55$ nM).

Interestingly, **2a–g** show μ-affinity markedly greater than that of the isomers 9-propionyl DBN (**1a–g**), the most active terms (**2b–d**,**f**,**g**) exhibiting K_i values (from 5.8 to 7.6 nM) very close to that of morphine ($K_i = 2.8$ nM).

These results deserve some comments, taking into account the previous hypothesis [3] of the existence on μ receptors of two hydrophobic pockets (HP-1 and

Table 1
Binding affinities to μ, δ and κ receptors

Comp.	Binding affinities (K_i (nM)) [a]		
	μ	δ	κ
1a	29 ± 2.0	12 000 ± 1152	> 50 000
2a	13 ± 1.5	1750 ± 144	2000 ± 180

[a] K_i values were calculated with the LIGAND program [8]. Each value represents the mean ± SEM from three experiments, each performed in triplicate ($n = 3$).

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))			
	DBN (**1**)	DBO (**I**)	DBN (**2**)	DBO (**II**)
a	29.0	55.0	13.0	160.0
b	70.0	25.0	7.66	5.1
c	48.33	6.2	8.66	16.3
d	246.66	139.0	5.83	47.0
e	220.0	48.0	18.0	14.0
f	1000.0	30.0	6.0	112.0
g	1750.0	55.0	6.0	87.0
Morphine	2.8			

[a] See footnote to Table 1.

HP-2) able to accommodate the endoethylenic bridge of DBO-**I** and DBO-**II**.

Considering that, in DBN the preferred chair–chair geometry of the bicyclic system involves the trimethylene bridge oriented towards N-3 (Fig. 1), two explanations are possible for the lower μ-affinity of compounds **1** with respect to the reverted isomers **2**.

As the trimethylene bridge of DBN-**1** and of DBN-**2** are supposed to accommodate, respectively, into HP-1 and HP-2 pockets, steric reasons could determine an interaction DBN-**1**–HP-1 less efficient than that DBN-**2**–HP-2. The alternative explanation could be related to the fact that the protonated nitrogen of DBO or DBN is supposed to interact with the Asp147 carboxylate group of the receptor [12]; the additional CH_2 in compounds **1** pointing towards the N-3 hydrogen atom could disturb this interaction while in compounds **2** the additional CH_2 cannot have any influence on this interaction as it is located on the opposite side of the molecule with respect to the N-9 hydrogen atom.

6. Experimental

6.1. Chemistry

Melting points (m.p.) were obtained on an electrothermal IA 9100 digital melting point apparatus or on a Köfler melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer

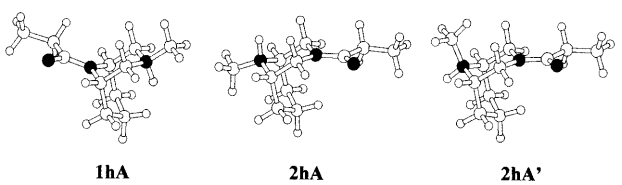


Fig. 1. Three-dimensional plots of the most populated conformations of compounds **1h** and **2h**.

Table 3
Calculated and experimental ^{1}H NMR coupling constants (Hz) [a] of the vicinal hydrogen atoms of the bicyclic system of compounds **1a** and **2a**

J	**1a** (calc.)	**1a** (exp.)	**2a** (calc.)	**2a** (exp.)
1–2ax	3.8	3.5	4.3	4.0
1–2eq	2.0	1.5	1.7	1.5
1–8a	2.4	2.5	2.4	n.d. [b]
1–8b	4.3	n.d. [b]	4.3	5.0
8a–7a	5.8	6.0	5.1	5.5
8a–7b	1.6	2.0	1.7	n.d. [b]
8b–7a	12.2	12.0	12.4	13.5
8b–7b	5.5	6.0	5.5	6.5
5–4ax	3.7	3.5	4.3	4.0
5–4eq	2.1	1.5	1.7	1.5
5–6a	2.5	2.5	2.3	n.d. [b]
5–6b	4.2	n.d. [b]	4.3	5.0
6a–7a	5.8	6.0	5.1	5.5
6a–7b	1.6	2.0	1.7	n.d. [b]
6b–7a	12.2	12.0	12.5	13.5
6b–7b	5.5	6.0	5.4	6.5

[a] The spectra were recorded in $CDCl_3$ solutions on a Bruker AM500 spectrometer.
[b] n.d., not determined.

781 (nujol mulls or films). All NMR spectra were taken on a Varian XL-200 NMR spectrometer with ^{1}H and ^{13}C being observed at 200 and 50 MHz, respectively. Chemical shifts for ^{1}H and ^{13}C NMR spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4Si$]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), dq (doublet of quartets), m (multiplet). Elemental analyses were performed by Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Padova, Italy and are within $\pm 0.4\%$ of the calculated values. All reactions involving air or moisture-sensitive compounds were performed under an atmosphere of argon 'S'.

Unless otherwise specified, all materials were obtained from commercial suppliers and used without purification.

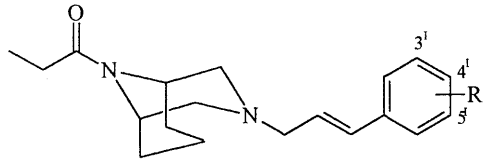
Flash chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM). Thin layer chromatography (TLC) was performed with Polygram® SIL N-HR/HV_{254} precoated plastic sheets (0.2 mm).

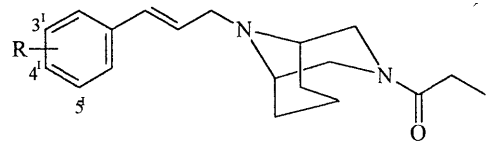
Precursors **14** were prepared by standard procedures (**14b**,**g**) or obtained from commercial suppliers (**14a**).

6.2. General arylpropenylation procedure for compounds *1a–g* and *2a–g*

A mixture of the appropriate propionyl-3,9-diazabicyclo[3.3.1]nonane **3** or **4** (2.30 mmol), the required cinnamyl halide **14** (2.30 mmol) and K_2CO_3 (2.30 mmol) in acetone or butanone (13.5 ml) was refluxed for 4–12 h. The inorganic salts were filtered off, the

Table 4
Physicochemical properties of compounds **1** and **2**





Compound	R	Yield (%)	M.p. [a] (°C)	Formula (analysis [b])	IR [c] (ν (cm^{-1}))	^1H NMR (δ (ppm))
1a	H	72	oil	$C_{19}H_{26}N_2O$ (C,H,N)	1635	1.16 (t, 3H); 1.40–1.60 (m, 1H); 1.70–1.95 (m, 4H); 2.20–2.40 (m, 4H); 2.70–3.15 (m, 5H); 3.88 (br s, 1H); 4.70 (br s, 1H); 6.20–6.40 (dt, 1H); 6.50 (d, 1H); 7.20–7.40 (m, 5H)
1b	4′-NO_2	34	oil	$C_{19}H_{25}N_3O_3$ (C,H,N)	1350, 1510, 1620	1.17 (t, 3H); 1.50–1.70 (m, 1H); 1.70–1.92 (m, 4H); 2.20–2.40 (m, 4H); 2.65–3.20 (m, 5H); 3.95 (br s, 1H); 4.73 (br s, 1H); 6.40–6.60 (m, 2H); 7.55 (d, 2H); 8.20 (d, 2H)
1c	3′-Cl	64	oil	$C_{19}H_{25}ClN_2O$ (C,H,N)	1640	1.18 (t, 3H); 1.40–1.60 (m, 1H); 1.70–1.93 (m, 4H); 2.20–2.40 (m, 4H); 2.80–3.10 (m, 5H); 3.88 (br s, 1H); 4.68 (br s, 1H); 6.10–6.30 (dt, 1H); 6.50 (d, 1H); 7.20–7.30 (m, 4H)
1d	3′,4′-Cl_2	72	oil	$C_{19}H_{24}Cl_2N_2O$ (C,H,N)	1635	1.11 (t, 3H); 1.42–1.63 (m, 1H); 1.70–1.90 (m, 4H); 2.20–2.40 (m, 4H); 2.80–3.10 (m, 5H); 4.05 (br s, 1H); 4.65 (br s, 1H); 6.10–6.30 (dt, 1H); 6.40 (d, 1H); 7.10–7.50 (m, 3H)
1e	3′-NO_2, 4′-Cl	76	oil	$C_{19}H_{24}ClN_3O_3$ (C,H,N)	1335, 1524, 1630	1.15 (t, 3H); 1.50–1.70 (m, 1H); 1.75–1.95 (m, 4H); 2.22–2.42 (m, 4H); 2.85–3.25 (m, 5H); 3.89 (br s, 1H); 4.73 (br s, 1H); 6.15–6.24 (dt, 1H); 6.40–6.50 (m, 2H) 7.40 (br s, 2H); 7.80 (s, 1H)
1f	2′-NO_2, 5′-Cl	25	130–134 [a]	$C_{19}H_{24}ClN_3O_3 \cdot H$ Cl (C,H,N)	1340, 1520, 1630	1.17 (t, 3H); 1.50–1.70 (m, 1H); 1.70–1.95 (m, 4H); 2.23–2.45 (m, 4H); 2.65–3.20 (m, 5H); 3.90 (br s, 1H); 4.72 (br s, 1H); 6.17–6.24 (dt, 1H); 7.05 (d, 1H); 7.30 (dd, 1H); 7.56 (d, 1H); 7.92 (d, 1H)
1g	2′-Cl, 5′-NO_2	31	208–210 [a]	$C_{19}H_{24}ClN_3O_3 \cdot H$ Cl (C,H,N)	1345, 1525, 1640	1.17 (t, 3H); 1.50–1.70 (m, 1H); 1.70–1.95 (m, 4H); 2.25–2.45 (m, 4H); 2.80–3.20 (m, 5H); 3.95 (br s, 1H); 4.72 (br s, 1H); 6.34–6.48 (dt, 1H); 6.95 (d, 1H); 7.53 (d, 1H); 8.03 (dd, 1H); 8.40 (d, 1H)
2a	H	36	oil	$C_{19}H_{26}N_2O$ (C,H,N)	1525, 1635	1.19 (t, 3H); 1.46–1.66 (m, 2H); 1.72–2.20 (m, 4H); 2.21–2.40 (m, 2H); 2.92 (br s, 2H); 3.18 (dd, 1H); 3.50–3.80 (m, 4H); 4.40 (d, 1H); 6.20–6.30 (dt, 1H);6.60 (d, 1H); 7.20–7.40 (m, 5H)
2b	4′-NO_2	22	oil	$C_{19}H_{25}N_3O_3$ (C,H,N)	1360, 1515, 1630	1.19 (t, 3H); 1.47–1.70 (m, 2H); 1.72–2.20 (m, 4H); 2.21–2.40 (m, 2H); 3.01 (br s, 2H); 3.50–3.70 (m, 5H); 4.37 (d, 1H); 6.30–6.40 (dt, 1H); 6.60 (d, 1H); 7.50 (d, 2H); 8.20 (d, 2H)
2c	3′-Cl	27	oil	$C_{19}H_{25}ClN_2O$ (C,H,N)	1630	1.17 (t, 3H); 1.40–1.60 (m, 2H); 1.70–2.20 (m, 4H); 2.30–2.50 (m, 2H); 2.98 (br s, 2H); 3.10 (dd, 1H); 3.40–3.60 (m, 4H); 4.40 (d, 1H); 6.20–6.40 (dt, 1H); 6.45 (d, 1H); 7.01–7.40 (m, 4H)
2d	3′,4′-Cl_2	36	oil	$C_{19}H_{24}Cl_2N_2O$ (C,H,N)	1635	1.17 (t, 3H); 1.40–1.60 (m, 2H); 1.70–2.10 (m, 4H); 2.20–2.40 (m, 2H); 2.89 (br s, 2H); 3.40–3.60 (m, 5H); 4.20 (d, 1H); 6.20–6.30 (dt, 1H); 6.40 (d, 1H); 7.10–7.20 (m, 1H); 7.30–7.50 (m, 2H)
2e	3′-NO_2, 4′-Cl	60	oil	$C_{19}H_{24}ClN_3O_3$ (C,H,N)	1330, 1520, 1630	1.19 (t, 3H); 1.42–1.62 (m, 2H); 1.70–2.20 (m, 4H); 2.20–2.40 (m, 2H); 2.92 (br s, 2H); 3.15 (dd, 1H); 3.40–3.60 (m, 4H); 4.40 (d, 1H); 6.20–6.40 (dt, 1H); 6.52 (d, 1H); 7.40–7.60 (m, 2H); 7.80 (s, 1H)
2f	2′-NO_2, 5′-Cl	25	130 (dec) [a]	$C_{19}H_{24}ClN_3O_3 \cdot H$ Cl (C,H,N)	1340, 1520, 1635	1.17 (t, 3H); 1.42–1.65 (m, 2H); 1.70–2.20 (m, 4H); 2.37 (q, 2H); 2.93 (br s, 2H); 3.12 (dd, 1H); 3.50–3.75 (m, 4H); 4.40 (d, 1H); 6.15–6.30 (dt, 1H); 7.01 (d, 1H); 7.30 (dd, 1H); 7.56 (d, 1H); 7.92 (d, 1H)
2g	2′-Cl, 5′-NO_2	30	245 [a]	$C_{19}H_{24}ClN_3O_3 \cdot H$ Cl (C,H,N)	1340, 1520, 1560, 1635	1.17 (t, 3H); 1.48–1.68 (m, 2H); 1.72–2.18 (m, 4H); 2.34 (dq, 2H); 2.93 (br s, 2H); 3.15 (dd, 1H); 3.42–3.78 (m, 4H); 4.40 (d, 1H); 6.30–6.50 (dt, 1H); 7.01 (d, 1H); 7.65 (d, 1H); 8.05 (dd, 1H); 8.42 (d, 1H)

[a] As the hydrochloride.
[b] Compounds gave satisfactory analyses within $\pm 0.4\%$ of theoretical calculation unless otherwise stated.
[c] As the free base.

filtrate evaporated and the oily residue purified by flash chromatography (eluent: CH_2Cl_2:$(CH_3)_2CO$, 9:1) to give the desired **1a–g** and **2a–g** as oils; when the stable corresponding hydrochloride was obtained: see Table 4 for data.

6.3. Dimethyl α,α′-dibromopimelate (6)

A mixture of pimelic acid (**5**) (50 g, 312 mmol) in thionyl chloride (55 ml) was heated at 40°C for 18 h.

To the irradiated (300 W lamp) solution, bromine (117.3 g, 733 mmol) was slowly added (~ 8 h) under stirring at 95°C. After the addition of Br_2 was complete, the brown solution was heated at the same temperature for a further hour and cooled to room temperature (r.t.). Methanol (180 ml) was added and the resulting reaction mixture was poured into ice-water (180 ml) and the whole solution concentrated and extracted with Et_2O three times. The combined organic extracts were washed with 2% $NaHSO_3$, 5% $NaHCO_3$ and H_2O, then dried (Na_2SO_4), filtered, and concentrated to a yellow oil (102.78 g, 95.6%, b.p. 96–98°C/0.2) [4] which was used in the next step without further purification.

R_f 0.66 (benzene); IR (film, cm^{-1}) ν: 1740 (C=O); ^1H NMR ($CDCl_3$) δ_H: 1.32–1.85 (m, 2H), 1.98–2.20 (m, 4H), 3.79 (s, 6H), 4.24 (t, 2H, $J = 70$ Hz).

6.4. Dimethyl cis- and trans-N-benzyl-2,6-piperidinedicarboxylate (7)

A solution of **6** (1.50 g, 4.3 mmol) and benzylamine (1.47 g, 13 mmol) in benzene (5.5 ml) was refluxed for 24 h. After cooling, the salts were filtered off, the solvent evaporated and the oily residue was distilled at 114–150°C/0.01–0.1 to give **7** as a yellowish oil (0.61 g, 64%) [4]. Flash chromatography with 20% ethyl acetate in petroleum ether afforded in the order *trans*-**7** (0.21 g, 22.8%), as an oil; R_f 0.58 (petroleum ether 50–70:ethyl acetate, 8:2); IR (film, cm^{-1}) ν: 1740 (C=O); ^1H NMR ($CDCl_3$) δ_H: 1.50–1.62 (m, 2H), 1.75–1.98 (m, 4H), 3.65 (q, 2H, $J = 13.4$ Hz), 3.72 (s, 6H), 3.87 (t, 1H, $J = 5$ Hz), 3.90 (t, 1H, $J = 5$Hz), 7.26–7.41 (m, 5H); ^{13}C NMR ($CDCl_3$) δ_C: 19.16, 28.36, 51.34, 56.76, 59.02, 127.10, 128.11, 128.93, 137.98, 174.39; and *cis*-**7** (0.33 g, 35.4%), as a wax; R_f 0.45 (petroleum ether 50–70:ethyl acetate, 8:2); IR (nujol mull, cm^{-1}) ν: 1740 (C=O); ^1H NMR ($CDCl_3$) δ_H: 1.20–1.50 (m, 2H), 1.75–1.95 (m, 4H), 3.15–3.30 (m, 2H), 3.60 (s, 6H), 3.85 (s, 2H), 7.20–7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ_C: 20.27, 28.47, 51.19, 58.64, 62.01, 126.90, 127.71, 129.15, 136.81, 173.25.

6.5. 2,4-Dioxo-3,9-dibenzyl-3,9-diazabicyclo-[3.3.1]nonane (8) hydrochloride

A solution of **7** (as a *cis* + *trans* mixture, 19.45 g, 67 mmol) and benzylamine (7.15 g, 767 mmol) in toluene (41 ml) was refluxed for 18 h, and the solvent evaporated. The oily residue was heated at 170°C for 4 h removing methanol by distillation to provide crude **8** which was dissolved in dry ethanol (40 ml) and dry Et_2O (40 ml) and treated at 0–5°C with ethereal hydrochloric acid to separate 2,4-dioxo-3,9-dibenzyl-3,9-diazabicyclo[3.3.1]-nonane (**8**) hydrochloride as a white solid (3.64 g, 40%), m.p. 173–175°C. The solid crystallized from ethanol as white needles, m.p. 175–176°C.

R_f 0.58 (petroleum ether 50–70:ethyl acetate, 8:2); IR (nujol mull, cm^{-1}) ν: 1680 (C=O), 2200–2400 (N^+H); UV λ_{max} (log ε): 196.7 (4.21), 242.1 (2.86); ^1H NMR ($CDCl_3$) δ_H: 1.20–1.50 (m, 1H), 1.80–1.95 (m, 1H), 2.02–2.20 (m, 2H), 2.80–3.02 (m, 2H), 4.11 (br s, 4H), 5.07 (s, 2H), 7.15–7.55 (m, 10H); *Anal.* $C_{21}H_{23}ClN_2O_2$ (C, H, Cl, N).

6.6. 2,4-Dioxo-3-benzyl-3,9-diazabicyclo[3.3.1]nonane (9)

A solution of **8** · HCl (8.9 g, 24.1 mmol) in ethanol (215 ml) was hydrogenated at 60 psi at r.t. for 2 h with 10% Pd–C (0.89 g).

The catalyst was filtered off and the solution was concentrated to afford **9** · HCl (6.75 g, 100%) as white powder, m.p. 180–182°C.

Analytical sample was crystallized from ethanol, m.p. 182–184°C.

R_f 0.20 (petroleum ether 50–70:ethyl acetate, 6:4); IR (nujol mull, cm^{-1}) ν: 1690 (C=O), 2300–2600 (N^+H); ^1H NMR ($CDCl_3$) δ_H: 1.12–1.42 (m, 1H), 1.70–1.88 (m, 1H), 2.01–2.20 (m, 2H), 2.30–2.55 (m, 2H), 4.57 (br s, 2H), 4.95 (s, 2H), 6.80–8.01 (m, 7H).

The free base **9** was isolated from the HCl salt with 10% Na_2CO_3 (16 ml) and extracted with CH_2Cl_2. Evaporation of the solvent led to a viscous oil which on standing solidified, m.p. 92–94°C. Analytical sample: m.p. 94–96°C (ethyl ether–petroleum ether) [4].

R_f 0.24 (petroleum ether 50–70:ethyl acetate, 6:4); IR (nujol mull, cm^{-1}) ν: 1670 (C=O), 3240, 3300 and 3360 (NH); ^1H NMR ($CDCl_3$) δ_H: 1.25–1.50 (m, 1H), 1.65–1.82 (m, 1H), 1.82–2.05 (m, 4H), 2.50 (br s, 1H, exch with D_2O), 3.79 (br s, 2H), 4.97 (s, 2H), 7.23–7.51 (m, 5H).

6.7. 3-Benzyl-3,9-diazabicyclo[3.3.1]nonane (10)

To a stirred mixture of lithium aluminium hydride (7.9 g, 210 mmol) in dry Et_2O (269 ml) at 0°C a solution of **9** (7.04 g, 29 mmol) in dry benzene (101 ml) was added dropwise. The reaction mixture was refluxed for 6 h, then cooled at 5°C, decomposed with water (27 ml) and kept at r.t. for 1 h. The salts were filtered off and the filtrate was dried (Na_2SO_4) and evaporated to leave a dark oil. The oil was purified by distillation at 170–178°C/0.8 [4] to afford the desired compound **10** (5.48 g, 88%) as a brown oil.

R_f 0.27 (chloroform:methanol, 9:1); IR (film, cm^{-1}) ν: 3340 (NH); ^1H NMR ($CDCl_3$) δ_H: 1.56–1.95 (m, 6H), 2.25–2.43 (m, 2H), 2.75–2.95 (m, 3H, one which exch with D_2O), 2.97–3.08 (br s, 2H), 3.37 (s, 2H), 7.20–7.40 (m, 5H).

6.8. 3-Benzyl-9-propionyl-3,9-diazabicyclo[3.3.1]nonane (11)

To a chilled solution of **10** (3.65 g, 16.9 mmol) in dichloromethane (10.3 ml), propionic anhydride (4.5 g, 35 mmol) was added dropwise. When addition was complete, the mixture was refluxed for 1 h. After cooling at −5°C, 20% NaOH (15 ml) was added and the mixture was stirred overnight at r.t. and then extracted with CH_2Cl_2. The organic layers were dried (Na_2SO_4), filtered and evaporated to give **11** as a crude oil which was purified by distillation at 130°C/0.2 (4.0 g, 87%) [4].

R_f 0.58 (petroleum ether 50–70:ethyl acetate, 6:4); IR (film, cm^{-1}) ν: 1660 (C=O); ^1H NMR ($CDCl_3$) δ_H: 1.10–1.30 (m, 4H), 1.50–1.90 (m, 5H), 2.20–2.50 (m, 4H), 2.80–3.00 (m, 2H), 3.38 (AB_q, 2H), 3.89 (br s, 1H), 4.69 (br s, 1H), 7.20–7.40 (m, 5H).

6.9. 9-Propionyl-3,9-diazabicyclo[3.3.1]nonane (3)

A solution of **11** (3.1 g, 11.4 mmol) in ethanol (28 ml) was hydrogenated at 60 psi at 60°C for 7 h in the presence of 10% Pd–C (0.6 g). The catalyst was filtered off, the solution was evaporated and the oily residue was purified by flash chromatography ($CHCl_3$–MeOH, 9:1) to give **3** as a yellow oil (1.96 g, 94.5%), b.p. 110–115°C/0.2, which on standing, solidified and was crystallized from ligroine (m.p. 54–56°C) [4].

R_f 0.34 (chloroform:methanol, 9:1); IR (film, cm^{-1}) ν: 1630 (C=O), 3390 (NH); ^1H NMR ($CDCl_3$) δ_H: 1.00–1.20 (m, 4H), 1.60–1.75 (m, 1H), 1.80–2.00 (m, 4H), 2.20–2.65 (m, 3H), 3.05–3.20 (m, 4H), 3.87 (br s, 1H), 4.19 (br s, 1H, exch with D_2O), 4.64 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ_C: 43.78 and 49.16 (CH × 2), 170.80 (C=O) assigned with DEPT and HETCOR experiments.

6.10. 3-Propionyl-3,9-diazabicyclo[3.3.1]nonane (4)

The compound **3** (0.83 g, 4.56 mmol) was heated in an open round-bottom flask at 150°C for 2 h. The crude product was chromatographed (silica gel) eluting with $CHCl_3$–CH_3OH (8:2) to give in the order **3** (0.1 g, 12%) and **4** (0.71 g, 88%) as an oil, b.p. 125–130°C/0.4.

IR (film, cm^{-1}) ν: 1630 (C=O), 3390 (NH); ^1H NMR ($CDCl_3$) δ_H: 1.16 (t, 3H), 1.50–1.70 (m, 2H), 1.80–2.20 (m, 4H), 2.35 (q, 2H), 3.15 (dd, 1H), 3.33 (br s, 2H), 3.65 (dd, 1H), 3.88 (d, 1H), 4.62 (d, 1H), 4.79 (br s, 1H exch with D_2O). ^{13}C NMR ($CDCl_3$) δ_C: 9.05 (CH_3), 18.24, 26.64, 29.48, 29.49, 45.08 and 49.22 (CH_2 × 6), 46.53 and 46.61 (CH × 2), 172.58 (C=O) assigned by DEPT (135°) and HETCOR experiments. *Anal.* $C_{10}H_{18}N_2O$ (C, H, N).

6.11. General procedure for arylpropenyl alcohols (13)

The required 3-arylacrylic acid (**12b**,**d**) (10 mmol) or ester (**12c**,**e**,**f**,**g**) in dry Et_2O (30 ml for **12b**) or dry toluene (50 ml for **12c–g**) was treated under argon with 1.5 M DIBALH (17.3 ml, 26 mmol) in toluene at −5°C. The mixture was stirred for 1 h, the temperature reaching 20°C. The reaction was cooled at 0–5°C, quenched by dropwise addition of saturated solution of potassium sodium tartrate (24.3 ml) and stirred overnight at r.t. To the mixture Et_2O (60 ml) was added, the organic layers separated, washed (H_2O), dried (Na_2SO_4), the solvent was evaporated and the crude **13** was purified by flash chromatography or by crystallization.

6.11.1. 13b

Yield: 34%; m.p. 129–130°C (ether–petroleum ether) (128–129°C [1]); ^1H NMR ($CDCl_3$) δ_H: 1.91 (br s, 1H), 4.30–4.40 (d, 2H), 6.20–6.40 (m, 1H), 6.50–6.70 (d, 1H), 7.50–7.60 (d, 2H), 8.20–8.30 (d, 2H).

6.11.2. 13c

Yield: 97%; b.p. 118–120°C/0.2 (125–130°C/0.4 [1]); ^1H NMR ($CDCl_3$) δ_H: 2.30–2.40 (br s, 1H), 4.30–4.40 (d, 2H), 6.20–6.40 (m, 1H), 6.50–6.70 (d, 1H), 7.10–7.30 (m, 3H), 7.30–7.40 (s, 1H).

6.11.3. 13d

Yield: 64%; b.p. 134–136°C/0.7; ^1H NMR ($CDCl_3$) δ_H: 1.60 (br s, 1H), 4.30–4.40 (t, 2H), 6.30–6.40 (m, 1H), 6.50–6.70 (d, 1H), 7.20–7.30 (m, 1H), 7.30–7.50 (d, 1H), 7.50 (d, 1H).

6.11.3.1. **13e**. Yield: 68%; m.p. 95–97°C (EtOH–H_2O); ^1H NMR ($CDCl_3$) δ_H: 1.69 (br s, 1H exch with D_2O), 4.38 (dd, 2H), 6.45 (dt, 1H), 6.63 (d, 1H), 7.49 (br s, 2H), 7.86 (s, 1H). *Anal.* $C_9H_8ClNO_3$ (C, H, Cl, N).

6.11.4. 13f

Yield: 96%; m.p. 64–66°C (EtOH–H_2O); ^1H NMR ($CDCl_3$) δ_H: 1.85 (br s, 1H exch with D_2O), 4.44 (d, 2H), 6.53 (dt, 1H), 7.03 (d, 1H), 7.53 (d, 1H), 8.00 (dd, 1H), 8.40 (d, 1H).

6.11.5. 13g

Yield: 78%; m.p. 85–86°C (EtOH–H_2O); ^1H NMR ($CDCl_3$) δ_H: 1.66 (br s, 1H exch with D_2O), 4.40 (d, 2H), 6.38 (dt, 1H), 7.13 (d, 1H), 7.38 (dd, 1H), 7.58 (d, 1H), 7.93 (d, 1H). *Anal.* $C_9H_8ClNO_3$ (C, H, Cl, N).

*6.12. General procedures for arylpropenyl chlorides (**14**)*

6.12.1. A

A solution of the appropriate **13b**,**e–g** (10 mmol) and dry pyridine (1 ml) in dry dichloromethane (37 ml) was added within 1 h at r.t. to a solution of thionyl chloride (2.7 ml, 37 mmol) in dry dichloromethane (13 ml). After a further 0.5 h stirring, the mixture was poured into an aqueous solution of $NaHCO_3$ (6.8 g, 81 mmol) and the layers were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated to give **14b**,**e–g** which were purified by flash chromatography.

*6.12.1.1. **14b***. Yield: 78%; m.p. 58.5–59.5°C; ^{1}H NMR ($CDCl_3$) δ_H: 5.3–5.6 (m, 3H), 6.0–6.2 (m, 1H), 7.5–7.6 (d, 2H).

*6.12.1.2. **14e***. M.p. 59–61°C [6].

*6.12.1.3. **14f***. Yield: 96%; m.p. 38–40°C; ^{1}H NMR ($CDCl_3$) δ_H: 4.26 (d, 2H), 6.31 (dt, 1H), 7.17 (d, 1H), 7.42 (dd, 1H), 7.60 (d, 1H), 8.00 (d, 1H). *Anal.* $C_9H_7Cl_2NO_2$ (C, H, Cl, N).

*6.12.1.4. **14g***. Yield: 98%; m.p. 159–161°C; ^{1}H NMR ($CDCl_3$) δ_H: 4.30 (d, 2H), 6.50 (dt, 1H), 8.10 (dd, 1H), 8.41 (d, 1H). *Anal.* $C_9H_7Cl_2NO_2$ (C, H, Cl, N).

6.12.2. B

A mixture of the appropriate **13c**,**d** (10 mmol) and concentrated HCl (15 ml) was heated at 78–80°C for 3 h with stirring. The mixture was cooled and extracted with Et_2O. The organic layer was washed several times with water, dried (Na_2SO_4) and then evaporated to provide the crude **14c**,**d** which were purified by bulb to bulb distillation.

*6.12.2.1. **14c***. Yield: 77%; b.p. 90°C/0.1; ^{1}H NMR ($CDCl_3$) δ_H: 4.2 (d, 2H), 6.2–6.4 (m, 1H), 6.5–6.7 (d, 1H), 7.2–7.4 (m, 4H).

*6.12.2.2. **14d***. B.p. 72–74°C/0.01 [13].

6.13. Biology

6.13.1. Membrane preparation

Male Wistar rats and guinea pigs were decapitated and the whole brain (minus brainstem, striatum and cerebellum) was dissected on ice. The tissue was disrupted in a Polytron (setting 5) in 20 vols of 50 nM Tris–HCl, pH 7.4. The homogenate was centrifuged at 34 000 × *g* for 10 min and the pellet was resuspended in the same buffer. After 30 min incubation at 37°C the membranes were centrifuged and pellets were used in binding experiments. An aliquot of homogenate was removed for protein assay [14].

6.13.2. Binding studies

Binding assays were carried out as described by Gillan and Kosterlitz [15] with small modifications.

Displacement experiments were performed in 250 μl of 50 nM Tris–HCl, pH 7.4 containing 1 nM [^{3}H]-DAMGO and 1 nM [^{3}H]-DELTORPHINE II to label μ and δ receptors in rat brain membranes (18 mg tissue); 1 nM [^{3}H]-U69 593 was used to label κ receptors in guinea pig brain membranes (15 mg of tissue). To determinate IC_{50} values (where IC_{50} is the inhibitor concentration displacing 50% of the labelled ligand), the test compounds were added in triplicate to the binding-assay samples at a minimum of six different concentrations. After 60 min incubation, bound and free radioactivity were separated by filtering the assay mixture through GF/B glass fibre filters (Whatman) using a MicroMate 196 Cell Harvester (Packard Instrument Company). The filter-bound radioactivity was counted on Top Count (efficiency 57%) with MicroScint-20 (30 ml in 96-well plates). Non-specific binding was defined as the binding in the presence of 10 μM bremazocine: this was always $<10\%$ of the total binding.

K_i values were calculated from IC_{50} values using the Cheng–Prusopff equation [16].

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