Eur J Med Chem (1995) 30, 515–520

Short communication

Synthesis and κ binding affinity of 1-(pyrrolidin-1-ylmethyl)-2-(*N*-methyl)-4-[(3,4-dichloro)phenyl]-1,2,3,4-tetrahydroisoquinolin-3(2*H*)-ones

GA Pinna[1], E Gavini[1], G Cignarella[2]*, S Scolastico[2], P Fadda[3]

[1]*Istituto di Chimica Farmaceutica, via Muroni, 23, 07100 Sassari;*
[2]*Istituto di Chimica Farmaceutica e Tossicologica, viale Abruzzi, 42, 20131 Milan;*
[3]*Centro CNR per la Neurofarmacologia, via Porcell, 4, 09124 Cagliari, Italy*

(Received 8 November 1994; accepted 20 February 1995)

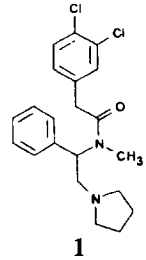
Summary — Diastereomeric forms of 1-(pyrrolidin-1-ylmethyl)-2-(*N*-methyl)-4-[(3,4-dichloro)phenyl]-1,2,3,4-tetrahydroisoquinolin-3(2*H*)-ones **3a** and its chloro analog **3c** were synthesized. Compounds **3a,c** are related to the κ-selective opiate ICI 199441 **1** by linking the benzylic CH_2 to the *ortho* position of the phenyl in **1**. Compared with morphine, these compounds had lost in κ and μ affinities; only ***cis*-3a** showed a modest κ affinity. 1-Pyrrolidin-1-ylmethyl-*N*-[2-(3,4-dichlorophenyl)acetyl]-1,2,3,4-tetrahydroisoquinoline **2**, which is also a cyclic congener of **1**, was reported to display high κ and μ affinity, and so a conformational study was undertaken on **1**, **2** and **3a**. This showed that, while active **2** extensively superposed on **1**, **3a** assumes another geometry which does not allow a fit with the pharmacophoric moieties of **1** and **2**.

tetrahydroisoquinolin-3(2*H*)-one / κ-opiate receptor / rigid congener

Introduction

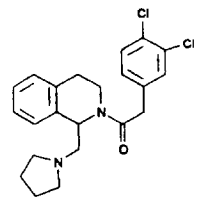
Morphine has been used through the centuries to control pain. However, side effects, most notably tolerance, physical dependence, respiratory depression and nausea [1], represent a serious drawback to its use. The existence of multiple subtypes of opioid receptors (μ, δ, κ) [2, 3], which modulate the analgesic potency to various extents [4], and the side effects, have been and still are the impetus for research devoted to the design of selective ligands [5]. In this context, it has been shown that κ agonists can induce centrally mediated analgesia, but side effects, such as sedation, dysphoria and diuresis, have been reported in animal studies [6].

In this class of compounds, benzeneacetamide amines like ICI 199441 **1** [7] and its rigid congener **2** [8] have emerged in recent years as potent and selective κ agonists. The promising results reported for **2**, a cyclic analog of **1**, prompted us to synthesize tetrahydroisoquinolinones **3** formally derived from **1** by linking the benzylic CH_2 to the phenyl group with a C-C bond.

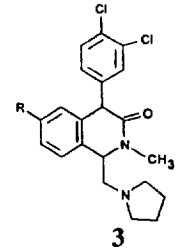


1

ICI 199441



2



3

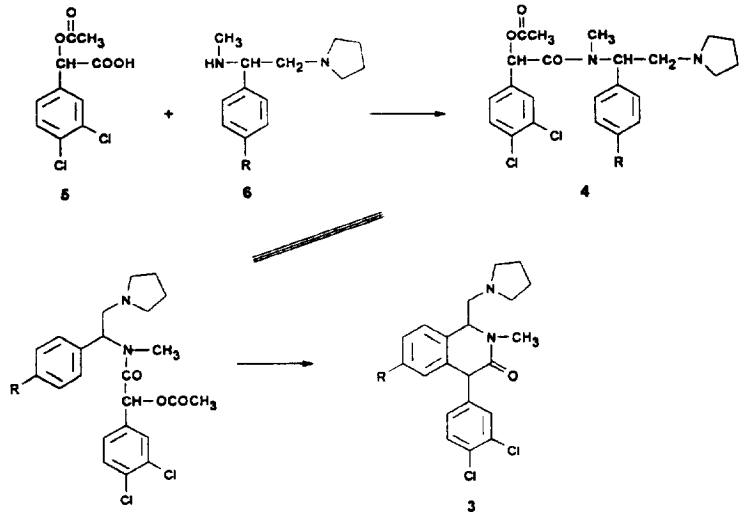
R = H **(a)**
OCH_3 **(b)**
Cl **(c)**

Chemistry

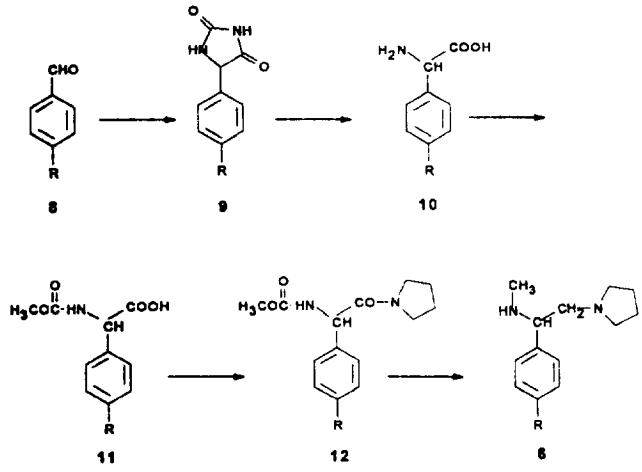
Compounds **3a,c** were synthesized by cyclization of amides **4a,c** (scheme 1) prepared in a convergent manner by condensing 3,4-dichloro-2-acetyloxybenzene acetic acid **5** with the appropriate amines **6a–c** in the presence of carbonyldiimidazole (CDI). In contrast, attempts to obtain **3b** from **5** and **4b** failed.

The acid **5** was synthesized by acetylation of the α-oxyacid **7** [9–11] which was obtained from 3,4 dichlorobenzaldehyde [12].

*Correspondence and reprints



Scheme 1. R = H (**a**), OCH_3 (**b**), Cl (**c**).



Scheme 2. R = H (**a**), OCH_3 (**b**), Cl (**c**).

Amines **6a–c** were synthesized as shown in scheme 2 from phenylglycines **10a–c** by *N*-carbomethoxylation to **11a–c** [13] followed by condensation with pyrrolidine in the presence of CDI. Simultaneous reduction of both carbamoyl and amide functions of **12a–c** with excess lithium aluminium hydride (LAH) gave the corresponding diamines **6a–c** in good yield. Phenylglycines **10b,c** [14] were not commercially available and were obtained from benzaldehydes **8b,c** *via* hydantoines **12b,c** through alkaline hydrolysis. Condensation of the acid **5** with amines **6a–c** to the desired amides **4a–c** was carried out with CDI.

Both **3a** and **3c** were obtained as diastereomeric mixtures, which were separated by flash chromatography to give in order *trans*-**3a** (R_f = 0.60; mp = 98–100°C), **3c** (R_f = 0.78; mp = 123–126°C) and *cis*-**3a** (R_f = 0.42; mp = 117–118°C), **3c** (R_f = 0.50; oil) (table I). The assignment of the configuration was made on the basis of NOE experiments and NMR spectra (table II). In particular the spectra of *trans*-isomers by irradiation of the C_4- proton signal exhibited a strong NOE effect on methyl, methylenic and pyrrolidinic protons, in agreement with a *trans* relationship between the C_1 and C_4 protons. In contrast, this effect was absent in the spectra of the *cis*-isomers.

In addition, the ^{1}H-NMR spectra of *trans*- and *cis*-isomers of **3a,c** differed in the signals attributed to the pyrrolidinic α-methylenic protons. In fact, although they appeared as narrow multiplets centered at 2.40 δ for *trans*-**3a,c**, in the *cis*-**3a,c** these signals are split into two multiplets centered at 2.37 and 2.47 δ.

Receptor binding

Compounds *cis*-**3a,c** and *trans*-**3a,c** were examined for their ability to displace [^{3}H]bromazocine and [^{3}H]DAGO [15] universal ligands with significant κ and μ receptor affinities, respectively, from binding sites on cell membranes prepared from whole rat brain minus cerebellum. The results obtained are shown in table III. The activity of the morphine is also reported for comparative purposes.

Molecular modelling

A conformational analysis of compound **1**, **2** and **3** was performed using the MM2 force field included in the software package Macromodel [16] and the Monte-Carlo method. The superpositions were performed considering the following 4 atoms: the nitrogen of the pyrrolidine ring; the nitrogen of the amidic moiety; the carbon of the chlorinated benzenic ring joining the side chain; and the chlorine in the *para* position.

Results and discussion

Biological data for *trans*-**3a,c** and *cis*-**3a,c** (table III) show a dramatic reduction in κ- and μ-affinities with respect to the model compounds **1** and **2**; only *cis*-**3a** retains a κ-affinity in the micromolar range. Figure 1 shows the superposition between the global minimum of **2** (E = 25.44 kcal/mol) and the conformer of compound **1** with an energy 0.41 kcal/mol superior to the global minimum (E = 18.69 kcal/mol).

The loss of affinity could be explained on the basis of molecular mechanics calculations. The superposition between **2** and **1** as their minimum energy conformers (25.44 kcal/mol for **2**, 19.10 kcal/mol for **1**) shows the great similarity between the compounds, the only difference being the orientation of the unsubstituted aromatic rings. However, superposition of minimum energy conformers of **3a** (E = 20.9 kcal/mol) and **1** (fig 2) and of **3a** and **2** (fig 3) focused on the pharmacophore >NCH_2CH_2NCOR evidenced marked differences in the orientation of the chlorine atoms in the *meta* position of the dichlorophenyl groups, and in the regions occupied by the unsubstituted aromatic moiety and the pyrrolidinic rings.

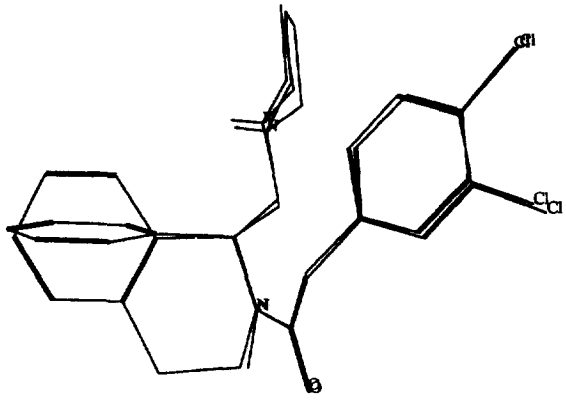


Fig 1. Superposition of **1** and **2**.

Table I. Characterization of compounds prepared.

Compound (salt)	*Mp (°C) (solvent)*	*Bp (°C/mmHg)*	*Yield (%)*
9b	190–192 ($EtOH/H_2O$)		86.1
9c	166–167 ($EtOH/H_2O$)		72.5
10b[a]	267–269[b]		86.0
10c[a]	270–271[b]		70.3
11a[c]	97–98 (benzene)		70.1
11b	65–67 (benzene)		63.2
11c	58–60 (benzene)		76.8
12a[d]	120–121 (EtOAc/lygroin)		74.6
12b	Oil[b]		95.7
12c	Oil[b]		76.6
6a	Oil	95/1.8 x 10^{-3}	94.6
6b	Oil	92–94/1.33 x 10^{-3}	93.6
6c	Oil	95/1.33 x 10^{-3}	88.0
4a[e]	Oil		96.4
4b[e]	Oil		87.0
4c[e]	Oil		91.6
3a[e]	Oil		93.7
trans-**3a**	98–100 (hexane)		68.3
cis-**3a**	117–118 (hexane/EtOAc)		31.7
3c	Oil		83.6
trans-**3c**	123–126 (EtOAc/lygroin)		64.6
trans-**3c**•HCl	145–147 ($EtOAc/CH_3OH$)		
cis-**3c**	Oil		35.4
cis-**3c** HCl	193–195 ($EtOAc/CH_3OH$)		

[a]This compound is described in reference [9]; [b]this compound was used in the next step without further purification; [c]this compound is described in reference [13]; [d]this compound is described in reference [7]; [e]diastereomeric mixture.

Experimental protocols

Chemistry

Melting points were determined on a Kofler apparatus and are uncorrected. Elemental analyses are within ± 0.4% of the theoretical values. ^{1}H-NMR spectra were obtained from $CDCl_3$ solutions using a Varian XL-200 spectrometer. Proton chemical shifts are given in δ (ppm) relative to Me_4Si (= 0 ppm). Infrared spectra were recorded as nujol mulls on a Perkin Elmer 781 spectrometer and are expressed in ν cm^{-1}. Analytical thin-layer chromatography (TLC) was performed on precoated silica-gel 60 F254 plastic sheets (Merck). Chemical and spectroscopic data of the new compounds are reported in tables I and II.

3,4-Dichloro-α-hydroxybenzeneacetic acid 7

This compound was obtained using the same procedure described in references [9–11]. mp 113°C (lit [9–11] mp 113°C).

Table II. Spectroscopic data of compounds.

Compound	*IR ν (cm⁻¹)*	*¹H-NMR (δ, ppm)*[a]
9b	3510–3220 (NH); 1805–1720 (CO)	3.80 (s, 3H, OCH_3); 4.94 (s, 1H, CH); 7.15 (qAB, 4H, Ar-H); 7.98 (brs, 1H, exch with D_2O, NH); 10.55 (brs, 1H, exch with D_2O, NH)
9c	3480–3080 (NH); 1780–1690 (CO)	5.19 (s, 1H, CH); 7.38 (qAB, 4H, Ar-H); 8.43 (s, 1H, exch with D_2O, NH); 10.84 (s, 1H, exch with D_2O, NH)
10b	1670 (CO)	3.81 (s, 3H, OCH_3); 5.00 (s, 1H, CH); 7.25 (qAB, 4H, Ar-H)
10c	1660–1630 (CO)	5.18 (s, 1H, CH); 7.45–7.56 (m, 4H, Ar-H)
11a	3480 (OH); 3380 (NH); 1700 (CO)	3.66 (s, 3H, CH_3); 5.07 (brs, 1H, exch with D_2O); 5.37 (d, 1H, exch with D_2O); 7.26–7.36 (m, 5H, Ar-H)
11b	3540 (OH); 3400 (NH); 1700 (CO)	3.67 (s, 3H, CH_3); 3.80 (s, 3H, OCH_3); 4.25 (brs, 1H, exch with D_2O); 5.32 (d, 1H, CH); 5.67 (d, 1H, exch with D_2O); 7.10 (qAB, 4H, Ar-H)
11c	3360 (NH, OH); 1730 (CO); 1690–1660 (CO)	3.41 (brs, 1H, exch with D_2O); 3.56 (s, 3H, CH_3); 5.18 (d, 2H, CH); 7.38–7.55 (m, 4H, Ar-H); 8.04 (d, 1H, exch with D_2O)
12a	3320 (NH); 1710 (CO); 1640 (CO)	5.18 (d, 2H, CH); 7.38–7.55 (m, 4H, Ar-H); 8.04 (d, 1H, exch with D_2O); 1.76–1.91 (m, 4H, CH_2 x 2); 3.03–3.08 (m, 2H, CH_2); 3.38–3.60 (m, 2H, CH_2); 3.63 (s, 3H, CH_3); 5.38 (d, 1H, CH); 5.27 (d, 1H, exch with D_2O); 7.27–7.39 (m, 5H, Ar-H)
12b	3500–3300 (NH); 1730 (CO); 1750 (CO)	1.78–1.90 (m, 4H, CH_2 x 2); 3.08–3.10 (m, 2H, CH_2); 3.50–3.54 (m, 2H, CH_2); 3.63 (s, 3H, CH_3); 3.79 (s, 3H, CH_3); 5.32 (d, 1H, CH); 6.24 (d, 1H, exch with D_2O); 7.12 (qAB, 4H, Ar-H)
12c	3400–3300 (NH); 1720 (CO); 1650 (CO)	1.80–1.92 (m, 4H, CH_2 x 2); 3.04–3.06 (m, 2H, CH_2); 3.37–3.50 (m, 2H, CH_2); 3.63 (s, 3H, CH_3); 5.34 (d, 1H, CH); 6.34 (d, 1H, exch with D_2O); 7.27–7.39 (m, 4H, Ar-H)
6a	3320 (NH)	1.74–1.80 (m, 4H, CH_2 x 2); 2.23–2.32 (m, 1H, CH_2); 2.29 (s, 3H, CH_3); 3.39–2.47 (m, 2H, CH_2); 2.57–2.65 (m, 2H, CH_2); 2.83 (dd, 1H, CH); 7.26–7.35 (m, 5H, Ar-H)
6b	3325 (NH)	1.73–1.78 (m, 4H, CH_2 x 2); 2.19–2.40 (m, 4H, CH_3 and CH); 2.40–2.46 (m, 2H, CH_2); 2.59–2.63 (m, 2H, CH_2); 2.61 (t, 1H, CH); 3.52 (dd, 1H, CH); 3.80 (s, 3H, CH_3); 7.07 (qAB, 4H, Ar-H)
6c	3320 (NH)	1.73–1.80 (m, 4H, CH_2 x 2); 2.18–2.33 (m, 4H, CH_3 and CH); 2.42–2.46 (m, 2H, CH_2); 2.58–2.63 (m, 2H, CH_2); 2.76 (t, 1H, CH); 5.54 (dd, 1H, CH); 7.26–7.30 (m, 4H, Ar-H)
4a[b]	1740 (CO); 1750 (CO); 1655 (CO); 1650 (CO)	1.69–1.72 (m, CH_2 x 2); 1.75–1.85 (m, CH_2 x 2); 2.18 (s, CH_3); 2.21 (s, CH_3); 2.37–2.42 (m, CH_2); 2.51–2.55 (m, CH_2); 2.65 (s, CH_3); 2.67 (s, CH_3); 2.66–2.70 (m, CH_2, CH); 2.70–2.82 (m, CH_2, CH); 3.07–3.19 (m, CH); 5.09–6.04 (dd, CH); 6.06–6.14 (dd, CH); 6.16 (s, CH); 6.18 (s, CH); 7.15–7.62 (m, Ar-H)
4b[b]	1750 (CO); 1660 (CO)	1.67–1.71 (m, CH_2 x 2); 2.22 (s, CH_3); 2.31–2.45 (m, CH_2); 2.59 (s, CH_3); 2.60–2.70 (m, CH_2 x 2 and CH); 3.10 (m, CH); 3.80 (s, CH_3); 6.10 (dd, CH); 6.17 (s, 1H, CH); 6.66–7.70 (m, Ar-H)
4c[b]	1750 (CO); 1660 (CO)	1.70 (m, CH_2 x 2); 1.78 (m, CH_2 x 2); 2.22 (s, CH_3); 2.27 (s, CH_3); 2.33–2.43 (m, CH_2); 2.44–2.55 (m, CH_2); 2.64 (s, CH_3); 2.65 (s, CH_3); 3.08 (m, CH_2, CH); 3.10 (m, CH_2, CH); 5.90 (dd, CH); 6.08 (dd, CH); 6.15 (s, CH); 7.10–7.70 (m, Ar-H)

Table II. *(Continued)*

Compound	*IR ν (cm⁻¹)*	*¹H-NMR (δ, ppm)*[a]
trans-**3a**	1640 (CO)	1.64–1.70 (m, 4H, CH_2 x 2); 2.37–2.40 (m, 4H, CH_2 x 2); 2.48–2.70 (m, 2H, CH_2); 3.17 (s, 3H, CH_3); 4.40–4.46 (t, 1H, CH); 4.89 (s, 1H, CH); 7.10–7.36 (m, 7H, Ar-H)
cis-**3a**	1640 (CO)	1.65–1.75 (m, 4H, CH_2 x 2); 2.37 (m, 2H, CH_2); 2.47 (m, 2H, CH_2); 2.82 (qq, 2H, CH_2); 3.18 (s, 3H, CH_3); 4.47 (t, 1H, CH); 4.81 (s, 1H, CH); 6.60–7.46 (m, 7H, Ar-H)
trans-**3c**	1640 (CO)	1.65–1.72 (m, 4H, CH_2 x 2); 2.38–2.48 (m, 2H, CH_2); 2.60 (qq, 2H, CH_2); 3.14 (s, 3H, CH3); 4.40 (t, 1H, CH); 4.84 (s, 1H, CH); 7.10–7.70 (m, 6H, Ar-H)
cis-**3c**	1640 (CO)	1.69 (m, 4H, CH_2 x 2); 2.37 (m, 2H, CH_2); 2.47 (m, 2H, CH_2); 2.92 (qq, 2H, CH_2); 3.17 (s, 3H, CH_3); 4.47 (t, 1H, CH); 4.80 (s, 1H, CH); 7.20–7.70 (m, 6H, Ar-H)

[a]All spectra were obtained in $CDCl_3$ except for compounds **9b** ($CDCl_3$/$(CD_3)_2SO$), **9c** $(CD_3)_2SO$, **10b** ($(CD_3)_2SO$ + TFA), **11c** $(CD_3)_2SO$. [b]Diastereomeric mixture.

3,4-Dichloro-α-acetyloxybenzeneacetic acid **5**
A mixture of 1.1 g (0.005 mol) of **7** 1.07 g (0.01 mol) of acetyl chloride was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness providing 1.17 g of a crude yellow solid which was purified by crystallization from xylene obtaining 1 g (77.5%) of **5** as crystals, mp 110–111°C. TCL ($CHCl_3$/MeOH 8:2) R_f 0.38. IR 1750–1710 (broad band CO). ¹H-NMR: 2.21 (s, 3H, CH_3), 5.87 (s, 1H, CH); 7.31–7.61 (m, 3H, aromatics); 9.20 (s, 1H exchange with D_2O).

5-Arylhydontoins **9b,c**. *General procedure*
A slurry of 0.015 mol arylaldehyde **8**, 0.019 mol potassium cyanide and 0.048 mol ammonium carbonate in 16 ml aqueous ethanol was stirred for 12 h at 60°C. After dissolution, a solid precipitated which was collected after cooling.

Arylglycines **10b,c**. *General procedure*
A mixture of 0.024 mol **9** in 38 ml of 10% hydroxide solution was refluxed with stirring for 24 h, and then the resulting solution was treated with charcoal under heating for 30 min and filtered. The filtrate was brought to pH 6 with concentrated HCl and kept at 5°C for 24 h. The crystallized solid was filtered off to give **10b,c**.

Table III. κ and μ opioid receptor affinity *in vitro*.

Compound	*Opioid receptor affinity (K_d, nM)*[a]	
	[³H]Bremazocine (κ)	*[³H]DAGO (μ)*
trans-**3a**	> 10 000	> 10 000
cis-**3a**	7407	> 10 000
trans-**3c**	> 10 000	> 10 000
cis-**3c**	> 10 000	> 10 000
Morphine	260	2.08

[a]K_d values represent the means from concentration–response curves performed in triplicate.

N-Carbomethoxyarylglycines **11a–c**. *General procedure*
To a solution of 0.0066 mol arylglycine **10** in 3.3 ml 4 N NaOH was added dropwise at 0°C 0.0066 mol of methylchloroformate. The slurry was kept under stirring at room temperature for 20 min and acidified with 5 N HCl. The mixture was extracted with ether. The organic layer was dried over sodium sulfate and concentrated to dryness to afford **11** as a colorless oil which solidified on standing as white glassy solid.

Methyl N-[(1-pyrrolidinylcarbamyl)arylmethyl]carbamate **12a–c**. *General procedure*
A solution of 0.0029 mol **11**, 0.0036 mol *N,N'*-carbonyldiimidazole in 5.5 ml dry DMF was stirred at 40–45°C until gas evolution ceased (10 min). The solution was cooled, 0.0072 mol pyrrolidine was added, and after stirring at 20°C for 30 min, the mixture was poured into excess 0.5 N aqueous Na_2CO_3 and

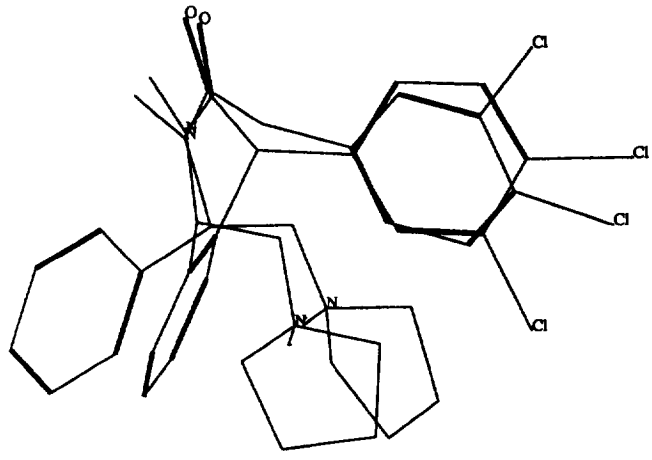


Fig 2. Superposition of **1** and **3a**.

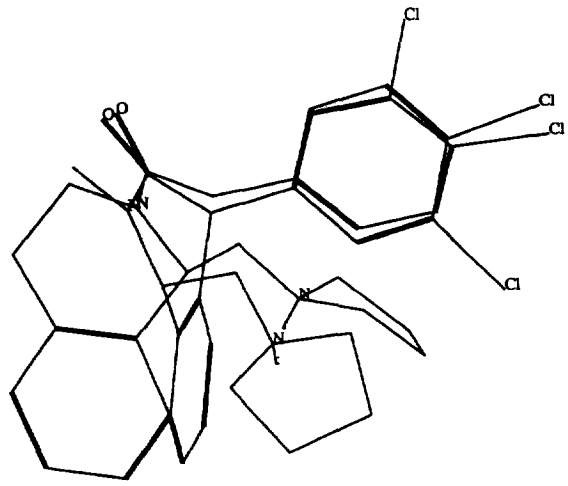


Fig 3. Superposition of **2** and **3a**.

the whole was extracted with ethylacetate. The organic layer was washed with diluted HCl and water, dried (Na_2SO_4) and concentrated to dryness to give **12** as white solid.

N-[(2-Methylamino-2-aryl)ethyl]pyrrolidine ***6a–c****. General procedure*
To a slurry of 0.019 mol **12** in 317 ml dry diethyl ether was added with caution 0.076 mol of LAH. The stirred reaction mixture was heated at reflux for 24–36 h, cooled, and quenched by successive addition of 3.6 ml water, 9 ml NaOH and 3.6 ml water. The mixture was filtered on celite pad and washed with diethyl ether. The organic filtrate was concentrated to give **6** as colorless oil.

2-(3,4-Dichlorophenyl)-2-acetyloxy-N-methyl-N-[1-aryl-2(1-pyrrolidynyl)ethyl]acetamide ***4a–c****. General procedure*
To a solution of 0.0049 mol **5** in 10 ml dry dichloromethane was added portionwise 0.0049 mol N,N^1-carbonyldiimidazole at room temperature and under nitrogen. The resulting solution was stirred for 1.5 h and then 0.0025 mol of the appropriate amine **6** was added, and stirred for 22 h. The reaction mixture was washed with 2 M Na_2CO_3 solution and water. The organic phase was dried (Na_2SO_4) and concentrated to dryness to give diastereomeric mixture of **4** as a yellowish oil. TLC showed two spots, each representing a diastereomeric form. For **4a** the diastereomeric mixture was chromatographed over silica eluting with 95:5 dichloromethane/methanol to yield 62.7% of a faster moving oily product, mp 204–207°C (AcOEt/MeOH as a hydrochloride). Further elution yielded 37.3% of the slower moving diastereomer as an oil which solidified on standing, mp 70–72°C (AcOEt/ligroin). Its hydrochloride melted at 218–220°C (AcOEt/MeOH).

1-(Pyrrolidin-1-ylmethyl)-2-(N-methyl)-4-[(3,4-dichloro)phenyl]-1,2,3,4-tetrahydroisoquinolin-3(2H)-ones ***3a,c***
A solution of 0.0045 mol of the diastereomeric mixture of **4a,c** in 10 ml concentrated sulfuric acid was heated at 60°C for 5 h under stirring. The solution was poured into crushed ice and basified with concentrated NH_4OH and extracted with ether which was dried (Na_2SO_4) and evaporated to give diastereomeric mixture of **3** as thick brown oil. The crude product was chromatographed over silica gel eluting with 85:15 benzene/acetone to yield 68.3% (for **3a**) and 64.57% (for **3c**) of the faster moving *trans*-diastereomer, followed by the slower moving *cis*-diastereomer. Attempts to convert **4b** into **3b** by the same procedure failed and an untractable mixture of products was isolated.

Receptor binding studies

Male Sprague-Dawley (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. For each receptor assay one whole brain minus cerebellum was homogenized in 50 volumes (w/v) of 50 mM Tris-HCl pH 7.4 with polytron, centrifuged at 48 000 *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 *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/prot/ml. [^{3}H]DAGO (2 nM), and [^{3}H]bremazocine (1 nM) (New England Nuclear, Germany) were used to label μ and κ receptor respectively.

Brain membranes were incubated with the appropriate ^{3}H ligand in 50 mM Tris HCl pH 7.4 at 0°C for 60 min in the absence or presence of 10 μM naloxone. [^{3}H]Bremazocine binding was carried out in the presence of unlabelled DAGO (100 nM) to prevent binding at μ sites.

Final protein concentration were determined by the method of Lowry *et al* [17]. K_d values were calculated with the Ligand program [18], from displacement curves of each compound at a concentration range between 10^{10} M and 10^4 M. Values are means ± SEM from two assays.

References

1 Jaffe JH, Martin WR (1990) *The Pharmacological Basis of Therapeutics*, 8th ed (Goodman A, Gilman LS, eds) Pergamon press, New York, USA, 485–521
2 Martin WR, Eades CG, Thompson TA, Huppler RE, Gilbert PE (1976) *J Pharmacol Exp Ther* 197, 517–532
3 Load JAH, Waterfield AA, Hughes J, Kosterlitz HW (1977) *Nature (Lond)* 267, 495–499
4 Martin WR (1983) *Pharmacol Rev* 35, 283–323
5 Millan MJ (1990) *Trends Pharmacol Sci* 11, 70–76
6 Von Voigtlander PF, Lahti RA, Ludens JH (1983) *J Pharmacol Exp Ther* 224, 7–12
7 Costello GF, James R, Shaw JS, Slater AM, Stutchbury NCT (1991) *J Med Chem* 34, 181–189
8 Vecchietti V, Clarke G, Colle R, Giardina G, Petrone G, Sbacchi M (1991) *J Med Chem* 34, 2624–2633
9 Reeve W, Pickert PE (1957) *J Amer Chem Soc* 79, 1932–1934
10 Pagani G, Baruffini A, Borgna P, Gialdi F (1967) *II Farmaco Ed Sci* 22, 1019–1034
11 (1962) NV Nederlands Combinatie Voor Chemische Industrie Belg, 619, 160; *Chem Abstr* (1963), 59, P9905c
12 Barthel WF, Leon J, Hall SA (1954) *J Org Chem* 19, 485–489
13 Leuchs H, Geiger W (1908) *Chem Ber* 41, 1722–1726
14 Doyle FP, Fosker GR, Nayler JHC, Smith H (1962) *J Chem Soc* 1440–1444
15 Gillan MGC, Kosterlitz HW (1982) *Br J Pharmacol* 77, 461–468
16 Mohamadi F, Richards NGJ, Guida WG *et al* (1990) *J Comp Chem* 11, 440–467
17 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) *J Biol Chem* 193, 265–275
18 Munson PJ, Rodbard D (1980) *Anal Biochem* 107, 220–239