J. Pharm. Pharmacol. 1997, 49: 835–842
Received December 6, 1996
Accepted May 7, 1997

Synthesis and Structure-Opioid Activity Relationships of *trans*-(±)-3,4-Dichloro-*N*-methyl-*N*-[4- or 5-hydroxy-2-(1-pyrrolidiny)cyclohexyl]benzeneacetamides

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Abstract

To explore the effects of attaching a hydroxy function to the cyclohexane ring of κ-selective opioid *N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamides, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[4- or 5-hydroxy-2-(1-pyrrolidiny)cyclohexyl]benzeneacetamides (**1–4**) and their benzoates (**5–8**) have been synthesized in a divergent and stereoselective manner.

When compared with the parent compound U-50488, hydroxy derivatives **1–4** maintained high selectivity towards the κ-opioid receptor (μ/κ ratio = 24 to > 91); while displaying significant reduction in binding affinity ($K_{i,\kappa}$ = 75–218 nM). The lowest κ-affinity was observed with compound **4**, where the hydroxy group is attached at the 5-axial or 5-β position. Further reduction in κ-affinity was observed when the hydroxy function was benzoylated. However, the 4β, 5α, and 5β isomers (**6–8**) maintained varying degrees of κ-selectivity; the 4α-isomer compound **5**, with its benzoate moiety situated at the 4-axial position is now a moderately potent μ-selective opioid ($K_{i,\mu}$ = 168 nM, μ/κ = 0·076).

The results suggest the importance of lipophilicity in binding to opioid receptors and the presence of a specific lipophilic binding site on the μ-opioid receptor.

In the search for centrally acting analgesics, selective non-peptide κ-opioid receptor agonists have received considerable attention in recent years (Scopes 1993, 1994) because they have been demonstrated to provide effective analgesia with minimal morphine-like side effects such as physical dependence and respiratory depression (Millan 1990). During our previous efforts (Cheng et al 1992; Chen et al 1993) in the discovery of selective and irreversible ligands for the κ-opioid receptor based on the structure of the prototype κ-opioid U-50488 (Szmuszkovicz & Von Voigtlander 1982), (±)-(1α, 2β, 4α)-3,4-dichloro-*N*-methyl-*N*-[4-hydroxy-2-(1-pyrrolidiny)cyclohexyl]benzeneacetamide (**1**) (Fig. 1) was synthesized as an intermediate, and found to retain moderate affinity and selectivity towards the κ-opioid receptor. Therefore, we decided to further explore the structure-activity relationships of U-50488 analogues with a hydroxy substituent on the cyclohexane ring. Reported here are the synthesis and opioid-receptor binding affinities of four isomeric *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[4- or 5-hydroxy-2-(1-pyrrolidiny)cyclohexyl]-benzeneacetamides (**1–4**) and their benzoate esters (**5–8**) (Fig. 1). Halfpenny et al (1990) have reported a series of 4- or 5-methoxy substituted derivatives (**Ia–d**) (Fig. 1) of the benzofuran analogue of the potent κ-selective opioid PD 117302. However, their synthetic strategy starting from methoxybenzenes cannot be adopted for the preparation of compounds **1–4**.

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Materials and Methods

General procedures

Melting points were taken in a capillary tube by using the Laboratory Devices, MEL-TEMP II melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AMX-400 or AM-300 FT-NMR spectrometer; chemical shifts were recorded in parts per million downfield from Me_4Si. IR spectra were determined with a Perkin-Elmer 1760-X FT-IR spectrometer. Mass spectra were recorded on a Jeol JMS-D300 or Finnigan TSQ-46C mass spectrometer; high-resolution mass spectra were obtained with a Jeol JMS-HX110 spectrometer. Elemental analysis was performed with a Perkin-Elmer 2400-CHN instrument. TLC was performed on Merck (Art. 5715) silica gel plates and visualized under UV light (254 nm), upon treatment with iodine vapour, or upon heating after treatment with 5% phosphomolybdic acid in ethanol. Flash column chromatography was performed with Merck (Art. 9385) 40–63 mm silica gel 60.

Syntheses

*3-Cyclohexen-1-ol (**9**).* To a stirred solution of 1,4-cyclohexanediol (80 g, 0·69 mol) in dry pyridine (800 mL) at 0°C under N_2, was added dropwise a solution of *p*-toluenesulphonyl chloride (118·2 g, 0·62 mol) in dry pyridine (400 mL). The resulting mixture was stirred continuously at 0°C overnight, and evaporated. The residue was then treated with 18% aqueous HCl (200 mL), and extracted with CH_2Cl_2 (300 mL). The combined organic layers were dried ($MgSO_4$), and evaporated to give a mixture of 1,4-cyclohexanediol *mono*-toluenesulphonate and 1,4-cyclohexanediol *di*-toluenesulphonate as a yellow liquid (105·4 g, 54%, *mono-/di-* = 9 : 1 based on HPLC, Merck Lichrospher 100 RP-18 (5 mM) 0·4 × 25 cm, $CH_3OH : H_2O$ = 60 : 40).

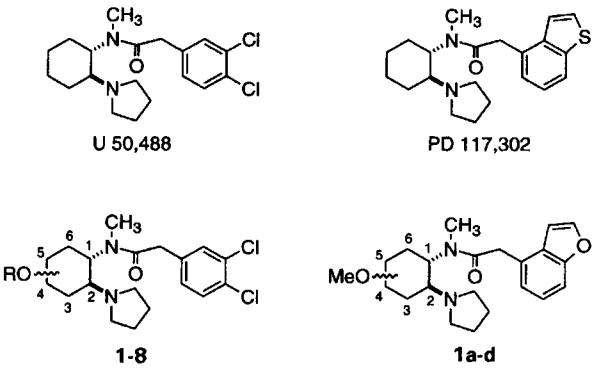


FIG. 1. Structures of U 50,488, PD 117,302 and compounds **1–8** (R = H: **1** (4α); **2** (4β); **3** (5α); **4** (5β). R = Bz: **5** (4α); **6** (4β); **7** (5α); **8** (5β)) and **Ia-d** (**Ia**: 4α; **Ib**: 4β; **Ic**: 5α; **Id**: 5β).

The above mixture of toluenesulphonates was mixed with 1,8-diazabicyclo[5,4,0]-undec-7-ene (60 g, 0·39 mol), degased, and heated at 120°C overnight. The resulting mixture was distilled (30 mbar, 86–87°C) to give **9** as a colourless liquid (19·7 g, 60%): IR (neat) 3338, 3026, 2921, 2841, 1651, 1439, 1364, 1072, 1052 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm) 5·7–5·6 (m, 1 H), 5·6–5·5 (m, 1 H), 3·9–3·8 (m, 1 H), 2·4–2·2 (m, 2 H), 2·2–1·9 (m, 2 H), 1·9–1·8 (m, 1 H), 1·6–1·5 (m, 1 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 126·7, 124·0, 66·8, 34·2, 30·8, 23·6; MS m/e 98 (M^+), 97 (base peak), 79, 67, 55.

*Cyclohex-3-enol t-butyldimethylsilyl ether (**10**).* To a refluxed solution of **9** (2·0 g, 20 mmol) and imidazole (1·8 g, 27 mmol) in dry THF (8 mL) under N_2, was added slowly a solution of *t*-butyldimethylsilyl chloride (3·4 g, 23 mmol) in dry THF (9 mL). The resulting mixture was refluxed for another 2 h, cooled to room temperature (21°C), and evaporated. The residue was treated with H_2O (20 mL), and extracted with CH_2Cl_2 (20 × 3 mL). The combined organic layers were dried ($MgSO_4$), and evaporated to give **10** as a colourless liquid (3·95 g, 91%): bp, 68°C at 7 mbar, IR (neat) 3027, 2955–2858, 1652, 1256, 1106, 1093 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm) 5·6–5·5 (m, 2H), 3·9–3·8 (m, 1 H), 2·3–1·9 (m, 4 H), 1·8–1·7 (m, 1 H), 1·6–1·5 (m, 1 H), 0·9 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 126·6, 124·7, 68·0, 35·2, 31·8, 25·9, 24·4, 18·2, − 4·6; MS m/e 211 (M^+–1), 197, 155, 101, 75 (base peak).

*(±)-(1α,3α,6α)-3-t-Butyldimethylsilyloxy-7-oxabicyclo[4,1,0]-heptane (**11**) and (±)-(1α,3β,6α)-3-t-butyldimethylsilyloxy-7-oxabicyclo[4,1,0]heptane (**12**).* To a stirred solution of **10** (4·44 g, 20·9 mmol) in CH_2Cl_2 (50 mL), cooled in ice-water bath, was added 70% *m*-CPBA (5·66 g, 23·0 mmol). The cooled mixture was stirred for another 2 h, and then allowed to warm to room temperature. After the addition of 20% aqueous Na_2SO_3 solution (3·6 mL) and further stirring for 1 h, saturated aqueous $NaHCO_3$ solution (20 mL) was added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layers were dried ($MgSO_4$) and evaporated to give a mixture of **11** and **12** as a colourless oil (4·63 g, 97%, **11**/**12** = 57 : 43 based on GC, SE-30 3% on Chrom-WHP 80/100 mesh 0·4 × 300 cm). The mixture (0·5 g) was chromatographed (silica gel; *n*-hexane : $CHCl_3$: ether = 100 : 10 : 1) to give pure **11** and **12** in a molar ratio of 59 : 41 (R_f = 0·36, 0·48, $CHCl_3$). **11**: ^{1}H-NMR ($CDCl_3$) δ (ppm) 3·6–3·5 (m, 1 H), 3·1–3·0 (m, 2 H), 2·2–2·1 (m, 2 H), 1·8–1·7 (m, 2 H), 1·5–1·4 (m, 2 H), 0·8 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 67·3, 51·6, 51·1, 34·2, 27·7, 25·8, 24·0, 18·1, − 4·7; MS m/e 228 (M^+), 211, 171 (base peak), 167, 149, 97. **12**: ^{1}H-NMR ($CDCl_3$) δ (ppm) 3·8–3·7 (m, 1 H), 3·1 (s, 2 H), 2·2–2·0 (m, 2 H), 1·9–1·6 (m, 2 H), 1·6–1·4 (m, 1 H), 1·4–1·2 (m, 1 H), 0·8 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 64·3, 51·9, 51·8, 34·0, 27·6, 25·8, 20·7, 18·0, − 4·8; MS m/e 228 (M^+), 213, 171 (base peak), 129, 101, 79, 75.

*(±)-(1α,2β,4β)-2-(1-Pyrrolidinyl)cyclohexane-1,4-diol 4-t-butyldimethylsilyl ether (**13**), (±)-(1α,2β,4α)-2-(1-pyrrolidinyl)cyclohexane-1,4-diol 4-t-butyldimethylsilyl ether (**14**) and (±)-(1α,2β,5β)-6-(1-pyrrolidinyl)cyclohexane-1,3-diol 3-t-butyldimethylsilyl ether (**15**).* A mixture of **11** + **12** (4·0 g, 17·5 mmol) and pyrrolidine (14·5 mL, 175 mmol) was refluxed under N_2 overnight. The excess pyrrolidine was evaporated to give an orange-coloured liquid (5·23 g, crude yield 99%). A fraction of the crude (1 g) was chromatographed (silica gel; CH_2Cl_2 : CH_3OH : NH_4OH = 100 : 10 : 1) to give pure **13**, **14** and **15** in a molar ratio of 8 : 69 : 23 (R_f = 0·7, 0·6 and 0·5, respectively). **13**: IR (neat) 3366, 2932–2857, 1255, 1088 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm) 3·6–3·5 (m, 1 H), 3·4–3·3 (m, 1 H), 2·9–2·7 (m, 4 H), 2·6-2·5 (m, 1 H), 2·1–1·7 (m, 7 H), 1·4–1·1 (m, 3 H), 0·8 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 70·6, 69·8, 62·5, 47·3, 33·6, 30·9, 29·7, 25·8, 23·5,18·0, − 4·7, − 4·8; MS m/e 299 (M^+), 284, 242, 240 (base peak); HRMS m/e (M^+) calculated 299·2281, observed 299·2279; nOe: irra. 3·6 ppm, δ (ppm) 2·6 (1·85%) 1·9 (3·66%). **14**: IR (KBr) 3418, 2952–2857, 1255, 1041 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm) 4·2–4·1 (m, 1 H), 4·0 (s, 1 H), 3·4–3·3 (m, 1 H), 3·0–2·9 (m, 1 H), 2·7–2·5 (m, 4 H), 1·9–1·4 (m, 8 H), 1·4–1·2 (m, 2 H), 0·8 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 70·5, 66·8, 59·5, 47·5, 31·4, 29·1, 27·4, 25·7, 23·5, 17·9, − 4·9, − 5·0; MS m/e 299 (M^+), 284, 242, 240 (base peak); HRMS m/e (M^+) calculated 299·2281, observed 299·2284; nOe: irra. 4·1 ppm, δ (ppm) 1·8 (7·91%), 1·7 (3·84%), 1·4 (8; 84%), 0·0 (8·34%). **15**: IR (neat) 3369, 2953–2857, 1255, 1040 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm) 4·4 (s, 1 H), 4·1–4·0 (m, 1 H), 3·8–3·7 (m, 1 H), 2·9–2·7 (m, 4 H), 2·6–2·5 (m,1 H), 2·1–2·0 (m, 1 H), 1·9–1·5 (m, 7 H), 1·5–1·3 (m, 2 H), 0·8 (s, 9 H), 0·0 (d, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 66·8, 66·1, 65·5, 47·8, 40·6, 32·3, 25·6, 23·5, 17·8, 16·0, − 5·0, − 5·1; MS m/e 299 (M^+), 284, 242, 110 (base peak); HRMS m/e (M^+) calculated 299·2281, observed 299·2276; nOe: irra. 4·0 ppm, δ (ppm) 2·1 (3·64%), 1·7 (1·81%), 1·4 (7·56%), 0·0 (4·28%).

*(±)-(1α,2β,4α)-3,4-Dichloro-N-methyl-N-[4-t-butyldimethylsilyloxy-2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (**16**), (±)-(1α,2β,5α)-3,4-dichloro-N-methyl-N-[5-t-butyldimethylsilyoxy-2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (**17**) and (±)-(1α,2β,5β)-3,4-dichloro-N-methyl-N-[5-t-butyldimethylsilyoxy-2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (**18**).* To a stirred solution of a mixture of **13** + **14** + **15** (2·21 g, 7·38 mmol), obtained from the previous step, and Et_3N (1·84 mL, 13·3 mmol) in dry CH_2Cl_2 (50 mL) under N_2 at 0°C, CH_3SO_2Cl (0·81 mL, 10·4 mmol) was added slowly. After stirring for 1 h, saturated aqueous $NaHCO_3$ solution was added. The organic layer was separated, and the aqueous layer

was extracted with CH_2Cl_2 (10 mL × 2). The organic layers were combined, dried ($MgSO_4$), and evaporated to give a crude mixture of the corresponding mesylates (2·78 g).

Without further separation, the crude mesylates were dissolved in dry THF (20 mL), together with a solution of 40% CH_3NH_2 in CH_3OH (14 mL). The mixture was heated at 120°C in a sealed vessel for 4 h, then cooled to room temperature, and evaporated. The residue was treated with H_2O (20 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were dried ($MgSO_4$) and evaporated to give a crude mixture of diamines as a brown-coloured liquid (2·33 g).

To a stirred solution of the above crude mixture (2·33 g) and Et_3N (1·44 mL, 10·4 mmol) in dry CH_2Cl_2 (20 mL), was added slowly 3,4-dichlorophenylacetyl chloride (1·54 mL, 9·7 mmol). After the mixture was stirred continuously overnight, saturated aqueous $NaHCO_3$ solution (10 mL) was added. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2). The combined organic layers were dried ($MgSO_4$) and evaporated to give a crude mixture, which was chromatographed (silica gel; $CH_2Cl_2 : C_2H_5OH : NH_4OH = 100 : 4 : 1$) to give **16** and its two isomers **17** and **18** (total yield, 2·62 g, 71·0%; **16** : **17** : **18** = 52 : 12 : 36; R_f = 0·5, 0·4 and 0·3, respectively, $CH_2Cl_2 : CH_3OH : NH_4OH = 100 : 5 : 1$). **16**: mp 94·5–95·5°C (white crystals from *n*-hexane); HCl salt: mp 240–241°C (white crystals from isopropanol); IR (KBr) 2952–2856, 1638, 1255, 1036 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm) 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 4·5 (td, J = 11·8 & 3·8 Hz, 1 H), 4·1 (s, 1 H), 3·7 (d, J = 15·5 Hz, 1 H), 3·6 (d, J = 15·5 Hz, 1 H), 3·3 (td, J = 11·5 & 3·4 Hz, 1 H), 2·8 (s, 3 H), 2·7–2·6 (m, 4 H), 2·0–1·3 (m, 10 H), 0·8 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 170·1, 135·8, 132·3, 131·0, 130·9, 130·2, 128·4, 66·6, 54·1, 52·8, 47·3, 40·5, 32·2, 30·0, 29·9, 25·7, 23·9, 17·9, − 4·9, − 5·0; MS m/e 498 (M^+), 483, 441, 281, 240 (base peak); HRMS m/e (M^+) calculated 498·2236, observed 498·2209; Anal. calculated for $C_{26}H_{40}N_2O_2SiCl_2$: C 60·10, H 8·07, N 5·61, found: C 59·71, H 7·90, N 5·70; HCl salt; Anal. calculated for $C_{25}H_{40}N_2O_2SiCl_2$ HCl: C 56·01, H 7·71, N 5·23, found: C 56·00, H 7·64, N 5·11; **17**: mp 100·5–101°C (white crystals from *n*-hexane); ^{1}H-NMR ($CDCl_3$) δ (ppm) 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 4·6 (s, 1 H), 3·8–3·4 (m, 4 H), 2·8 (s, 3 H), 2·8–2·4 (m, 4 H), 1·9–1·2 (m, 10 H), 0·8 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 172·7, 136·0, 131·8, 131·7, 130·3, 129·9, 129·6, 65·3, 57·0, 52·1, 47·9, 40·3, 31·3, 30·3, 25·6, 24·9, 24·4, 23·0, 17·8, − 4·6; MS m/e 498 (M^+), 483, 441, 388, 366, 281, 110 (base peak); HRMS m/e (M^+) calculated 498·2236, observed 498·2226; Anal. calculated for $C_{26}H_{40}N_2O_2SiCl_2$: C 60·10, H 8·07, N 5·61, found: C 60·16, H 8·22, N 5·47; **18**: ^{1}H-NMR ($CDCl_3$) δ (ppm) 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 4·1–4·0 (m, 2 H), 3·7–3·5 (m, 2H), 2·8 (s, 3 H), 2·8–2·4 (m, 5 H), 1·9–1·2 (m, 10 H), 0·8 (s, 9 H), 0·0 (s, 6 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 169·9, 136·1, 131·3, 130·6, 130·1, 128·8, 128·3, 66·7, 59·7, 55·0, 48·0, 40·9, 38·9, 38·7, 36·9, 32·3, 27·2, 25·8, 24·0, 23·8, 18·4, − 4·9, − 5·0; MS m/e 498 (M^+), 483, 441, 388, 366, 281, 110 (base peak); HRMS m/e (M^+) calculated 498·2236, observed 498·2230.

*(±)-(1α, 2β, 4α)-3,4-Dichloro-N-methyl-N-[[4-hydroxyl-2-(1-pyrrolidinyl)]-cyclohexyl]benzeneacetamide (**1**).* A mixture of **16** (0·70 g, 1·40 mmol), 37% aqueous HCl (3·7 mL), and C_2H_5OH (10 mL) was stirred at room temperature for 5 h. After evaporation of C_2H_5OH, the residue was treated with saturated aqueous $NaHCO_3$ solution (30 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined extracts were dried ($MgSO_4$), and evaporated to give **1** as a white solid (0·52 g, 96%): IR (neat) 3323 (br.), 2937–2869, 1645, 1032 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm) 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 4·6–4·4 (m, 1 H), 4·2 (s, 1 H), 3·7 (d, J = 15·6 Hz, 1 H), 3·6 (d, J = 15·8 Hz, 1 H), 3·3–3·2 (m, 1 H), 2·8 (s, 3 H), 2·7–2·4 (m, 4 H), 2·0–1·4 (m, 10 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 170·1, 135·7, 132·3, 131·0, 130·8, 130·2, 128·3, 66·1, 54·5, 52·6, 47·2, 40·5, 31·7, 30·1, 29·3, 23·9, 23·4; MS m/e 385 (M^+ + 1), 167 (base peak), 126, 84; HRMS m/e (M^+) calculated 384·1372, observed 384·1371; HCl salt: mp. 254–255°C (white crystals from ethyl acetate); Anal. calculated for $C_{19}H_{26}N_2O_2Cl_2$, HCl: C 54·10, H 6·45, N 6·64, found: C 53·99, H 6·39, N 6·51.

*(±)-(1α,2β,5α)-3,4-Dichloro-N-methyl-N-[[5-hydroxy-2-(1-pyrrolidinyl)]-cyclohexyl]benzeneacetamide (**3**).* Compound **17** (0·5 g, 100 mmol) was subjected to the same procedure as described above to give **3** (0·37 g, 96%): ^{1}H-NMR ($CDCl_3$) δ (ppm) 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 4·6–4·5 (m, 1 H), 3·7–3·5 (m, 4 H), 2·8 (s, 3 H), 2·7–2·6 (m, 1 H), 2·6–2·4 (m, 4 H), 2·1–1·3 (m, 10 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 169·7, 135·5, 132·4, 131·0, 130·7, 130·3, 128·6, 128·2, 68·9, 59·3, 58·2, 57·8, 52·4, 48·8, 47·2, 40·5, 39·5, 38·5, 33·9, 29·9, 23·9, 18·7; MS m/e 384 (M^+), 366, 314, 274, 167, 110 (base peak), 97; HRMS m/e (M^+) calculated 384·1372, observed 384·1394; HCl salt: mp 260·5–261·5°C (white crystals from ethyl acetate); Anal. calculated for $C_{19}H_{26}N_2O_2Cl_2$, HCl: C 54·10, H 6·45, N 6·64, found: C 53·79, H 6·20, N 6·27.

*(±)-(1α,2β,5β)-3,4-Dichloro-N-methyl-N-[[5-hydroxy-2-(1-pyrrolidinyl)]-cyclohexyl]benzeneacetamide (**4**).* Compound **18** (0·5 g, 100 mmol) was subjected to the same procedure as described for **1** to give **4** (0·37 g, 97%): ^{1}H-NMR ($CDCl_3$) δ (ppm) 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 4·9–4·8 (m, 1 H), 4·1–4·0 (m, 1 H), 3·7–3·5(m, 3 H), 2·8 (s, 3 H), 2·7–2·6 (m, 1 H), 2·6–2·4 (m, 4 H), 1·9–1·6 (m, 10 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 170·2, 169·9, 136·1, 135·6, 132·3, 132·1, 131·4, 130·7, 130·6, 130·5, 130·3, 130·1, 129·0, 128·2, 66·0, 65·9, 59·8, 58·6, 54·8, 50·6, 48·0, 47·0, 40·6, 39·3, 37·4, 36·4, 31·7, 31·3, 30·1, 27·2, 24·0, 23·8, 18·5, 16·3; MS m/e 384 (M^+), 366, 314, 274, 167, 110 (base peak), 97; HRMS m/e (M^+) calculated 384·1372, observed 384·1375; HCl salt: mp 260–261°C (white crystals from ethyl acetate); Anal. calculated for $C_{19}H_{26}N_2O_2Cl_2$, HCl: C 54·10, H 6·45, N 6·64, found: C 53·94, H 6·32, N 6·56.

*(±)-(1α,2β,4α)-3,4-Dichloro-N-methyl-N-[4-benzoyloxy-2-(1-pyrrolidiny)-cyclohexyl]benzeneacetamide (**5**).* To a stirred solution of **1** (280 mg, 72·7 mmol) in dry pyridine (5 mL) at 70°C under N_2, was added benzoyl chloride (1 mL). The mixture was stirred for 1 h, cooled to room temperature, and evaporated, The residue was treated with saturated aqueous $NaHCO_3$ solution (10 mL), and extracted with CH_2Cl_2 (10 ml × 3). The combined extracts were dried ($MgSO_4$), and evaporated to give a crude product, which was chromatographed (silica gel; $EtOAc : HOAc = 100 : 1$, followed by $EtOAc : CH_3OH : HOAc = 100 : 40 : 1$) to give **5** (200 mg, 56%): HCl salt: mp 230·5–232·5°C (white crystals from isopropanol); ^{1}H-NMR ($CDCl_3$) δ (ppm) 8·0–7·9 (m, 2 H),

7·6–7·5 (m, 1 H), 7·5–7·4 (m, 2 H), 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 5·4 (s, 1 H), 4·7–4·6 (m, 1 H), 3·7–3·6 (m, 2 H), 3·2–3·1 (m, 1 H), 2·8 (s, 3 H), 2·7–2·4 (m, 4 H), 2·3–2·2 (m, 1 H), 2·1–2·0 (m, 1 H), 1·9–1·6 (m, 8 H); ^{13}C-NMR (HCl salt, $DMSO_{d\text{-}6}$) δ (ppm) 172·5, 165·7, 138·6, 134·2, 133·0, 131·5, 131·1, 130·7, 130·5, 129·5, 69·6, 57·5, 52·5, 48·4, 31·2, 28·5, 27·9, 25·2, 24·9, 24·2; MS m/e 488 (M^+), 383, 367, 271, 230, 149 (base peak), 105, 97, 84, 77, 70, 55; HRMS m/e (M^+) calculated 488·1634, observed 488·1633.

(±)-(1α,2β,5α)-3,4-Dichloro-N-methyl-N-[[5-benzoyloxy-2-(1-pyrrolidinyl)]-cyclohexyl]benzeneacetamide (7). Compound **3** (200 mg, 519 mmol) was subjected to the same procedure as described above to give **7** (147 mg, 58%): HCl salt: mp 150–154°C (white crystals from isopropanol); ^{1}H-NMR ($CDCl_3$) δ (ppm) 8·0–7·9 (m, 2 H), 7·6–7·5 (m, 1 H), 7·5–7·4 (m, 2 H), 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 5·1–5·0 (m, 1 H), 4·9–4·7 (m, 1 H), 3·9–3·6 (m, 3 H), 2·9 (s, 3 H), 2·8–2·4 (m, 4 H), 2·4–1·2 (m, 10 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 170·0, 165·6, 135·5, 132·9, 130·8, 130·4, 129·5, 128·3, 71·5, 59·3, 58·0, 48·7, 47·6, 40·7, 39·5, 36·0, 34·8, 31·0, 27·5, 24·0, 19·0; MS m/e 418, 366, 149 (base peak), 136, 110, 97; HRMS m/e (M^+) calculated 488·1634, observed 488·1627.

(±)-(1α,2β,5β)-3,4-Dichloro-N-methyl-N-[5-benzoyloxy-2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (8). Compound **4** (200 mg, 519 mmol) was subjected to the same procedure for **5** to give **8** (157 mg, 62%): HCl salt: mp 215–218°C (white crystals from isopropanol); ^{1}H-NMR ($CDCl_3$) δ (ppm) 8·0–7·9 (m, 2 H), 7·6–7·5 (m, 1 H), 7·5–7·4 (m, 2 H), 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 5·4–5·3 (m, 1 H), 5·2–5·0 (m, 1 H), 3·7–3·4 (m, 3 H), 2·9 (s, 3 H), 2·8–2·4 (m, 4 H), 2·4–1·2 (m, 10 H); ^{13}C-NMR ($CDCl_3$) δ (ppm) 169·9, 165·6, 165·3, 156·8, 135·6, 133·2, 133·0, 132·3, 131·1, 130·7, 130·6, 130·2, 130·1, 129·5, 129·3, 128·7, 128·57, 128·4, 128·2, 69·9, 69·5, 59·6, 58·8, 55·8, 48·9, 48·3, 40·5, 39·4, 34·8, 33·9, 33·5, 30·4, 29·0, 28·7, 27·2, 25·6, 24·8, 24·1, 23·7, 19·8, 17·8; MS m/e 488 (M^+), 366, 149 (base peak), 136, 110; Anal. calculated for $C_{26}H_{30}N_2O_3Cl_2$, HCl: C 59·72, H 5·94, N 5·33, found: C 59·50, H 5·99, N 5·65.

(±)-(1α,2β,4β)-3,4-Dichloro-N-methyl-N-[4-benzoyloxy-2-(1-pyrrolidiny)-cyclohexyl]benzeneacetamide (6). To a stirred solution of **1** (2·13 g, 5·53 mmol), PPh_3 (2·90 g, 11·1 mmol) and benzoic acid (1·35 g, 11·1 mmol) in dry THF (80 mL) under N_2 at room temperature, was added a solution of diethyl azodicarboxylate (1·93 g, 11·1 mmol) in dry THF (20 mL). The resulting mixture was stirred continuously overnight. After evaporation of THF, the residue was chromatographed (silica gel, EtOAc : HOAc = 100 : 1, followed by EtOAc : CH_3OH = 10 : 1) to give **6** as a white solid (444 mg, 16·4%): HCl salt: mp 146–149°C (white crystals from isopropanol); ^{1}H-NMR ($CDCl_3$) δ (ppm) 8·0–7·9 (m, 2 H), 7·6–7·5 (m, 1 H), 7·5–7·4 (m, 2 H), 7·4-7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 5·0–4·9 (m, 1 H), 5·7–5·5 (m, 1 H), 3·8–3·6 (m, 2 H), 2·9–2·8 (m, 1 H), 2·8 (s, 3 H), 2·7–2·5 (m, 4 H), 2·5–1·9 (m, 3 H), 1·9–1·5 (m, 7 H); ^{13}C-NMR (HCl salt, $DMSO_{d\text{-}6}$) δ (ppm) 172·6, 165·8, 138·5, 134·4, 132·9, 131·5, 131·1, 130·7, 130·5, 130·1, 129·6, 71·3, 58·3, 52·5, 48·6, 30·1, 29·2, 25·5, 25·1, 24·8, 23·7; MS m/e 383, 367, 271, 230, 159, 149 (base peak), 105, 97, 84, 77, 70, 55; HRMS m/e (M^+) calculated 488·1634, observed 488·1629.

(±)-(1α,2β,4β)-3,4-Dichloro-N-methyl-N-[4-hydroxy-2-(1-pyrrolidiny)-cyclohexyl]benzeneacetamide (2). To a stirred solution of **6** (211 mg, 0·43 mmol) in CH_3OH (40 mL) at room temperature was added 40% NaOH (2 mL). The resulting mixture was kept stirring for 2 h. After evaporation of CH_3OH, the residue was treated with H_2O (10 mL), and extracted with CH_2Cl_2. The combined extracts were dried ($MgSO_4$) and evaporated to give **2** as a yellow solid (152 mg, 92%): ^{1}H-NMR ($CDCl_3$) δ (ppm) 7·4–7·3 (m, 2 H), 7·1–7·0 (m, 1 H), 4·5–4·4 (m, 1 H), 3·7–3·5 (m, 3 H), 2·7 (s, 4 H), 2·6–2·4 (m, 5 H), 2·4–1·9 (m, 4 H), 1·7–1·5 (m, 4 H), 1·4–1·3 (m, 2 H); ^{13}C-NMR (HCl salt, $DMSO_{d\text{-}6}$) δ (ppm) 172·5, 138·5, 132·9, 131·5, 131·4, 131·1, 130·7, 129·6, 67·6, 67·0, 60·1, 58·7, 52·3, 51·7, 25·1, 24·8, 22·2, 21·4; MS m/e 384 (M^+), 367, 314, 271, 230, 179, 167, 126 (base peak), 97, 84, 70, 56; HRMS m/e ($M^+ + 2$) calculated 386·1343, observed 386·1342.

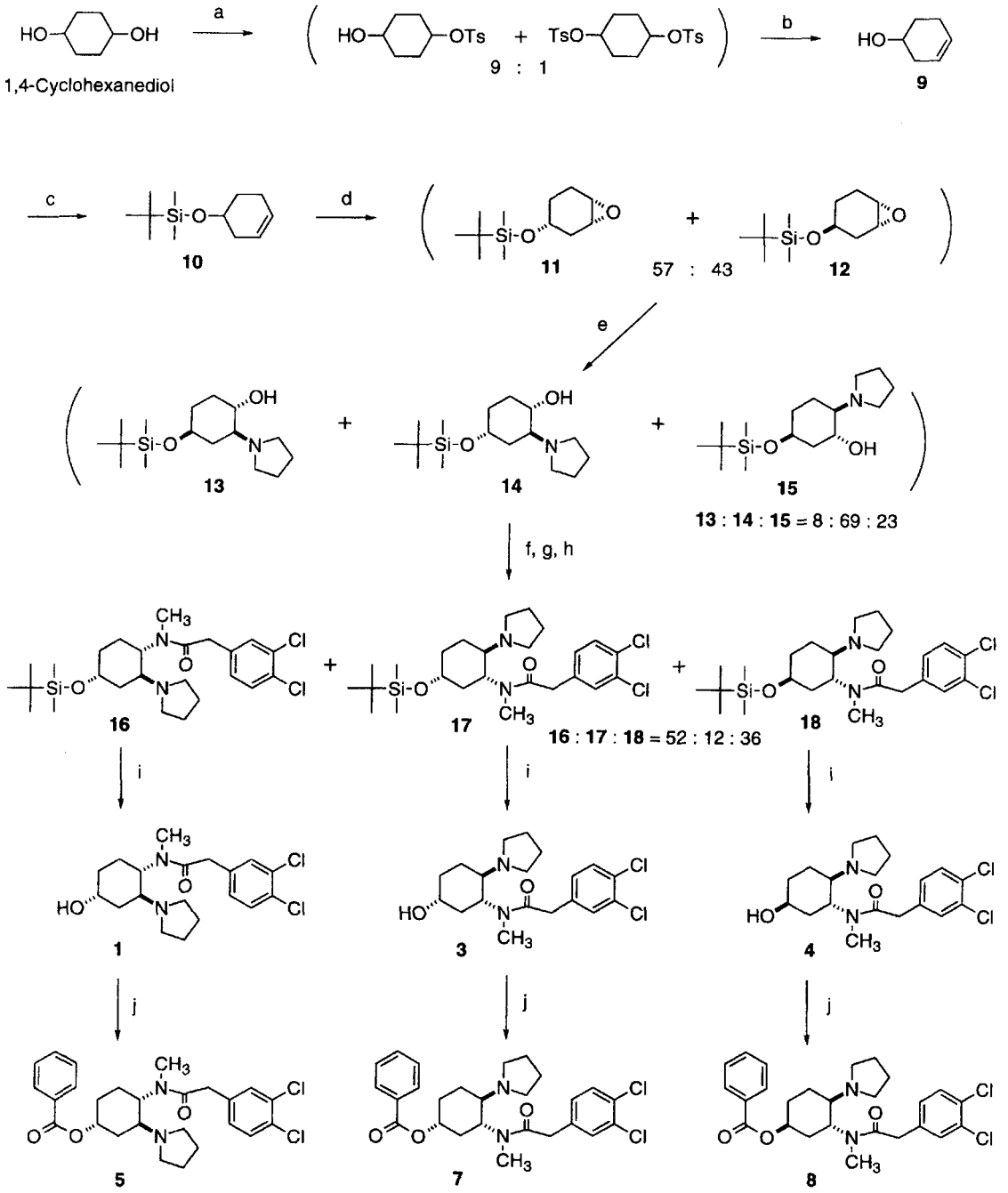
Opioid-receptor binding assay

Brain membranes were prepared from male Hartley guinea-pigs, and binding was performed by literature procedures (Tam 1985) with modification. The following labelled ligands were used: 1·0 nM [^{3}H]DAMGO (μ-binding); 2·0 nM [^{3}H]ethylketocyclazocine with 500 nM DADLE and 500 nM DAMGO (κ-binding); 2·0 nM [^{3}H]DADLE with 100 nM morphiceptin (δ-binding); nonspecific binding was determined with 1 mM DAMGO (μ-binding), 10 mM naloxone and U-50,488 (κ-binding), and 10 mM naloxone and DADLE (δ-binding). Radioactivity was determined by scintillation counting. Protein was determined by the method of Lowry et al (1951). The IC50 and K_i values were determined with the program by McPherson (1983), which is a modification of the LIGAND program originally written by Munson & Rodbard (1980).

Results and Discussion

Syntheses

Three out of the four hydroxy derivatives, namely compounds **1**, **3** and **4**, and their benzoate esters **5**, **7** and **8** were synthesized in a divergent manner according to Scheme 1. The starting 1,4-cyclohexanediol was subjected to tosylation under carefully controlled reaction conditions to give predominantly the mono-tosylate as shown, which underwent E2 elimination reaction effected by DBU to give 3-cyclohexen-1-ol (**9**) in 60% yield. Protection of the hydroxy function in **9** with a *tert*-butyldimethylsilyl group, followed by epoxidation with *m*-CPBA resulted in the formation of a pair of diastereomeric epoxides **11** and **12** in a ratio of 57 : 43, as determined by GC analysis. A mixture of epoxides **11** and **12** was then subjected to ring-cleavage reaction with pyrrolidine to give, after chromatography, *trans*-aminoalcohols **13**, **14**, and **15** in a molar ratio of 8 : 69 : 23. Compounds **13** and **15** were derived from epoxide **12**, while out of the two possible isomers from epoxide **11**, only compound **14** was obtained. The regiochemistry of these aminoalcohols can be easily determined via the use of EI-mass spectrometry, with the corresponding azadienium ion fragments appearing as base peaks (Fig. 2); while the assignment of their relative stereochemistry is supported by NMR nOe experiment. Only compound **13** showed measurable dipolar coupling between its protons at C-2 and C-4 (Fig. 3). A



SCHEME 1. Synthesis of compounds **1**, **3**, **4**, **5**, **7** and **8**. Reagents and conditions: a, TsCl, pyridine, 0°C; b, DBU, 120°C; c, TBDMSCl, imidazole, THF, reflux; d, *m*-CPBA, CH_2Cl_2, 0°C; e, pyrrolidine, reflux; f, CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0°C; g, 40% CH_3NH_2 in CH_3OH, THF, reflux; h, $ClOCCH_2C_6H_3Cl_2$, NEt_3z, CH_2Cl_2, r.t.; i, 10% HCl in C_2H_5OH, r.t.; j, C_6H_5COCl, pyridine, 70°C.

mixture of **13**, **14**, and **15** was then subjected to a sequence of three transformations, namely mesylation, displacement with methylamine, followed by amide formation with 3,4-dichlorophenylacetylchloride. Since the displacement reaction proceeds via the intermediate aziridinium ions through participation of the neighbouring pyrrolidine nitrogen, as shown in Scheme 2, only *trans* amino-acetamides **16**, **17** and **18** were obtained in a ratio of 52 : 12 : 36. Again, the regiochemistry of **16–18** as assigned was determined by EI-mass spectrometry. The stereochemistry of compound **16** has been unambiguously determined by X-ray structure analysis on its hydrochloride salt. As shown in Fig. 4, the cyclohexane ring of **16** assumes a chair conformation. The pyrrolidine ring and the amide side chain are both in the equatorial position and thus *trans* to each other; while the *tert*-butyldimethylsilyloxy group, despite its bulkiness, positions itself in the axial position and *trans* to the pyrrolidine ring. Compounds **16–18** were then subjected to desilylation via treatment with aqueous hydrochloric acid to give target compounds **1**, **3** and **4** respectively, which were further reacted with benzoyl chloride to provide the corre-

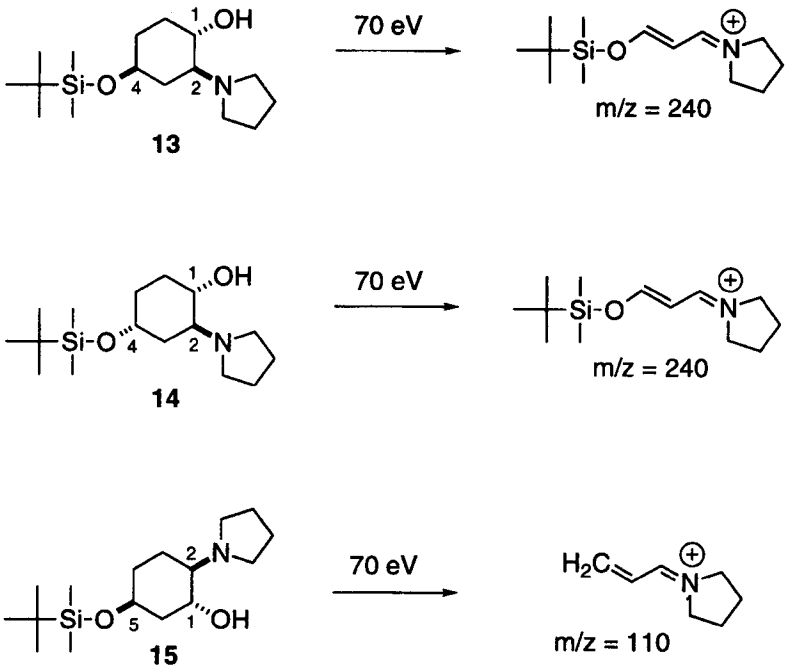
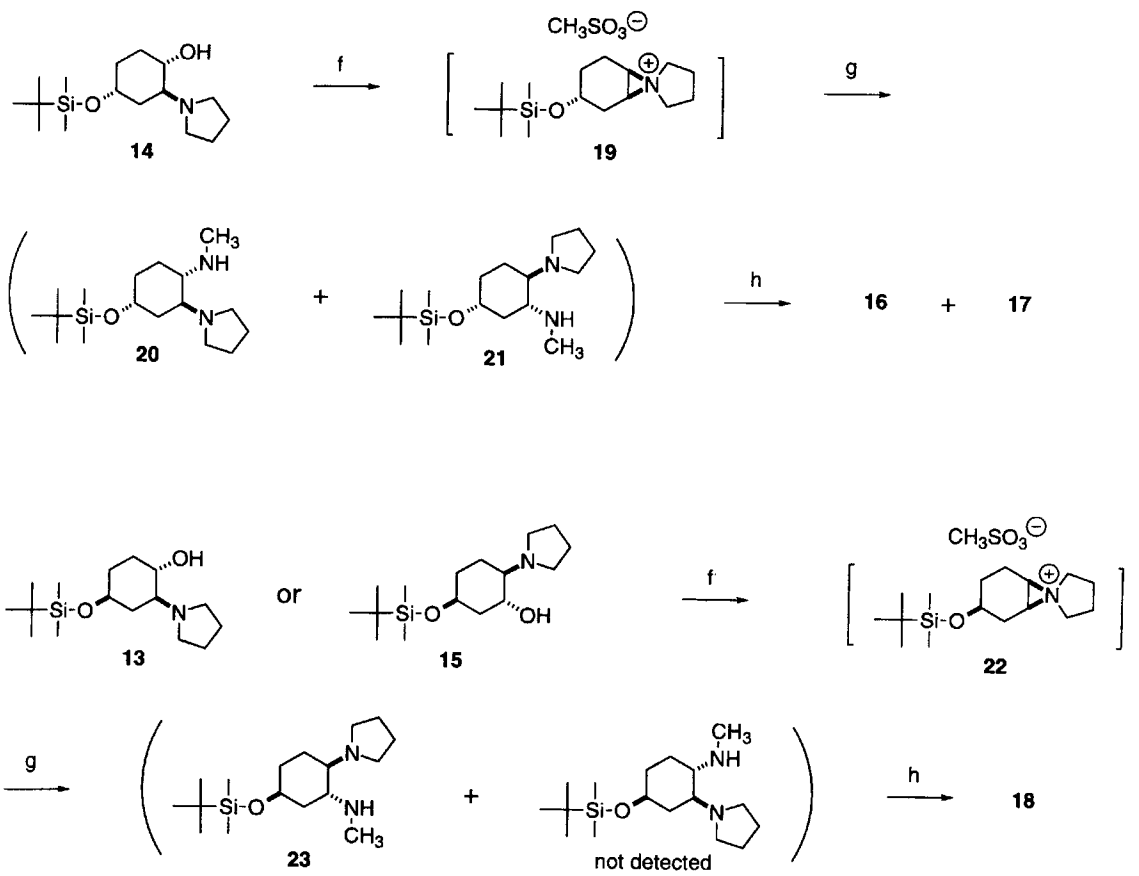


FIG. 2. Major EI-mass fragments from compounds **13–15**.



SCHEME 2. Mechanism for the formation of compounds **16–18** from **13–15**. Reagents and conditions: as defined in Scheme 1.

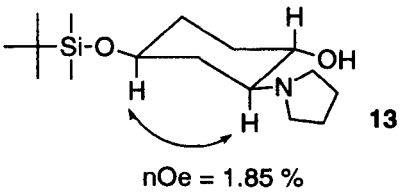


FIG. 3. nOe coupling between protons at C-2 and C-4 of compound **13**.

sponding benzoates **5**, **7** and **8**. Finally, the 4-β isomer compound **2**, which cannot be obtained via Scheme 1, was prepared from its epimer compound **1** via a Mitsunobu reaction (Mitsunobu 1981) with benzoic acid followed by alkaline hydrolysis, as shown in Scheme 3.

Pharmacology

Table 1 lists the μ- and κ-opioid receptor binding affinities of our target compounds **1–8** and that of U-50488. All four monohydroxy derivatives **1–4** maintained good selectivity towards the κ-opioid receptor, the μ/κ ratio ranging from 24 to >91. The 4-α, 4-β, and 5-α isomers (compounds **1–3**) are about equipotent at the κ-opioid receptor ($K_{i,\kappa}$ = 75–110 nM) and approximately one order of magnitude less potent than U-50488. The 5-β isomer compound **4**, with its 5-hydroxyl group oriented preferentially in an axial position, is the least potent among the four hydroxy derivatives ($K_{i,\kappa}$ = 218 nM) and about 30 times less potent than U-50488. The above observation is consistent with earlier findings by Halfpenny et al (1990) that the 5-β methoxy derivative **Id** is also the least potent among a series of monomethoxy derivatives of PD-117302 (**Ia-d**).

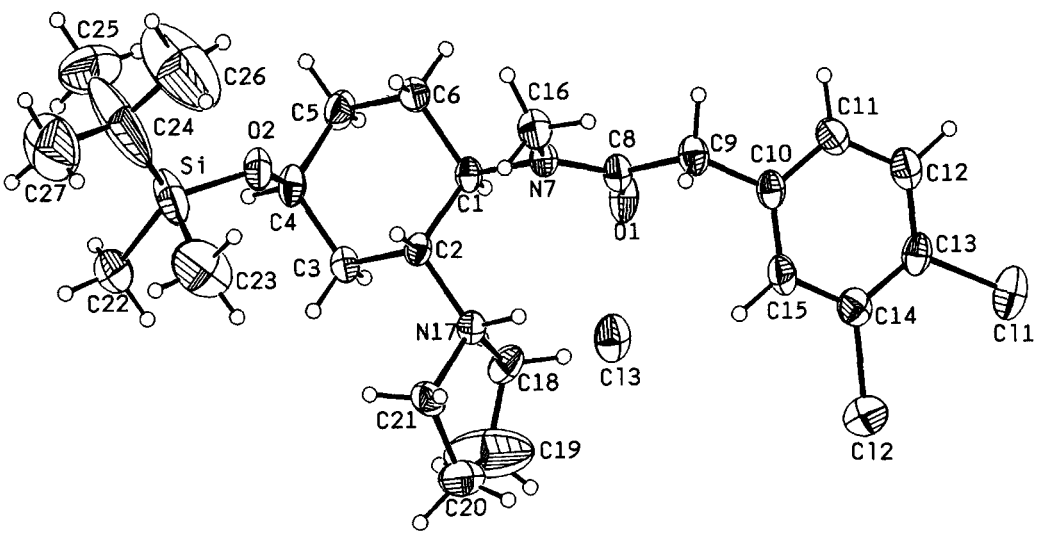
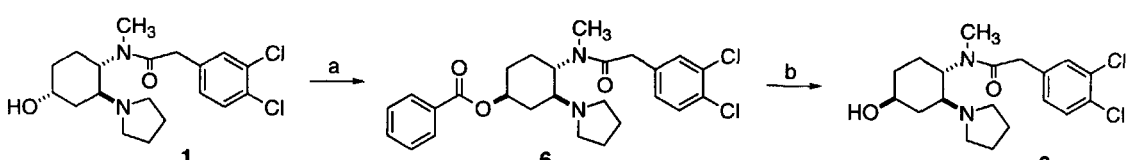


FIG. 4. X-ray crystal structure of the HCl salt of compound **16**.



SCHEME 3. Synthesis of compounds **2** and **6** from **1**. Reagents and conditions: a, C_6H_5COOH, $P(C_6H_5)_3$, diethylazodicarboxylate, THF, r.t.; b, $NaOH_{(aq)}$, CH_3OH, r.t.

Table 1. μ- and κ-Opioid receptor binding affinities of 4- or 5-monohydroxy derivatives of U-50,488 and their benzoates.

Compound	Substitution	Binding affinity [K_i (nM)][a] μ	κ	μ/κ Ratio[b]
1	4-α-OH	4893±673	75·0±10·7	65
2	4-β-OH	>10 000	110·0±6·7	>91
3	5-α-OH	2097±491	86·9±9·7	24
4	5-β-OH	13 330±1900	218±36	61
5	4-α-OBz	167·9±62·6	2204±404	0·076
6	4-β-OBz	954·7±261·0	333·4±46·9	2·9
7	5-α-OBz	20 000±2800	282±35	71
8	5-β-OBz	5692±1576	476±98	12
U-50,488		762±9·5	7·5±1·3	102

[a]Data represents the mean ± s.e.m. of three experiments, each performed in duplicate. [b]μ/κ ratio = $K_i(\mu)/K_i(\kappa)$.

The effects of benzoylating the hydroxyl functions in compounds **1–4** on their opioid receptor affinity are dependent on the receptor type and the position of the hydroxyl function. With the 4-β, 5-α and 5-β isomers (compounds **6–8**), a 2- to 3-fold reduction in κ-affinity was observed; while a dramatic 30-fold reduction in κ-affinity was observed with the 4-α isomer **5**. At the μ-opioid receptor, only the 5-α or 5-equatorial isomer **7** showed reduced binding; while the 5-β (**8**), 4-β (**6**), and 4-α (**5**) isomers demonstrated respectively a 2-fold, > 10-fold and 30-fold increase in binding affinity. It is particularly noteworthy that the 4-α isomer **5**, with its benzoate moiety at the 4-axial position, is now a moderately potent and selective ligand at the μ-opioid receptor ($K_{i,\mu} = 168$ nM, $\mu/\kappa = 0{\cdot}076$).

In conclusion, the opioid activity of κ-selective *N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamides such as U-50488 is significantly reduced when a hydroxyl group is attached to the C-4 or C-5 position. The observed reduction in opioid affinity is likely to result from decreased lipophilicity since the analogous substitution with a methoxy group resulted in comparable or enhanced opioid activity (Halfpenny et al 1990). Substitution at the 4-α or 4-axial position with a benzoate moiety resulted in reversal of opioid selectivity, producing a moderately potent and selective ligand (**5**) for the μ-opioid receptor, indicating the presence of a specific lipophilic binding site on the μ-opioid receptor.

Acknowledgments

We thank the National Science Council of the Republic of China for financial support (NSC82-0420-B-002-462).

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