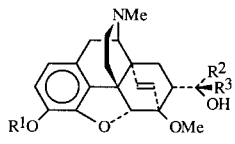
Derivatives of Flavonepenthone: Kappa Opioid Receptor Selectivity in an *N*-Methylmorphinan

by **Fernando Martinez Bermejo**, **Stephen M. Husbands** and **John W. Lewis***

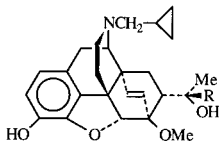
School of Chemistry, University of Bristol, Cantock's Close, BS81TS, U.K.

To extend our investigation of analogs of the orvinols incorporating phenyl groups constrained in appropriately located fixed conformations, a series of analogs of flavonepenthone have been prepared and evaluated in opioid binding assays. Acid-catalyzed rearrangement of phenyldihydrothevinone gave a 7:1 mixture of (*E*)- and (*Z*)-isomers; the corresponding orvinol gave a corresponding 9:1 mixture. The 3,4-substitution pattern in the major isomer was manipulated to give a series of analogs for evaluation. Surprisingly, the flavonepenthone analogs showed selectivity for κ opioid receptors, and the (*E*)-4-hydroxy-3-methoxy isomer with $K_i(\kappa) = 0.14$ nM and selectivity of $\kappa/\delta = 40$ and $\kappa/\mu = 32$ is, to our knowledge, the most selective *N*-methylmorphinan derivative so far reported. It will be the subject of a full pharmacological evaluation.

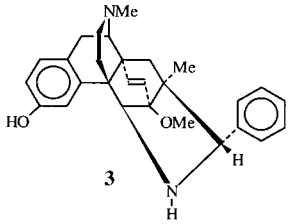
Introduction. – The orvinols, *e.g.*, **1a** and **2**, an extensively investigated series of potent opioids offering a range of pharmacological profiles, include the extremely potent agonist etorphine (**1a**) and the antagonist diprenorphine (**2a**), which are used in veterinary anaesthesia and as pharmacological tools [1]. Buprenorphine (**2b**) has attracted the greatest interest as it has become increasingly used as a treatment for opiate abuse, in addition to its established use as a clinical analgesic [2]. In our search for new pharmacotherapies for drug abuse, we have investigated analogues of the orvinols in which a Ph group is conformationally constrained. One such analogue, the morphinan derivative **3** has received particular attention [3]. In *in vitro* and *in vivo* functional assays, **3** showed very potent high efficacy μ agonist effects. The μ agonist effect in mouse antinociception tests was of long duration, after which antagonism of morphine was observed [4].



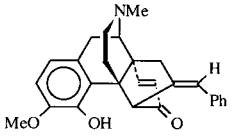
1a R^1 = H, R^2 = Me, R^3 = $CH_2CH_2CH_3$
b R^1 = Me, R^2 = H, R^3 = Ph
c $R^1 = R^2$ = Me, R^3 = Ph



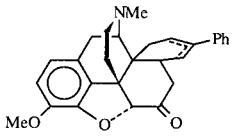
2a R = Me
b R = $C(CH_3)_3$



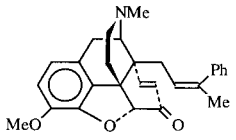
3



4



5



6

This unusual profile of **3** was thought to be due to the position of the Ph group with respect to the other primary binding centres, *i.e.*, the phenolic OH group and the piperidine N-atom.

A similar location of a Ph group is found in flavonepenthone, the product of acid-catalyzed rearrangement of nepenthol (**1b**) [5]. The product was a single geometrical isomer to which the (*Z*)-configuration (see **4**) was tentatively assigned. The tertiary alcohol **1c**, corresponding to nepenthol, gave rise to cyclohexenodihydrocodeinones **5** by acid treatment *via* cyclisation of the intermediate alkenylcodeinone (**6**) [6]. This cyclisation was not possible in the rearrangement of the dihydrothevinols **7**, which were converted to bridged dihydrothebainones **8** [7] (*Scheme 1*). This latter rearrangement was used to provide a series of dihydroflavonepenthone analogues **10**–**15** with particular manipulation of the 3,4-substituents, which were evaluated in opioid-receptor binding assays.

Scheme 1

7 **8**

Results. – *Chemistry.* In the only previous report of the acid-catalyzed rearrangement of dihydrothevinols [7], it was recognised that mixtures of geometrical isomers of bridged dihydrothebainones were produced. However, no attempt to separate or assign configuration to any of these mixtures was reported. We now report that treatment of phenyldihydrothevinol (**9**) with concentrated HCl gave a 7:1 mixture of **11** and **10**, respectively, in 84% combined yield, and similarly phenyldihydroorvinol gave a 9:1 mixture of **13** and **12** (*Scheme 2*). In each case, separation was achieved by gravity column chromatography. Structures were assigned to **12** and **13** by NOE studies. Irradiation of the protons of the Ph–C(20) group in **13** gave a clear enhancement of H_α–C(19) and H_β–C(19), while irradiation of the protons of the Me–C(20) group gave enhancement of H–C(5). Thus, the major isomer **13** was assigned the (*E*)-configuration. This was confirmed by NOE experiments on **12**, in which enhancements of 2 H–C(19) and H–C(5) resulted from irradiation of the Me–C(20) and Ph–C(20) protons, respectively.

The 3,4-dimethoxy analog **15** was prepared from **11** in 56% yield with trimethyl(phenyl)ammonium chloride [8].

The 3-OH and 4-MeO substituted derivative **14** was prepared from catechol (**13**) by selective tritylation at C(3) to give **17**, followed by methylation (NaH/MeI) to give **18** and final deprotection with dilute AcOH (*Scheme 2*).

Opioid-Receptor Binding. The dihydrothebainone derivatives were evaluated in opioid-receptor displacement binding assays in transfected chinese hamster ovary (CHO) cells expressing recombinant μ, δ and κ human receptor proteins. The displaced radioligands were [^{3}H]DAMGO (μ), [^{3}H]Cl-DPDPE (δ), [^{3}H]U69,593 (κ). Binding data are shown in the *Table*.

Scheme 2

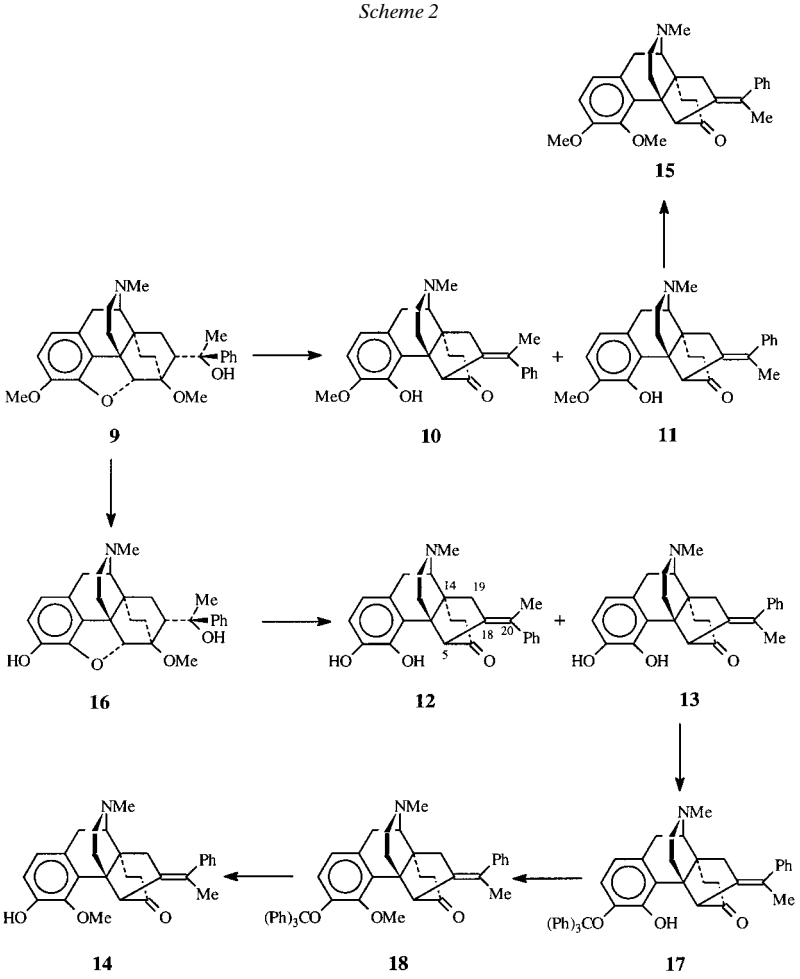


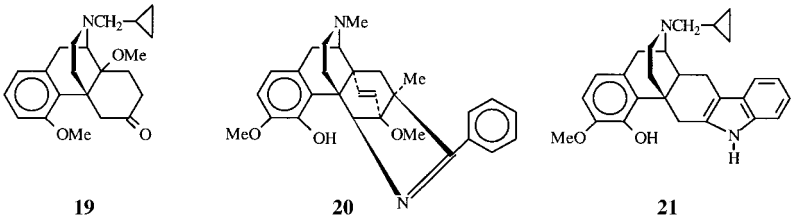
Table 1. *Binding Affinities of Recombinant Human Opioid Receptors from Transfected Chinese Hamster Ovary Cells*

Compound	K_i [nM]		
	μ	δ	κ
	[^{3}H]DAMGO	[^{3}]Cl-DPDPE	[^{3}H]U69,593
10	29.6 ± 2.8	115 ± 2.2	5.3 ± 2.7
11	4.5 ± 0.2	5.6 ± 0.2	0.1 ± 0.1
12	3.6 ± 1.0	9.3 ± 1.2	2.0 ± 1.0
14	0.8 ± 0.3	2.6 ± 1.2	0.2 ± 0.1
15	3.3 ± 0.8	9.8 ± 4.0	0.3 ± 0.2

The new ligands displayed moderate to high affinities for opioid receptors, with highest affinity for the κ and lowest for the δ receptor types. The dihydrothebainone with an (*E*)-configured bridge, **11**, had substantially higher affinity than the correspond-

ing (*Z*)-isomer **10**, and also the highest κ affinity and greatest κ selectivity ($\kappa/\delta = 40$; $\kappa/\mu = 32$) of the new ligands. The effect of methylation of the 4-OH group (**11** → **15**) was to reduce κ and δ, but not μ affinity. 3-*O*-Demethylation (**15** → **14** and **10** → **12**) resulted in increased μ and δ affinity, and loss of κ selectivity.

Discussion. – It is very unusual among morphinan and benzomorphan derivatives for *N*-methyl derivatives to display κ selectivity in binding assays. We believe that the (*E*) configured flavonepenthone analogue **11** is the most κ-selective *N*-methylmorphinan or benzomorphan ligand reported to date. The effect of a 4-OH or 4-MeO group in other series, *e.g.*, cyprodime (**19**) [9] and **20** [3], is to confer μ selectivity. However, in the indole derivative **21** δ selectivity was higher than in naltrindole, the prototype δ-selective antagonist [10]. It seems that a 4-OH or 4-MeO group is able to enhance selectivity for μ, κ and δ receptors depending on the presence of appropriate structural features in the remainder of the molecule that favour one of other receptor types. In this situation, the 4-OH or 4-MeO group acts as a selectivity-enhancing auxiliary structure. This will be explored in studies of other derivatives of dihydrothebainone.



19 **20** **21**

This work was supported by *NIDA* (*OTDP Medications Development Division*, grant No. DA 07315).

Experimental Part

General. Solvents and reagents were purchased and used as supplied by commercial sources unless otherwise stated. All extracted solns. were washed with brine and dried over $MgSO_4$, and concentrated to dryness on a rotary evaporator under reduced pressure. Preparation of salts: dry MeOH was added to the free base until it was dissolved. Dry HCl was then added slowly until a slight precipitation started to take place. The suspension was left until no further precipitation was observed (30 min – 3 days). The salt was then filtered and washed with a small quantity of cold MeOH, before drying in air, followed by high vacuum pump and drying pistol. M.p.: *Reichert* hot-stage cap. microscope; uncorrected. Gravity-elution column chromatography (CC): flash silica gel (*Fluka*; silica gel *60*, mesh 220–240). TLC: aluminium sheets coated with silica gel *60* F_{254} (*Merck*). IR Spectra: *Perkin-Elmer 881* spectrometer; in cm^{-1}. ^1H- (300 MHz) and ^{13}C- (75 MHz)-NMR Spectra: *JEOL JNM-GX FT 300* spectrometer; in $CDCl_3$ solvent. NOE Spectra: *JEOL JNMX-GX FT400* spectrometer; δ in ppm rel. to Me_4Si as internal reference, *J* in Hz. All compounds exhibited NMR data consistent with those of the structures assigned. MS: *Fissions Autospectrometer*, *m*/*z*, rel. int. [%]. Elemental analyses were obtained with a *Carlo Erba EA 1108* analyser, and the results were within ±0.3% of the theoretical values.

6,7,8,14-Tetrahydro-7α-(α-hydroxy-α-methylbenzyl)-6,14-endo-*ethanothebaine* (**9**). Compound **9** was prepared according to the method of *Bentley et al.* [11]. Yield: 78%. R_f (CH_2Cl_2/MeOH 95:5) 0.77. IR ($CHCl_3$): 3430 (br., HO–C(20)). ^1H-NMR: 1.79 (*s*, Me–C(21)); 2.16 (*s*, MeN); 3.05 (*d*, *J* = 18.5, H_β–C(10)); 3.58 (*s*, MeO–C(6)); 3.85 (*s*, MeO–C(3)); 4.44 (*s*, H_β–C(5)); 6.56 (*d*, *J* = 8.2, H–C(1)); 6.72 (*d*, *J* = 8.2, H–C(2)); 7.22–7.28 (*m*, 1 arom. H); 7.32–7.39 (*m*, 2 arom. H); 7.49–7.54 (*m*, 2 arom. H). ^{13}C-NMR: 18.0; 29.9; 32.3; 36.4; 43.2; 52.4; 56.8; 118.6; 126.0; 126.7; 127.9; 147.8. MS: 461 (80, M^+), 446 (15, [*M* – Me]$^+$), 340 (100, [*M* – PhC(OH)Me]$^+$).

6,7,8,14-Tetrahydro-7α-(α-hydroxy-α-methylbenzyl)-6,14-endo-*ethanooripavine* (**16**). To a mixture of sodium propane-1-thiolate (5.12 g, 52.2 mmol) and **9** (6.01 g, 14.6 mmol), hexamethylphosphoric triamide

(13 ml) was added dropwise, and the mixture was heated to 120°. After 3 h, TLC showed that the starting material had been consumed, and the reaction mixture was worked up by adding sat. NH_4Cl and extracting the org. phase with Et_2O. The crude product **16** was purified by CC (CH_2Cl_2/MeOH 95:5) 4.69 g (80%) of **16**. R_f (CH_2Cl_2/MeOH 95:5) 0.42. IR ($CHCl_3$): 3581 (br., HO–C(3)), 3444 (br., HO–C(20)). ^{1}H-NMR: 1.79 (*s*, Me–C(21)); 2.2 (*s*, MeN); 3.18 (*d*, J = 18.5, H_β–C(10)); 3.55 (*s*, MeO–C(6)); 4.4 (*s*, H_β–C(5)); 6.58 (*d*, J = 8.1, H–C(1)); 6.69 (*d*, J = 8.1, H–C(2)); 6.95–6.85 (*m*, 2 arom. H); 7.46–7.34 (*m*, 3 arom. H). MS: 447 (60, M^+), 432 (25, $[M-Me]^+$), 326 (94, $[M-PhC(OH)Me]^+$).

*3,4-Dihydroxy-17-methyl-18-[(*E*)-α-methylbenzylidene]-5,14-ethanomorphinan-6-one* (**13**). Compound **16** (4.90 g, 9.13 mmol) was mixed with conc. HCl (30 ml) and heated over boiling water for 2 h. The soln. was then diluted with a small quantity of H_2O, made alkaline with NH_4OH and extracted with AcOEt. The aq. phase was extracted with CH_2Cl_2, and the org. layers were combined. The crude product was triturated in AcOEt and the resulting white solids filtered. Additionally the mother liquor was purified by CC (AcOEt/hexane 6:4) to yield further pure **13**. Combined yield: 2.62 g (56%). R_f (AcOEt/hexane 6:4) 0.24. IR ($CHCl_3$): 3519, 3329 (br., HO–C(3), HO–C(4)), 1689 (*s*, C=O). ^{1}H-NMR: 2.05 (*s*, Me–C(21)); 2.15 (*d*, J = 16.5, H_α–C(19)); 2.35 (*s*, MeN); 3.05 (*d*, J = 18.2, H_β–C(10)); 3.50 (*d*, J = 16.5, H_β–C(19)); 4.34 (*s*, H_β–C(5)); 6.55 (*d*, J = 8.1, H–C(1)); 6.77 (*d*, J = 8.1, H–C(2)); 7.3 (*m*, 5 arom. H); 7.78 (*s*, OH); 8.85 (*s*, OH). ^{13}C-NMR: 14.1; 20.9; 21.0; 24.2; 31.9; 32.8; 34.6; 35.4; 43.1; 44.7; 45.5; 47.5; 48.6; 48.9; 49.2; 49.5; 49.8; 58.3; 60.7; 64.7; 113.5; 118.8; 126.5; 127.3; 128.3; 133.3; 135.5; 142.3; 142.8; 143.8; 208.3. MS: 415 (100, M^+), 400 (3, $[M-Me]^+$).

*3,4-Dihydroxy-17-methyl-18-[(*Z*)-α-methylbenzylidene]-5,14-ethanomorphinan-6-one* (**12**). The mother liquor from the crystallisation of the isomer (*E*)-**13** according to the procedure described above was purified by CC (AcOEt/hexane 6:4) and recrystallised from AcOEt to yield 287 mg (6%) of (*Z*)-**12**.

R_f (AcOEt/hexane, 6:4) 0.16. IR ($CHCl_3$): 3519, 3303*s* (br., HO–C(3), HO–C(4)), 1693*s* (C=O). ^{1}H-NMR: 2.03 (*s*, Me–C(21)); 2.27 (*s*, MeN); 2.36 (*d*, J = 16.8, H_α–C(19)); 2.65 (*dd*, J = 5.8, 18.3, H_α–C(10)); 2.84 (*d*, J = 5.8, H_α–C(9)); 2.93 (*d*, J = 18.3, H_β–C(10)); 3.45 (*d*, J = 16.8, H_β–C(19)); 3.85 (*s*, H_β–C(5)); 6.42 (*d*, J = 8.1, H–C(1)); 6.51 (*d*, J = 8.1, H–C(2)); 7.24 (*m*, 5 arom. H); 7.78 (*s*, OH); 8.85 (*s*, OH). ^{13}C-NMR: 13.9; 20.6; 21.2; 38.5; 38.8; 39.1; 42.7; 43.4; 47.6; 57.2; 59.6; 64.0; 112.7; 117.2; 124.7; 126.1; 127.5; 127.8; 128.6; 131,3; 136.5; 142.2; 142.8; 208.2. MS: 415 (100, M^+), 400 (4, $[M-Me]^+$). HR-MS: 415.2147 ($C_{27}H_{29}NO_3^+$; calc. 415.2150).

Compound **5** was recrystallised from AcOEt. M.p. 164–168° (AcOEt, dec.). Anal. calc. for $C_{27}H_{29}N_1O_3 \cdot 4/3\ H_2O$: C 73.72, H 7.65, N 3.36; found: C 73.77, H7.26, N 3.18.

4-Hydroxy-17-methyl-18-(α-methylbenzylidene)-3-(triphenylmethoxy)-5,14-ethanomorphinan-6-one (**17**). Et_3N (0.04 g, 0.396 mmol) was added to a mixture of **13** (100 mg, 0.24 mmol), Ph_3CCl (0.07 g, 0.264 mmol), 4-(dimethylamino)pyridine (2.9 mg, 0.02 mmol) and dry CH_2Cl_2 (5 ml), and stirred for 15 h. Once the reaction was complete (monitored by TLC), it was quenched with H_2O. The mixture was then extracted with CH_2Cl_2 (3×) and the crude product purified by CC (AcOEt/hexane 6:4) to yield **17** (123 mg, 77%). R_f 0.37 (AcOEt/hexane 6:4). IR ($CHCl_3$): 3506*s* (OH), 1705*s* (C=O). ^{1}H-NMR: 2.03 (*s*, Me–C(21)); 2.25 (*s*, MeN); 2.87 (*d*, J = 18.6, H_β–C(10)); 4.14 (*s*, H_β–C(5)); 6.0 (OH); 6.2 (*d*, J = 8.5, H–C(1)); 6.42 (*d*, J = 8.5, H–C(2)); 7.26 (*m*, 20 arom. H). ^{13}C-NMR: 14.1; 20.9; 21.0; 24.2; 31.5; 32.0; 34.1; 35.5; 43.1; 44.2; 45.2; 46.5; 60.3; 64.6; 81.9; 92.7; 117.2; 119.5; 126.3; 127.1; 127.3; 127.3; 127.8; 127.8; 127.9; 128.1; 128.2; 128.7; 130.0; 140.8; 143.5; 146.3; 146.8; 208.4. MS: 657 (79, M^+), 414 (62, $[M-Ph_3C]^+$).

4-Methoxy-17-methyl-18-(α-methylbenzylidene)-3-(triphenylmethoxy)-5,14-ethanomorphinan-6-one (**18**). To a soln. of **17** (100 mg, 0.15 mmol) in THF (3 ml), NaH (3.6 mg, 0.15 mmol) was added at 0°. After the addition was completed, a suspension of 18-crown-6 (0.04 g, 0.15 mmol) in THF (0.3 ml) was added to the mixture. The mixture was stirred for 0.5 h before adding MeI (0.01 ml, 0.9 mmol). The reaction was then allowed to warm to r.t. and stirred for 7 h, after which H_2O (10 ml) was added. The aq. phase was then extracted with AcOEt and the crude product purified by CC (CH_2Cl_2/MeOH 99:1) to yield **18** (46 mg, 45%).

R_f (AcOEt/hexane 6:4) 0.57, R_f (CH_2Cl_2/MeOH 98:2) 0.69. IR ($CHCl_3$): 1708*s* (C=O). ^{1}H-NMR: 2.05 (*s*, Me–C(21)); 2.3 (*s*, MeN); 2.85 (*d*, J = 18.5, H_β–C(10)); 3.87 (*s*, H_β–C(5)); 4.05 (*s*, MeO); 6.40 (*d*, J = 8.4, H–C(1)); 6.50 (*d*, J = 8.4, H–C(2)); 7.25 (*m*, 20 arom. H). ^{13}C-NMR: 20.9; 31.5; 32.0; 34.1; 35.5; 43.1; 44.3; 45.2; 45.5; 46.6; 60.3; 120.6; 126.9; 127.2; 127.3; 127.5; 128.2; 128.9; 143.5; 208.4. MS: 671 (40, M^+), 243 (70, $[M-Ph_3C]^+$).

3-Hydroxy-4-methoxy-17-methyl-18-(α-methylbenzylidene)-5,14-ethanomorphinan-6-one (**14**). A soln. of (**18**) (328 mg, 0.489 mmol) and CH_2Cl_2 (13 ml) with 50% aq. AcOH (40 ml) was stirred vigorously at ambient temp. for 6 h. The mixture was then poured onto ice/NH_3 and extracted with CH_2Cl_2. The solvent was removed under reduced pressure. The crude product was purified by CC (CH_2Cl_2/MeOH 98:2) to afford 81 mg (39%) of **14**. R_f (AcOEt/hexane 6:4) 0.36, R_f (CH_2Cl_2/MeOH 98:2) 0.40. IR: 3690*s* (OH), 1714*s* (C=O). ^{1}H-NMR: 2.07

(*s*, Me–C(21)); 2.33 (*s*, MeN); 3.05 (*d*, *J* = 18.0, H_β–C(10)); 3.90 (*s*, MeO); 4.18 (*s*, H_β–C(5)); 6.79 (*s*, H–C(1), H–C(2)); 7.24 (*m*, 5 arom. H). ^{13}C-NMR: 20.9; 24.1; 32.0; 34.1; 35.3; 43.0; 44.5; 45.1; 47.0; 60.9; 64.5; 115.0; 123.4; 126.3; 127.2; 128.2; 131.8; 136.4; 143.8; 145.6; 147.4; 208.8. MS: 429 (100, M^+), 414 (4, $[M - Me]^+$), 325 (7, $[M - PhCMe]^+$). HR-MS: 429.2303 ($C_{28}H_{31}NO_3^+$; calc. 429.2298).

Compound **14** was converted to the corresponding HCl salt. M.p. over 250° (MeOH, dec.).

*4-Hydroxy-3-methoxy-17-methyl-18-[(*E*)-α-methylbenzylidene]-5,14-ethanomorphinan-6-one* (**11**). A mixture of thevinol (**9**; 12.03 g, 26.1 mmol) with HCl (12M, 90 ml) was treated as described above and purified by CC (AcOEt/hexane 6:4) to yield 8.34 g (74%) of **11**. R_f (AcOEt/hexane, 6:4) 0.63. M.p. over 250° (AcOEt, dec.). IR ($CHCl_3$) 3526*s* (OH), 1707*s* (C=O). ^{1}H-NMR: 2.07 (*s*, Me–C(21)); 2.30 (*s*, MeN); 3.05 (*d*, *J* = 18.1, H_β–C(10)); 3.85 (*s*, MeO); 4.34 (*s*, H_β–C(5)); 5.91 (*s*, OH); 6.53 (*dd*, *J* = 8.4, 8.4, H–C(1), H–C(2)); 7.30 (*m*, 5 arom. H). ^{13}C-NMR: 20.9; 24.0; 32.4; 34.4; 35.4; 43.1; 44.4; 45.4; 46.8; 47.0; 55.9; 57.9; 65.8; 108.6; 118.0; 126.3; 127.3; 128.2; 143.1; 144.0; 146.2; 208.8. MS: 429 (100, M^+), 325 (10, $[M - PhCMe]^+$). HR-MS: 429.2303 ($C_{28}H_{31}NO_3^+$; calc. 429.2302). Anal. calc. for $C_{28}H_{31}N_1O_3 \cdot ½\ H_2O$: C 76.83, H 7.27, N 3.36; found: C 76.68, H 7.35, N 3.19.

*4-Hydroxy-3-methoxy-17-methyl-18-[(*Z*)-α-methylbenzylidene]-5,14-ethanomorphinan-6-one* (**10**). The mother liquor from the crystallisation of **11** (see above) was purified by CC (AcOEt/hexane 6:4) and recrystallised from AcOEt to yield 123 mg (10%) of **10**. R_f (AcOEt/hexane 6:4) 0.53. M.p. 222–225° (AcOEt, dec.). IR ($CHCl_3$): 3528*s* (OH), 1709*s* (C=O). ^{1}H-NMR: 2.07 (*s*, Me–C(21)); 2.34 (*s*, MeN); 3.07 (*d*, *J* = 18.3, H_β–C(10)); 3.75 (*s*, MeO); 3.95 (*s*, H_β–C(5)); 5.68 (*s*, OH); 6.61 (H–C(1), H–C(2)); 7.25 (*m*, 5 arom. H). ^{13}C-NMR: 21.7; 23.6; 32.0; 33.4; 35.3; 35.8; 43.2; 44.0; 45.4; 47.5; 55.8; 57.3; 64.4; 108.7; 117.9; 126.4; 127.7; 128.1; 131.4; 134.9; 135.7; 143.0; 143.1; 209.7. MS: 429 (100, M^+). HR-MS: 429.2303 ($C_{28}H_{31}NO_3^+$; calc. 429.2300). Anal. calc. for $C_{28}H_{31}N_1O_3 \cdot ¾\ H_2O$: C 76.07, H 7.77, N 3.28; found: C 75.90, H 7.39, N 3.16.

3,4-Dimethoxy-17-methyl-18-(α-methylbenzylidene)-5,14-ethanomorphinan-6-one (**15**). A mixture of **11** (0.2 g, 0.45 mmol), anh. K_2CO_3 (0.186 g, 1.35 mmol) and $Me_3N(Ph)Cl$ (0.16 g, 0.93 mmol) in anh. DMF (5 ml) was stirred at 80° under N_2 for 2.5 h. The mixture was worked up by filtering the inorg. solid and washing it with CH_2Cl_2. The crude product was then purified by CC (CH_2Cl_2/MeOH 97:3) to yield 0.117 g (56%) of **15**. R_f (CH_2Cl_2/MeOH 95:5) 0.63. IR ($CHCl_3$): 1707*s* (C=O). ^{1}H-NMR: 2.08 (*s*, Me–C(21)); 2.29 (*s*, MeN); 3.06 (*d*, *J* = 17.5, H_β–C(10)); 3.80 (*s*, MeO); 3.99 (*s*, MeO); 4.26 (*s*, H_β–C(5)); 6.73 (*d*, *J* = 8.4, H–C(1)); 6.79 (*d*, *J* = 8.4, H–C(2)); 7.26 (*m*, 5 arom. H). ^{13}C-NMR: 21.0; 24.2; 32.1; 33.7; 34.4; 35.4; 43.1; 44.5; 45.4; 47.4; 55.8; 58.3; 60.7; 63.7; 111.4; 121.8; 126.3; 127.3; 128.2; 132.8; 135.1; 145.4; 147.4; 150.7; 208.3. MS: 443 (100, M^+), 339 (14, $[M - PhCMe]^+$). HR-MS: 443.2460 ($C_{29}H_{33}NO_3^+$; calc. 443.2459).

Compound **12** was recrystallised from MeOH. M.p. 198–201° (MeOH, dec.). Anal. calc. for $C_{29}H_{33}N_1O_3 \cdot ½\ H_2O$: C 77.25, H 7.81, N 3.16; found: C 76.96, H 7.57, N 3.09.

Pharmacological Tests [12]. *Binding Assays Method* Receptor binding studies were conducted on recombinant human opioid receptors from transfected Chinese hamster ovary (CHO) cells. The μ cell line was maintained in *Ham*'s *F-12* medium supplemented with 10% fetal bovine serum and 400 μg/ml *Geneticin* (*G418* sulfate). The δ cell line was maintained in *Ham*'s *F-12* medium supplemented with 10% fetal bovine serum and 500 μg/ml hygromycin B. The κ cell line was maintained in *Dulbecco*'s minimal essential medium (DMEM) supplemented with 10% fetal bovine serum, 400 μg/ml *GENETICIN* (*G418* sulphate) and 0.1 penicillin/streptomycin. All cell lines were grown to full confluency, then harvested for membrane preparation. The membrane for the binding assays was prepared in 50 mM *Tris* buffer, pH 7.7. Cells were harvested by scraping the plates with a rubber policeman and were centrifuged at 500 × g for 10 min. The cell pellet was suspended in *Tris* buffer, homogenised in a *Polytron* homogeniser, and centrifuged at 20000 × *g* for 20 min. The cell pellet was then washed in *Tris*, centrifuged at 20000 × *g* for another 20 min and finally suspended in a small amount of buffer to determine protein content. Membrane was aliquoted in small vials at a concentration of 6 mg/ml per vial and stored at −70° and used as needed.

Routine binding assays were conducted with [^{3}H]DAMGO, [^{3}]Cl-DPDPE, and [^{3}H]U69,593, which bind to μ, δ and κ receptors, respectively. For binding, cell membranes were incubated with the appropriate radioligand and unlabeled drug in a total volume of 200 μl in 96-well plates, usually for 1 h at 25°. For routine experiments, membranes were incubated with the test compounds at concentrations ranging from 10^{-5} to 10^{-10} M. After the incubation, samples were filtered through glass fiber filters by using a *Tomtec* cell harvester. Filters were dried overnight before radioactivity levels were determined. Non-specific binding is determined by using 1.0 μM of the unlabeled counterpart of each radioligand.

Full characterisation of compound included analysis of the data for IC_{50} values and *Hill* coefficients by the program RPIM. K_i values were calculated with the *Cheng Prusoff* transformation:

$$K_i = IC_{50}/1 + L/K_d$$

where L is radioligand concentration and K_d is the binding affinity of the radioligand, as determined previously by saturation analysis.

REFERENCES

[1] J. W. Lewis, K. W. Bentley, A. Cowan, *Annu. Rev. Pharmacol*. **1971**, *11*, 241.
[2] J. W. Lewis, A. Cowan, 'Buprenorphine: Combatting Drug Abuse with a Unique Opioid', John Wiley & Sons, New York, 1995.
[3] S. M. Husbands, J. W. Lewis, *Biorg. Med. Chem. Lett*. **1995**, *5*, 2969.
[4] C. L. Neilan, S. M. Husbands, S. Breeden, J. Broadbear, J. W. Lewis, J. H. Woods, J. R. Traynor, *NIDA Res. Monog.* **1998**, *179*, 93.
[5] K. W. Bentley, J. C. Ball, *J. Chem. Soc.* **1958**, *23*, 1720.
[6] K. W. Bentley, D. G. Hardy, C. F. Howell, W. Fulmor, J. E. Lancaster, J. J. Brown, G. O. Morton, R. A. Hardy, *J. Am. Chem. Soc.* **1967**, *89*, 3303.
[7] K. W. Bentley, D. G. Hardy, B. Meek, *J. Am. Chem. Soc*. **1967**, *89*, 3293.
[8] H. Schmidhammer, L. Aeppli, L. Atwell, F. Fritsch, A. E. Jacobson, M. Nebuchla, G. Sperk, *J. Med. Chem*. **1984**, *27*, 1575.
[9] H. Schmidhammer, W. Burkard, L. Aeppli, C. Smith, *J. Med. Chem.* **1989**, *32*, 418.
[10] A. Coop, R. B. Rothman, C. Dersch, J. Partilla, F. Porreca, P. Davis, A. E. Jacobson, K. Rice, *J. Med. Chem.* **1999**, *42*, 1673.
[11] K. W. Bentley, D. G. Hardy, B. Meek, *J. Am. Chem. Soc*. **1967**, *89*, 3273.
[12] J. R. Traynor, S. R. Nahorski, *Mol. Pharmacol*. **1995**, *47*, 848.

Received June 12, 1999