Synthesis and Biological Evaluation of 14-Alkoxymorphinans

Part 19

Effect of 14-*O*-Benzylation on the Opioid Receptor Affinity and Antagonist Potency of Naltrexone

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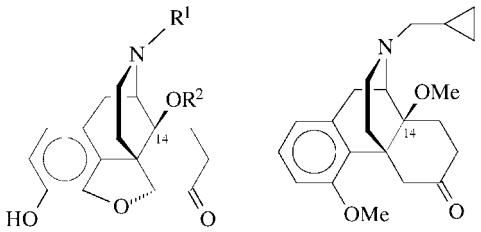
The 14-*O*-benzylnaltrexones **3**–**6** were prepared from naltrexone (**2**) in several steps. The novel compounds were biologically evaluated in radioligand binding and in [^{35}S]GTPγS functional assays in comparison to the reference compound naltrexone. In the binding assay, compounds **3**–**6** exhibited preference for κ opioid receptors, while the parent compound naltrexone shows preference for μ receptors. In the functional assay, μ antagonist potency of compounds **3**–**6** was in the range of naltrexone, while κ antagonist potency was considerably higher for most novel compounds in comparison to naltrexone.

Introduction. – Naloxone (**1**) was the first pure opioid antagonist detected, and it has become an indispensable tool in opioid research. Both, naloxone and its *N*-(cyclopropylmethyl) analogue naltrexone (**2**) are competitive antagonists at μ, κ, and δ opioid receptors with some preference for μ receptors. The major criterion for the classification of an agonist effect as being opioid-receptor-mediated is the ability of these antagonists to competitively antagonize this effect [1][2]. Naloxone (**1**) is being used to reverse the potentially lethal respiratory depression caused by neurolept analgesia or opioid overdose. Among other pharmacological actions, **1** antagonizes the blood-pressure drop in various forms of shock [3–7], reverses neonatal hypoxic apnea [1], counteracts chronic idiopathic constipation [8], reduces the food intake in humans [9][10], and shows beneficial effects in CNS injuries [11].

Naltrexone (**2**) appears also to be a relatively pure opioid antagonist but with higher oral efficacy and a longer duration of action than **1** [9]. All these properties make **2** suitable for the management of opioid dependence and provide a new effective modality for the physician treating addict patients [10][12]. Alcoholism is another addiction for which treatment with **2** is applied [12].

It was described that 14-*O*-alkylation (Me, Et) of naloxone (**1**) and naltrexone (**2**) did not significantly alter the *in vitro* and *in vivo* potencies of the parent compounds [13]. We have shown that introduction of a 14-(benzyloxy) group into the opioid antagonist cyprodime leads to an increase in the antagonist potency at all three opioid



cyprodime

1 $R^1 = CH_2{=}CHCH_2$, $R^2 = H$ (naloxone)
2 $R^1 =$ (cyclopropyl)CH_2, $R^2 = H$ (naltrexone)
3 $R^1 =$ (cyclopropyl)CH_2, $R^2 = PhCH_2$
4 $R^1 =$ (cyclopropyl)CH_2, $R^2 = 2\text{-}MeC_6H_4CH_2$
5 $R^1 =$ (cyclopropyl)CH_2, $R^2 = 2\text{-}ClC_6H_4CH_2$
6 $R^1 =$ (cyclopropyl)CH_2, $R^2 = 3\text{-}ClC_6H_4CH_2$

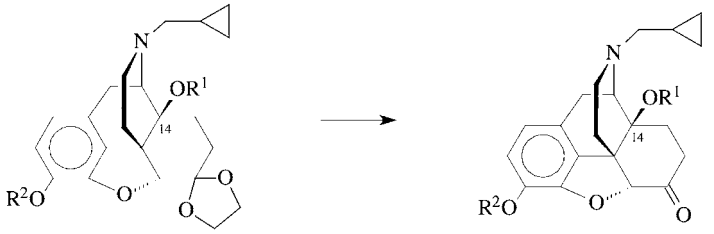
receptors by a factor of five to eight [14][15]. Based on the reported increase in the antagonist potency of cyprodime, it was of interest to prepare 14-*O*-benzyl derivatives of the opioid antagonist **2** and to evaluate these compounds biologically in comparison to its parent compound. Since benzylation increases lipophilicity, depot preparations (*e.g.*, transdermal systems) could be developed for therapeutic use.

Results and Discussion. – The 14-*O*-benzylnaltrexone (**3**) was prepared starting from 3-*O*,14-*O*-dibenzylated ketal **8**, which is readily accessible from dihydroxy ketal **7** [14] (*Scheme*). Compound **8** was hydrolyzed in MeOH/conc. HCl solution to afford both 3-*O*,14-*O*-dibenzylated ketone **13** [14] and 14-*O*-monobenzylated ketone **3**. For the synthesis of 14-*O*-(2-methylbenzyl)naltrexone (**4**), the 3-hydroxy group of ketal **7** was protected with a trityl group ($\rightarrow$ **10**) prior to 14-*O*-alkylation with 2-methylbenzyl bromide in DMF and NaH as base to give compound **14**. Acid hydrolysis afforded compound **4**. The 14-*O*-(2-chlorobenzyl) and 14-*O*-(3-chlorobenzyl) derivatives **5** and **6**, respectively, were also prepared from ketal **7**, which was protected with a benzyl group to give 3-*O*-benzylated ketal **9**. Compound **9** was then 14-*O*-alkylated with 2-chlorobenzyl bromide and 3-chlorobenzyl bromide, respectively, to yield **11** and **12**; acid hydrolysis yielded the desired 6-keto analogues **5** and **6**, respectively. The 4-substituted 14-*O*-benzyl analogues in another series of morphinans were found to have less affinity to opioid receptors than their 2- and 3-substituted counterparts (unpublished data). Therefore, we did not prepare 4-substituted derivatives.

Compounds **3**–**6** were evaluated in the radioligand binding assay and [^{35}S]GTPγS assay by means of human opioid receptors transfected into Chinese hamster ovary (CHO) cells. Receptor-binding studies were conducted employing [^{3}H]DAMGO (μ), [^{3}H]U69,593 (κ), and [^{3}H]Cl-DPDPE (δ), while in the [^{35}S]GTPγS assay, the prototypical full agonists DAMGO, U69,593, and DPDPE were used.

In opioid-receptor-binding experiments, compounds **3**–**6** exhibited preference for κ over μ and particularly δ opioid receptors, while the parent compound naltrexone (**2**)

Scheme



7 $R^1 = R^2 = H$
8 $R^1 = R^2 = PhCH_2$
9 $R^1 = H$, $R^2 = PhCH_2$
10 $R^1 = H$, $R^2 = Ph_3C$
11 $R^1 = 2\text{-}ClC_6H_4CH_2$, $R^2 = PhCH_2$
12 $R^1 = 3\text{-}ClC_6H_4CH_2$, $R^2 = PhCH_2$
14 $R^1 = 2\text{-}MeC_6H_4CH_2$, $R^2 = Ph_3C$

3 $R^1 = PhCH_2$, $R^2 = H$
4 $R^1 = 2\text{-}MeC_6H_4CH_2$, $R^2 = H$
5 $R^1 = 2\text{-}ClC_6H_4CH_2$, $R^2 = H$
6 $R^1 = 3\text{-}ClC_6H_4CH_2$, $R^2 = H$
13 $R^1 = R^2 = PhCH_2$

shows some preference for μ receptors (*Table 1*). With no exception, the 14-*O*-benzyl derivatives of naltrexone exhibited improved κ binding affinity, while μ and δ affinity was decreased considerably. This is in contrast to earlier findings where 14-*O*-benzylation of cyprodime resulted in an increase of affinity to all three opioid-receptor types [14][15]. In the present study, the 14-*O*-benzyl derivatives showed particularly high δ/κ selectivity ratios ranging from 183 to 636.

Table 1. *Opioid-Receptor Binding of Compounds* **3**–**6** *and Naltrexone* (**2**)

	K_i [nM] ± s.e.m.			Selectivity ratio	
	[^{3}H]DAMGO (μ)	[^{3}H]U69,593 (κ)	[^{3}H]Cl-DPDPE (δ)	μ/κ	δ/κ
3	0.79 ± 0.25	0.13 ± 0.10	23.82 ± 9.67	6.1	183
4	3.18 ± 0.15	0.29 ± 0.10	140.0 ± 38.88	11.0	483
5	3.54 ± 1.0	0.27 ± 0.10	171.6 ± 2.29	13.1	636
6	2.29 ± 0.36	0.10 ± 0.10	27.0 ± 0.97	22.9	270
Naltrexone (**2**)	0.20 ± 0.00	0.40 ± 0.10	8.70 ± 0.40	0.5	22

The binding to the δ receptor of the 2-Cl-substituted compound **5** was decreased by one order of magnitude ($K_i = 172$ nM) compared to the δ affinity determined for the 3-Cl-substituted compound **6** ($K_i = 27$ nM). However, the binding affinities of compounds **5** and **6** to the κ receptor were similar and in the low subnanomolar range, 0.27 and 0.10 nM, respectively. Based on the calculated selectivity ratios, compound **5** was found to exhibit the highest δ/κ selectivity ratio of 636, whereas the 3-Cl-substituted compound **6** showed the largest increase in the μ/κ selectivity ratio of 23, among the tested compounds.

A functional approach was used to establish the antagonist potency of the new compounds. In the [^{35}S]GTPγS functional assay, μ antagonist potency of compounds **3**–**6** was in the range of naltrexone (**2**), while κ antagonist potency was considerably higher for most of the novel compounds in comparison to **2** (*Table 2*). Interestingly, no

Table 2. *Antagonist* K_e *Values of Compounds* **3**–**6** *and Naltrexone* (**2**) *Determined in the [*^{35}*S]GTPγS Assay*

	K_2 [nM] ± s.e.m.			Selectivity ratio	
	DAMGO (μ)	U69,593 (κ)	DPDPE (δ)	μ/κ	δ/κ
3	0.17 ± 0.05	0.10 ± 0.03	5.41 ± 1.73	1.7	54
4	0.97 ± 0.26	0.44 ± 0.39	12.35 ± 2.36	2.2	28
5	0.22 ± 0.05	1.10 ± 0.13	45.0 ± 13.03	0.2	41
6	0.19 ± 0.02	0.12 ± 0.06	2.02 ± 0.61	1.6	17
Naltrexone (**2**)	0.59 ± 0.04	1.86 ± 0.16	5.44 ± 0.75	0.3	2.9

significant κ/μ selectivity was noted for the new series of compounds in the functional assay, while κ/δ selectivity was reduced in comparison to the findings in the binding assay.

In conclusion, 14-*O*-benzylation of naltrexone (**2**) leads to opioid antagonists with preference for κ opioid receptors, as determined in receptor-binding assay, and higher κ antagonist potency, as established in the functional assay when compared to the parent compound **2**. Since there is evidence that κ opioid receptor antagonists have a positive effect on cocaine addiction, it is feasible that 14-*O*-benzylnaltrexones may have therapeutic potential under such conditions.

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Experimental Part

General. TLC: *Polygram SilG/UV254* plates, solvent system CH_2Cl_2/MeOH/conc. NH_4OH soln. 90:9:1. Column chromatography (CC): silica gel 230–400 mesh. M.p.: *Kofler* melting-point microscope; uncorrected. ^{1}H-NMR Spectra: *Varian-Gemini-200* spectrometer; δ in ppm rel. to $SiMe_4$ as internal reference, *J* in Hz. Mass spectra (CI-MS): *Finnigan-MAT-44S* apparatus, in *m*/*z*. Elemental analyses were performed at the Institute of Physical Chemistry of the University of Vienna.

14β-(Benzyloxy)-17-(cyclopropylmethyl)-4,5α-epoxy-3-hydroxymorphinan-6-one (**3**) *and 3,14β-Bis(benzyloxy)-17-(cyclopropylmethyl)-4,5α-epoxymorphinan-6-one* (**13**). A soln. of **8** [14] (4.13 g, 7.3 mmol) in MeOH (52 ml) and conc. HCl soln. (23 ml) was refluxed for 3 h. After cooling with ice, the soln. was alkalinized with conc. NH_4OH soln. and extracted with CH_2Cl_2 (3 × 100 ml). The combined org. phase was washed with H_2O, dried (Na_2SO_4) and evaporated and the obtained yellow oil (3.64 g) purified by CC (CH_2Cl_2/MeOH/conc. NH_4OH soln. 250:2:0.5): 1.23 g (32%) of pure **13** [14] as a colorless foam and 1.41 g (45%) of **3** as a slightly yellow oil. A portion of this material (**3**) was converted into the HCl salt in the usual manner: **3**·HCl. M.p. 202° (dec.; Et_2O). ^{1}H-NMR ((D_6)DMSO): 9.61 (*s*, OH); 8.76 (*s*, NH^+); 7.61–7.29 (*m*, 5 arom. H); 6.75 (*d*, *J* = 8.2, 1 arom. H); 6.68 (*d*, *J* = 8.2, 1 arom. H); 5.07 (*s*, H–C(5)); 4.80 (*d*, *J* = 10.4, 1 H, PhCH_2O), 4.25 (*d*, *J* = 10.4, 1 H, PhCH_2O). CI-MS: 432 ($[M+1]^+$). Anal. calc. for $C_{27}H_{29}NO_4 \cdot HCl \cdot 1.3\ H_2O$ (491.42): C 65.99, H 6.69, N 2.85; found: C 65.95, H 6.57, N 2.88.

17-(Cyclopropylmethyl)-4,5α-epoxy-14β-hydroxy-3-(triphenylmethoxy)morphinan-6-one Ethane-1,2-diyl Acetal (**10**). A soln. of trityl bromide (3.8 g, 11.86 mmol), Et_3N (2.41 ml, 15.99 mmol), *N*,*N*-dimethylpyridin-4-amine (DMAP; 14 mg, 0.21 mmol), and **7** (3.0 g, 7.78 mmol) in CH_2Cl_2 (80 ml) was refluxed for 24 h, cooled, and washed with H_2O (2 × 100 ml). The org. layer was evaporated, and the obtained yellowish crystals (7.11 g) were recrystallized from MeOH (15 ml): 4.15 g (85%) of **10**. M.p. 203–205°. ^{1}H-NMR ($CDCl_3$): 7.50–7.15 (*m*, 15 arom. H); 6.60 (*d*, *J* = 8.2, 1 arom. H); 6.30 (*d*, *J* = 8.2, 1 arom. H); 6.13 (br. *s*, OH); 4.37 (*s*, H–C(5)); 4.12–3.80 (*m*, OCH_2CH_2O). CI-MS: 628 ($[M+1]^+$). Anal. calc. for $C_{41}H_{41}NO_5 \cdot 0.4$ MeOH (640.57): C 77.62, H 6.70, N 2.19; found: C 77.60, H 6.60, N 2.07.

17-(Cyclopropylmethyl)-4,5α-epoxy-14β-[(2-methylbenzyl)oxy]-3-(triphenylmethoxy)morphinan-6-one Ethane-1,2-diyl Acetal (**14**). NaH (312 mg, 13.34 mmol; from 520 mg of a 60% NaH dispersion in oil after washings with petroleum ether) was added to a soln. of **10** (2.0 g, 3.18 mmol) in anh. DMF (20 ml) under N_2 at 0° (bath temp.) while stirring. After 20 min, 2-methylbenzyl bromide (0.64 ml, 5.4 mmol) was added, and the resulting mixture was stirred at r.t. for 20 h. Excess NaH was destroyed carefully by addition of small pieces of ice. Then, the mixture was diluted with H_2O (80 ml) and extracted with CH_2Cl_2 (1 × 40 ml, 2 × 30 ml), the combined org. phase was washed with H_2O (3 × 100 ml), dried (Na_2SO_4), and evaporated. The orange oil (1.8 g) was purified by CC (CH_2Cl_2/MeOH/conc. NH_4OH soln. 250:2:0.5): 480 mg of yellowish oil. Crystallization from MeOH yielded 304 mg (13%) of **14**. M.p. 180–182°. ^{1}H-NMR ((D_6)DMSO): 7.30–7.15 (*m*, 19 arom. H); 6.31 (*d*, *J* = 8.2, 1 arom. H); 6.23 (*d*, *J* = 8.2, 1 arom. H); 4.68 (*d*, *J* = 10.2, 1 H, ArC*H*$_2$O); 4.22 (*d*, *J* = 10.2, 1 H, ArC*H*$_2$O); 4.17 (*s*, H–C(5)); 4.05–3.50 (*m*, OCH_2CH_2O); 2.33 (*s*, *Me*C_6H_4). CI-MS: 733 ($[M+1]^+$). Anal. calc. for $C_{49}H_{49}NO_5 \cdot 0.3$ MeOH (741.50): C 79.85, H 6.82, N 1.85; found: C 79.94, H 6.85, N 1.96.

17-(Cyclopropylmethyl)-4,5α-epoxy-14β-[(2-methylbenzyl)oxy]-3-hydroxymorphinan-6-one Hydrochloride (**4**·HCl). A soln. of **14** (300 mg, 0.67 mmol) in MeOH (5 ml) and conc. HCl soln. (0.5 ml) was refluxed for 10 h. After cooling with ice, the soln. was alkalinized with conc. NH_4OH soln. and extracted with CH_2Cl_2 (1 × 30 ml, 2 × 20 ml). The combined org. phase was washed with H_2O, dried (Na_2SO_4), and evaporated and the obtained yellowish oil (165 mg) purified by CC (CH_2Cl_2/MeOH/conc. NH_4OH soln. 250:2:0.5) to yield a colorless oil (80 mg) which was dissolved in Et_2O and treated with Et_2O/HCl: 70 mg (23%) of pure **4**·HCl. M.p. 198–200°. ^{1}H-NMR ((D_6)DMSO): 9.50 (*s*, OH); 8.60 (*s*, NH^+), 7.67–7.22 (*m*, 4 arom. H); 6.74 (*d*, *J* = 8.0, 1 arom. H); 6.69 (*d*, *J* = 8.0, 1 arom. H); 5.16 (*s*, H–C(5)); 2.31 (*s*, Me). CI-MS: 446 ($[M+1]^+$). Anal. calc. for $C_{28}H_{31}NO_5 \cdot$ HCl (498.00): C 69.77, H 6.69, N 2.91; found: C 69.53, H 6.76, N 2.56.

3-(Benzyloxy)-17-(cyclopropylmethyl)-4,5α-epoxy-14β-hydroxymorphinan-6-one Ethane-1,2-diyl Acetal (**9**). A mixture of **7** (6.9 g, 17.9 mmol), K_2CO_3 (6.7 g, 48.48 mmol), benzyl bromide (2.34 ml, 19.66 mmol), and anh. DMF (70 ml) was stirred for 22 h under N_2 at r.t. The inorg. material was filtered off and the filtrate evaporated. The residue was dissolved in CH_2Cl_2 (80 ml), the soln. washed with H_2O (7 × 50 ml), dried (Na_2SO_4), and evaporated. The residue (8.2 g) was recrystallized from MeOH: 7.37 g (87%) of **9**. Colorless crystals. M.p. 130–131°. ^{1}H-NMR ($CDCl_3$): 7.42–7.27 (*m*, 5 arom. H); 6.75 (*d*, *J* = 8.3, 1 arom. H); 6.54 (*d*, *J* = 8.3, 1 arom. H); 5.17 (*d*, *J* = 11.7, 1 H, PhC*H*$_2$O); 5.10 (*d*, *J* = 11.7, 1 H, PhC*H*$_2$O); 4.58 (*s*, H–C(5)); 4.19–3.73 (*m*, OCH_2CH_2O). CI-MS: 476 ($[M+1]^+$). Anal. calc. for $C_{29}H_{33}NO_5$ (477.58): C 73.24, H 6.99, N 2.95; found: C 72.91, H 7.09, N 2.94.

3-(Benzyloxy)-14β-[(2-chlorobenzyl)oxy]-17-(cyclopropylmethyl)-4,5α-epoxymorphinan-6-one Ethane-1,2-diyl Acetal (**11**). A mixture of **9** (5.82 g, 12.2 mmol), NaH (1.47 g, 61.3 mmol; from 2.45 g of 60% NaH dispersion in oil after washings with petroleum ether), and anh. DMF (100 ml) was stirred under N_2 at 0–5° for 25 min. Then, 2-chlorobenzyl bromide (3.16 ml, 24.3 mmol) was added, and the resulting mixture was stirred for 1 h at 0–5° and then for 3 h at r.t. Excess NaH was destroyed carefully by addition of small pieces of ice. The mixture was poured into 350 ml of H_2O and extracted with CH_2Cl_2 (1 × 250 ml, 3 × 100 ml). The combined org. phase was washed with H_2O (3 × 100 ml) and brine (2 × 50 ml), dried (Na_2SO_4), and evaporated. The obtained yellow oil (8.30 g) was purified by CC (CH_2Cl_2/MeOH/conc. NH_4OH soln. 250:4:0.5): 4.75 g (65%) of pure **11**. Colorless oil used as such for the next synthetic step. ^{1}H-NMR ((D_6)DMSO): 7.71–7.31 (*m*, 9 arom. H); 6.79 (*d*, *J* = 8.0, 1 arom. H); 6.57 (*d*, *J* = 8.0, 1 arom. H); 5.14 (*d*, *J* = 11.6, 1 H, ArC*H*$_2$O); 5.07 (*d*, *J* = 11.6, 1 H, ArC*H*$_2$O); 4.81 (*d*, *J* = 12, 1 H, ArC*H*$_2$O); 4.50 (*s*, H–C(5)); 4.46 (*d*, *J* = 12, 1 H, ArC*H*$_2$O); 4.01–3.74 (*m*, OCH_2CH_2O).

14β-[(2-Chlorobenzyl)oxy]-17-(cyclopropylmethyl)-4,5α-epoxy-3-hydroxymorphinan-6-one Hydrochloride (**5**·HCl). A soln. of **11** (5.50 g, 9.16 mmol) in MeOH (52 ml) and conc. HCl soln. (23 ml) was refluxed for 2.5 h. After cooling with ice, the soln. was alkalinized with conc. NH_4OH soln. and extracted with CH_2Cl_2 (1 × 200 ml, 1 × 100 ml, 2 × 50 ml). The combined org. phase was washed with H_2O and brine, dried (Na_2SO_4), and evaporated. The obtained yellow oil (5.54 g) was purified by CC (CH_2Cl_2/MeOH/conc. NH_4OH soln. 250:3:0.5): 2.69 g (63%) of pure **5**. Yellow oil. A small amount of **5** was dissolved in Et_2O and treated with Et_2O/HCl: **5**·HCl. Colorless crystals. M.p. 165–168°. ^{1}H-NMR ((D_6)DMSO): 8.63 (br. *s*, OH); 7.96 (*s*, NH^+); 7.50–7.30 (*m*, 4 arom. H); 6.74 (*d*, *J* = 8.0, 1 arom. H); 6.69 (*d*, *J* = 8.0, 1 arom. H); 5.19 (*s*, H–C(5)); 4.87 (*d*, *J* = 11.6, 1 H, ArC*H*$_2$O); 4.81 (*d*, *J* = 11.6, 1 H, ArC*H*$_2$O). CI-MS: 466 ($[M+1]^+$). Anal. calc. for $C_{27}H_{28}ClNO_4 \cdot$ HCl · 3.5 H_2O (529.44): C 57.35, H 6.42, Cl 12.54, N 2.48; found: C 57.72, H 6.43, Cl 12.37, N 2.09.

3-(Benzyloxy)-14β-[(3-chlorobenzyl)oxy]-17-(cyclopropylmethyl)-4,5α-epoxymorphinan-6-one Ethane-1,2-diyl Acetal Hydrochloride (**12**·HCl). As described for **11**, with **9** (0.50 g, 1.1 mmol), NaH (0.14 g, 5.8 mmol), and anh. DMF (15 ml) for 15 min. Then with 3-chlorobenzyl bromide (400 µl, 3 mmol) for 12 h at r.t. Workup with H_2O (350 ml), CH_2Cl_2 (3 × 40 ml), H_2O (5 × 40 ml), and brine (1 × 50 ml). The crude yellow oil

(0.92 g) gave, after CC, 310 mg of a colorless oil that was dissolved in Et_2O and treated with Et_2O/HCl: 300 mg (45%) of **12**·HCl. M.p. 124–125°. ^{1}H-NMR ((D$_6$)DMSO): 8.50 (*s*, NH^+); 7.57–7.30 (*m*, 9 arom. H); 6.92 (*d*, *J* = 8.1, 1 arom. H); 6.69 (*d*, *J* = 8.1, 1 arom. H); 5.12 (*s*, PhCH_2O); 4.66–4.45 (*m*, H–C(5), ArCH_2O); 4.00–3.74 (*m*, OCH_2CH_2O). CI-MS: 600 ($[M+1]^+$). Anal. calc. for $C_{36}H_{38}ClNO_5 \cdot HCl \cdot 4\ H_2O$ (708.63): C 65.33, H 6.37, N 2.12; found: C 65.09, H 6.07, N 1.97.

14β-[(3-Chlorobenzyl)oxy]-17-(cyclopropylmethyl)-4,5α-epoxy-3-hydroxymorphinan-6-one Hydrochloride (**6**·HCl). As described for **5**·HCl, with **12** (500 mg, 0.8 mmol) and MeOH/H_2O/conc. HCl soln. 4:5:1 (50 ml) for 27 h. Extraction with CH_2Cl_2 (3 × 100 ml) and washing with H_2O (4 × 100 ml) and brine (1 × 100 ml). The crude yellow oil (850 mg) was purified by CC (CH_2Cl_2/MeOH/conc. NH_4OH 250:2:0.5): 302 mg (76%) of **6**. Colorless oil. A portion was dissolved in Et_2O and treated with Et_2O/HCl: **6**·HCl. Colorless crystals. M.p. 147–150°. ^{1}H-NMR ((D$_6$)DMSO): 9.54 (br. *s*, OH); 8.69 (*s*, NH^+); 7.67–7.37 (*m*, 4 arom. H); 6.73 (*d*, *J* = 8.1, 1 arom. H); 6.67 (*d*, *J* = 8.1, 1 arom. H); 5.10 (*s*, H–C(5)); 4.78 (*s*, ArCH_2O). CI-MS: 466 ($[M+1]^+$). Anal. calc. for $C_{27}H_{28}ClNO_4 \cdot HCl \cdot 0.6\ Et_2O$ (511.42): C 63.59, H 6.14, Cl 12.77, N 2.52; found: C 63.68, H 5.95, Cl 12.73, N 2.48.

Receptor-Binding Studies. They were conducted on human opioid receptors transfected into Chinese hamster ovary (CHO) cells. The μ cell lines were maintained in *Ham*'s F-12 medium supplemented with 10% of fetal bovine serum and 400 μg/ml of geneticin (G418 sulfate). The δ and the κ cell lines were maintained in *Dulbecco*'s minimal essential medium (DMEM) supplemented with 10% of fetal bovine serum, 400 μg/ml of geneticin (G418 sulfate), and 0.1% of penicillin/streptomycin. All cell lines were grown to full confluency, then harvested for membrane preparation. The membranes used for functional assays were prepared in buffer *A* (20 mM HEPES, 10 mM $MgCl_2$, and 100 mM NaCl at pH 7.4), and the membranes for binding assays were prepared in 50 mM *Tris* buffer, pH 7.7. Cells were harvested by scraping the plates with a rubber policeman and then centrifuged at 500 × *g* for 10 min. The cell pellet was suspended in buffer *A* or *Tris* buffer, homogenized in a *Polytron* homogenizer, and centrifuged at 20000 × *g* for 20 min. The cell pellet was washed in buffer *A* or *Tris*, centrifuged at 20000 × *g* for another 20 min, and finally suspended in a small amount of buffer to determine the protein content. Membranes were placed in small vials at a concentration of 3 mg/ml per vial, stored at −70°, and used as needed.

Routine binding assays were conducted with [^{3}H]DAMGO, [^{3}H]Cl-DPDPE, and [^{3}H]U69,593 to bind to μ, δ, and κ recetors, resp. For μ, δ, and κ 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. Nonspecific binding was determined by using 1.0 μM of the unlabeled counterpart of each radioligand.

Full characterization of compounds included analysis of the data for IC_{50} values and *Hill* coefficients by using the program PRISM. K_i Values were calculated by means of the *Cheng Prusoff* transformation $K_i = IC_{50}/(1 + L/K_d)$, where L is the radioligand concentration and K_d the binding affinity of the radioligand, as determined previously by saturation analysis.

[^{35}S]GTPγS Binding for Functional (Agonist/Antagonist) Determinations. Membranes were prepared as described above and incubated with [^{35}S]GTPγS (50 pM), GDP (usually 10 μM), and the desired compound, in a total volume of 200 μl, for 60 min at 25° [16]. Samples were filtered over glass-fiber filters and counted as described for the binding assays. A dose-response curve with a prototypical full agonist (DAMGO, DPDPE, and U69593, for μ, δ, and κ receptors, resp.) was conducted in each experiment to identify full and partial agonist compounds.

High-affinity compounds that demonstrate no agonist activity were tested as antagonists. For each compound, a full *Schild* analysis was conducted, utilizing a full agonist-dose–response curve in the presence of at least three concentrations of the antagonist. The equilibrium dissociation constant (K_e) is calculated from $K_e = a/(DR - 1)$, where a is the nanomolar concentration of antagonist and DR the virtual shift of the agonist-concentration–response curve to the right in the presence of a given concentration of antagonist.

REFERENCES

[1] D. M. Zimmerman, J. D. Leander, *J. Med. Chem.* **1990**, *33*, 895.
[2] I. Iijima, J. Minamikawa, A. E. Jacobson, A. Brossi, K. C. Rice, W. A. Klee, *J. Med. Chem.* **1978**, *21*, 398.
[3] M. D. Aceto, L. S. Harris, W. L. Dewey, E. L. May, *NIDA Res. Monogr.* **1979**, *27*, 330.

[4] N. J. Gurll, D. G. Reynolds, T. Vargish, R. Lechner, *J. Pharmacol. Exp. Ther*. **1982**, *220*, 621.
[5] J. W. Holaday, A. I. Faden, *Nature (London)* **1978**, *275*, 450.
[6] A. I. Faden, J. W. Holaday, *Science (Washington, D.C.)* **1979**, *205*, 317.
[7] A. I. Faden, J. W. Holaday, *J. Pharmacol. Exp. Ther*. **1980**, *212*, 441.
[8] M.-J. Kreek, R. A. Schaefer, E. F. Hahn, J. Fishman, *Lancet* **1983**, 261.
[9] W. R. Martin, D. R. Jasinski, P. A. Mansky, *Arch. Gen. Psychiat.* **1973**, *28*, 784.
[10] M. S. Gold, C. A. Dackis, A. L. Pottash, H. H. Sternbach, W. J. Annitto, D. Martin, M. P. Dackis, *Med. Res. Rev*. **1982**, *3*, 211.
[11] T. K. McIntosh, A. I. Faden, in 'Pharmacological Approaches to the Treatment of Brain and Spinal Cord Injury', Eds. G. Stein and B. A. Sabel, Plenum Publishing Corporation, New York, 1988, p. 89.
[12] M.-J. Kreek, in ' Handbook of Experimental Pharmacology', Eds. C. R. Schuler and M. J. Kuhar, Springer, Berlin, **1996**, Vol. 118, p. 563.
[13] R. J. Kobylecki, R. W. Carling, J. A. H. Lord, C. F. C. Smith, A. C. Lane, *J. Med. Chem*. **1982**, *25*, 116.
[14] H. Schmidhammer, R. Krassnig, E. Greiner, J. R. Traynor, *Heterocycles* **1998**, *49*, 489.
[15] H. Schmidhammer, W. P. Burkard, L. Eggstein-Aeppli, C. F. C. Smith, *J. Med. Chem*. **1989**, *32*, 418.
[16] J. R. Traynor, S. R. Nahorski, *Mol. Pharmacol*. **1995**, *47*, 848.

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