Synthesis and Biological Evaluation of 14-Alkoxymorphinans. 20.[1] 14-Phenylpropoxymetopon: An Extremely Powerful Analgesic

Johannes Schütz,[†] Mariana Spetea,[†,‡] Martin Koch,[†] Mario D. Aceto,[§] Louis S. Harris,[§] Andrew Coop,[||] and Helmut Schmidhammer*,[†]

Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria, Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Innsbruck, Peter-Mayr-Strasse 1, A-6020 Innsbruck, Austria, Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298-0613, Department of Pharmaceutical Sciences, University of Maryland, School of Pharmacy, Baltimore, Maryland 21201

Received April 22, 2003

The synthesis and the biological and pharmacological evaluation of several 14-phenylpropoxy analogues of 14-methoxymetopon are described. Most of the new compounds were nonselective and exhibited binding affinities in the subnanomolar or low nanomolar range at opioid receptors (μ, κ, δ), with 14-phenylpropoxymetopon (PPOM; **7**) displaying the highest affinity for all three opioid receptor types. The most striking finding of this study is that the derivatives from the novel series of *N*-methyl-14-phenylpropoxymorphinans acted as extremely powerful antinociceptives with potencies higher than that of 14-methoxymetopon (**1**) and even etorphine. 14-Phenylpropoxymetopon (PPOM; **7**) showed considerably increased potency in the in vivo assays in mice (25-fold in the tail-flick assay, 10-fold in the hot-plate assay, and 2.5-fold in the paraphenylquinone writhing test) when compared to etorphine, while it was equipotent to dihydroetorphine in the hot-plate assay and the paraphenylquinone writhing test and ca. twice as potent in the tail-flick assay than this reference compound. The 3-*O*-alkyl ethers of PPOM, compounds **6** and **8**, showed less potency in in vivo assays, but partly surpassed the potency of the 3-OH analogue 14-methoxymetopon (**1**).

Introduction

Opioid drugs such as morphine, hydromorphone, or fentanyl acting predominantly at the μ receptor type are potent analgesics that are employed in the management of severe pain. These analgesics share the same general pharmacological profiles including adverse effects, i.e., respiratory depression, inhibition of gastrointestinal motility, pruritus, hypotension, nausea, and sedation. With continued administration, these drugs all show variable degrees of tolerance and physical dependence.[2,3] In view of these adverse effects, a major aim in medicinal chemistry is the development of novel opioid analgesics exhibiting more favorable pharmacological features.

14-Methoxymetopon (HS 198; **1**), a member of the 14-alkoxymorphinan series of opioids, is a highly potent analgesic selectively labeling μ opioid receptors,[4,5] which is 24 to 20 000 times more potent than morphine following systemic administration depending upon the assay (hot-plate, tail-flick or acetic acid writhing test) and the animals (mice, rats) used.[4,6–9] This enhanced potency is markedly increased with either spinal or supraspinal administration, where its analgesic potency is more than 1 million-fold greater than that of morphine.[9] Moreover, 14-methoxymetopon was reported to elicit minimal physical dependence and tolerance compared to morphine.[4] It shows significantly lower potential to induce respiratory depression, hypotension, and bradycardia than sufentanil, another highly potent μ receptor selective opioid.[10] In addition, the effects of 14-methoxymetopon on gastrointestinal transit, a potential measure of the ability of a drug to produce constipation clinically, were far less than those seen with morphine.[9] Despite its classification as a μ opioid agonist which is strongly supported by receptor binding studies,[4,5] antagonism of its in vivo actions by μ selective opioid antagonists and antisense mapping studies,[9] its actions differ pharmacologically from those of morphine.

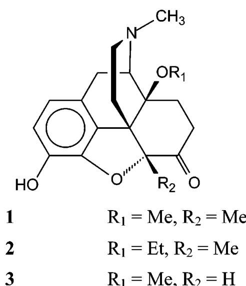


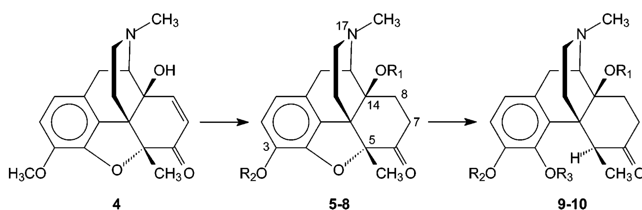
Figure 1.

The analogue 14-ethoxymetopon (**2**) was reported to exhibit slightly less analgesic potency and similar low physical dependence scores and tolerance,[4] while the 5-demethyl analogue 14-*O*-methyloxymorphone (**3**) shows also similar analgesic potency but more pronounced physical dependence.[11]

* To whom correspondence should be addressed. Phone: (43) 512-507-5248. Fax: (43) 512-507-2940. E-mail: helmut.schmidhammer@uibk.ac.at.
† Department of Pharmaceutical Chemistry, University of Innsbruck.
‡ Department of Pharmacology and Toxicology, University of Innsbruck.
§ Virginia Commonwealth University.
|| University of Maryland.

10.1021/jm030878b CCC: $25.00 © 2003 American Chemical Society
Published on Web 08/14/2003

Scheme 1



5	R_1 = cinnamyl, R_2 = Me, $\Delta^{7,8}$
6	R_1 = 3-phenylpropyl, R_2 = Me
7 (PPOM)	R_1 = 3-phenylpropyl, R_2 = H
8	R_1 = 3-phenylpropyl, R_2 = propargyl (= prop-2-ynyl)
9	R_1 = 3-phenylpropyl, R_2 = Me, R_3 = H
10	R_1 = 3-phenylpropyl, R_2 = Me, R_3 = Me

Very recent results on differently *N*-substituted 14-phenylpropoxymorphinan-6-one derivatives provide further evidence that the nature of the substituent at the oxygen in position 14 has a major impact on the ability of the compounds to interact with opioid receptors.[12] These compounds exhibit significantly increased binding affinities to all opioid receptors without any specific preference for one particular receptor type. Several structure−activity relationship and molecular modeling studies on opioid peptides and nonpeptides to establish the critical positions responsible for interaction with opioid receptors have been published.[13−19] As recently reported by our group[12] and also by others,[13,14,19] not the nature of the substituent at the nitrogen in morphine-like compounds but rather residues occupying a defined position in the vicinity to the morphinan nitrogen seem to be responsible for agonist/antagonist action. We have found that in the series of morphinan-6-ones having a cyclopropylmethyl or allyl group at the morphinan nitrogen, the presence of a 14-*O*-phenylpropyl substituent gives rise to highly potent analgesics.[12]

Therefore, it was of interest to prepare 14-phenylpropoxy analogues of 14-methoxymetopon in order to further study the influence of this substituent in this class of compounds. A 14-phenylpropoxy substituent would also increase lipophilicity of 14-methoxymetopon which might broaden the therapeutic scope, e.g., use in transdermal systems. The latter are known to be advantageous regarding the compliance of the patients to therapy and thus possibly increase the success of the treatment. Since it was shown that a 14-phenylpropoxy group also enhances opioid receptor affinity and antinociceptive potency in 3-*O*-alkyl-substituted morphinanones,[12] it was intended to study the influence of a 3-*O*-methyl and of a 3-*O*-propargyl group. A 3-*O*-propargyl group was shown to enhance the μ agonist character of clocinnamox to a higher extent than a 3-*O*-methyl group.[20] Since 3,4-dimethoxy-substituted *N*-methylmorphinan-6-ones are known to have considerably high oral bioavailability,[11,21,22] we also decided to prepare the corresponding 3,4-dimethoxy-substituted 14-phenylpropoxymetopon derivative.

Chemistry. Compound **5** (Scheme 1) was prepared from 14-hydroxy-5-methylcodeinone (**4**)[23] by 14-*O*-alkylation with cinnamyl bromide (3-bromo-1-phenyl-1-propene) in anhydrous DMF in the presence of NaH as a base. Compound **6** was obtained by hydrogenation of compound **5** in EtOH. The 3-*O*-methyl ether of compound **6** was cleaved by refluxing in HBr to obtain compound **7**. Compound **8** was prepared from compound **7** by 3-*O*-alkylation with propargyl bromide in acetone in the presence of K_2CO_3. 4−5-Ring opening of compound **6** with activated zinc in the presence of NH_4Cl in EtOH gave compound **9**, which was 4-*O*-methylated with dimethyl sulfate under phase transfer conditions (40% tetrabutylammonium hydroxide, CH_2Cl_2) to afford compound **10**.

Results and Discussion

Opioid Receptor Binding. The binding affinities of compounds **6**−**8** and **10** were determined as previously described[24] in homogenates of rat brain (μ, δ) and guinea-pig brain (κ). The following type selective opioid radioligands were used: [^{3}H][D-Ala^2,(N-Me)Phe^4,Gly^5-ol]enkephalin ([^{3}H]DAMGO, μ),[25] [^{3}H][Ile^{5,6}]deltorphin II (δ),[26] and [^{3}H]U69,593 (κ).[27] The binding data expressed as inhibition constant (K_i) values are shown in Table 1.

Compounds **6**−**8** and **10** exhibited very high affinity (in the subnanomolar range) to the μ opioid binding site. Among the tested ligands, the 3-hydroxy analogue **7** (PPOM) displayed the highest μ affinity (K_i = 0.20). Surprisingly, the 3-*O*-alkyl ethers **6**, **8**, and **10** showed only slightly less binding affinity to the μ receptor. PPOM also showed affinities in the subnanomolar range to δ and κ opoid receptors, while the 3-*O*-alkyl ethers had lower affinities to these receptors, except the 3,4-dimethoxy-substituted compound **10**, which exhibited δ opioid receptor affinity in the subnanomolar range. Thus, compounds **6** and **8** showed preference for μ over δ and κ opioid receptors, while PPOM had similar high affinities to all three opioid receptor types comparable to the reference compounds etorphine and dihydroetorphine (Table 1). When comparing PPOM to 14-methoxymetopon (**1**), it becomes apparent that a 14-phenylpropoxy group increases both δ and κ opioid affinity considerably, while μ affinity remains unchanged.

Antinociceptive Assays. All compounds of this series appeared to be very potent opioid receptor agonists in vivo (Table 2). They showed high antinociceptive potency in the hot-plate assay (HP), the tail-flick test (TF), and the paraphenylquinone writhing test (PPQ) in mice, and their potency was considerably greater than that of morphine.

The 3-hydroxy derivative **7** (PPOM) behaved as an opioid agonist with a potency which is 24 000-fold higher in the TF and 8500-fold higher in the HP compared to morphine. Compared to etorphine, PPOM shows 25, 10, and 2.5 times higher potency in the TF, HP, and PPQ, respectively, while, compared to dihydroetorphine, PPOM is similarly potent in the HP and PPQ and ca. twice as potent in the TF.

The 3-*O*-methyl ether of PPOM, compound **6**, showed unexpectedly high antinociceptive potency although its potency was ca. 10−55 times lower than that of PPOM. Thus, compound **6** is ca. 12- and 7-fold more potent than 14-methoxymetopon (**1**) and ca. 330- and 440-fold more potent than morphine in the HP and TF, respectively. The potency of the 3-*O*-propargyl ether **8** was similar to 14-methoxymetopon, but lower compared to compound **6** (5−15 times) and PPOM (60−380 times), depending on the assay used. The 3,4-dimethoxy deriva-

Table 1. Opioid Receptor Binding of Compounds **6**–**8**, **10**, and Reference Compounds

compd	K_i (nM) ± SEM		
	[³H]DAMGO (μ)[a]	[³H][Ile^{5,6}]deltorphin II (δ)[a]	[³H]U69,593 (κ)[b]
6	0.62 ± 0.13	6.33 ± 0.61	25.0 ± 4.9
7	0.20 ± 0.05	0.14 ± 0.02	0.40 ± 0.01
8	0.73 ± 0.06	13.1 ± 1.5	24.1 ± 3.3
10	0.65 ± 0.08	0.76 ± 0.17	2.26 ± 0.65
1[c]	0.15 ± 0.01	13.3 ± 0.2	26.9 ± 1.2
morphine[c]	6.55 ± 0.74	217 ± 19	113 ± 9
etorphine[d]	0.26 ± 0.07[e]	0.27 ± 0.09[f]	0.18 ± 0.07[g]
dihydroetorphine[d]	0.19 ± 0.05[e]	0.20 ± 0.06[f]	0.13 ± 0.05[g]

[a] Rat brain membranes were used. [b] Guinea pig brain membranes were used. [c] Taken from ref 5. [d] Taken from ref 34. [e] [³H]DAMGO and gerbil cerebellar membranes were used. [f] [³H]DPDPE-Cl and guinea pig forebrain membranes were used. [g] [³H]U69,593 and human placental P3 fraction were used.

Table 2. In Vivo Activities of Compounds **6**–**8**, **10**, and Reference Compounds in Mice

compd	ED_{50} (sc, μg/kg)[a]		
	HP[b]	TF[c]	PPQ[d]
6	2.6 (0.70–9.5)	4.4 (2.1–9.4)	1.7 (1.0–2.9)
7	0.10 (0.05–0.45)	0.08 (0.06–0.10)	0.16 (0.07–0.40)
8	38 (16–91)	21 (11–42)	9.0 (4.5–12)
10	22 (9.0–54)	14 (5.0–40)	2.3 (1.1–4.8)
1	30 (10–50)	30 (10–600)	9.0 (3.0–230)
morphine[e]	850 (390–1,860)	1,920 (890–4,140)	400 (200–800)
etorphine[f]	1.0 (0.40–3.0)	2.0 (1.0–4.0)	0.40 (0.20–0.90)
dihydroetorphine[f]	0.10 (0.05–0.30)	0.15 (0.06–0.40)	0.20 (0.06–0.40)

[a] Effective dose 50% (95% confidence limits). [b] HP = hot plate test. [c] TF = tail flick test. [d] PPQ = paraphenylquinone writhing assay. [e] Taken from ref 32. [f] Taken from ref 33.

tive **10** exhibits somewhat higher antinociceptive potency than compound **8**.

Conclusions

The present study on different 14-phenylpropoxymetopon derivatives provides further evidence that the nature of the substituent at position 14 has a major impact on the ability of morphinans to interact with opioid receptors. In agreement with a recent report,[12] introduction of a 14-phenylpropoxy group markedly increases binding affinities to δ and κ opioid receptors while retaining the high affinity to the μ receptor. Moreover, in case of the 3-*O*-alkyl ether derivatives a significant increase in μ affinity was noted. The presence of a 14-phenylpropoxy substituent leads to a profound alteration of the pharmacological profile in this class of compounds. The most striking finding is that this substituent is able to dramatically increase the antinociceptive potency. Thus, one compound, PPOM, possesses ca. 60 to 400 times higher antinociceptive potency than its 14-methoxy analogue **1**. Moreover, the 3-*O*-ether derivatives **6** and **10** show considerably higher potency than 14-methoxymetopon (**1**).

Experimental Section

The required reagents as well as anhydrous DMF were purchased from Fluka, Switzerland, in the highest purities available, and the solvents were distilled before usage. Melting points were measured using a Kofler melting point microscope and are uncorrected. IR spectra were recorded with a Mattson Galaxy Series FTIR 3000 spectrometer (in cm^{-1}). ¹H NMR (200 MHz) and ¹³C NMR (50.3 MHz) spectra were recorded using a Varian Gemini 200 spectrometer. Chemical shifts (δ) are reported in ppm (relative to $SiMe_4$ as internal standard), coupling constants (*J*) in hertz. Mass spectra were recorded with a Finnigan Mat SSQ 7000 apparatus. Elemental analyses were performed at the Institute of Physical Chemistry at the University of Vienna, Austria. For TLC, POLYGRAM SIL G/UV$_{254}$ precoated plastic sheets (Macherey-Nagel, Germany) were used (eluent: CH_2Cl_2/MeOH/concentrated NH_4OH solution, 90:9:1), and for column chromatography, silica gel 60 (230–400 mesh ASTM, Fluka, Switzerland) was used.

7,8-Didehydro-4,5α-epoxy-3-methoxy-5β,17-dimethyl-14β-{[(*E*)-3-phenylprop-2-enyl]oxy}morphinan-6-one (5). A mixture of **4**[23] (5.00 g, 15.27 mmol), NaH (0.72 g, 30.00 mmol, obtained from 1.20 g 60% NaH dispersion in oil by washing with hexane), and anhydrous DMF (30 mL) was stirred under N_2 at 0 °C. After 25 min, a solution of cinnamyl bromide (3.01 g, 15.27 mmol) in anhydrous DMF (20 mL) was added slowly, and stirring was continued for 1.5 h at 0 °C. Excess NaH was destroyed with ice and 500 mL of H_2O, the mixture was extracted with CH_2Cl_2/*i*-PrOH 4:1 (1 × 150 mL, 3 × 50 mL), and the combined organic layers were washed with H_2O (5 × 400 mL) and brine (250 mL), dried (Na_2SO_4), and evaporated. The residue (7.59 g orange oil) was crystallized from *i*-PrOH/Et_2O 1:1 (20 mL) to give 3.03 g (45%) of pure **5**: slightly yellow crystals; mp >150 °C (dec); IR (KBr) 1675 (C=O) cm^{-1}; ¹H NMR (DMSO-d_6) δ 7.44–7.23 (m, 5 arom H, *H*-C(8)), 6.70 (d, *J* = 8.2, 1 arom H), 6.62 (d, *J* = 8.2, 1 arom H), 6.56 (d, *J* = 15.7, PhC*H*CHCH$_2$), 6.33 (dt, *J* = 15.7, *J* = 5.0, PhCHC*H*CH$_2$), 6.10 (d, *J* = 10.0, *H*-C(7)), 4.35 (dd, ^2J = 10.6, ^3J = 5.0, PhCHCHC*H*$_2$), 4.16 (dd, ^2J = 10.6, ^3J = 5.0, PhCHCHC*H*$_2$), 3.70 (s, C*H*$_3$O), 2.36 (s, C*H*$_3$N), 1.61 (s, C*H*$_3$-C(5)); ¹³C NMR ($CDCl_3$) δ 198.4, 148.4, 144.7, 143.1, 137.5, 134.4, 133.2, 131.6, 129.2 (2C), 128.3, 127.9, 127.1 (2C), 126.4, 119.7, 114.8, 93.3, 74.3, 63.5, 59.1, 57.3, 49.0, 46.4, 43.3, 26.4, 23.3, 18.0; CI-MS *m*/*z* 444.3 (M^+ + 1). Anal. ($C_{28}H_{29}NO_4$) C, H, N.

4,5α-Epoxy-3-methoxy-5β,17-dimethyl-14β-[(3-phenylpropyl)oxy]morphinan-6-one (6). A mixture of **5** (3.44 g, 7.76 mmol), EtOH (80 mL), and 10% Pd/C (0.35 g) was hydrogenated at 50 °C and 50 psi for 2 h. The catalyst was filtered off and the filtrate evaporated. The residue (3.31 g brown oil) was crystallized from EtOH (20 mL) to give 2.52 g (73%) of pure **6**: white crystals; mp 126–128 °C; IR (KBr) 1720 (C=O) cm^{-1}; ¹H NMR ($CDCl_3$) δ 7.32–7.17 (m, 5 arom H), 6.67 (d, *J* = 8.2, 1 arom H), 6.58 (d, *J* = 8.2, 1 arom H), 3.87 (s, C*H*$_3$O), 2.33 (s, C*H*$_3$N), 1.65 (s, C*H*$_3$-C(5)); ¹³C NMR ($CDCl_3$) δ 212.7, 145.4, 143.2, 142.8, 131.6, 129.2 (2C), 128.9 (2C), 126.4, 119.6, 114.7, 96.8, 81.0, 76.7, 59.3, 57.2, 51.5, 46.2, 43.2, 35.2, 33.3, 32.7, 26.7, 26.6, 23.1, 17.8; CI-MS *m*/*z* 448.3 (M^+ + 1). Anal. ($C_{28}H_{33}NO_4$) C, H, N.

4,5α-Epoxy-3-hydroxy-5β,17-dimethyl-14β-[(3-phenylpropyl)oxy]morphinan-6-one Hydrobromide (7·HBr). A mixture of **6** (2.23 g, 4.98 mmol) and 48% HBr (13 mL) was refluxed for 20 min and then allowed to cool to room temperature. The mixture was treated with MeOH (20 mL) and evaporated almost to dryness. This procedure was repeated twice whereas the evaporation the last time was carried out until dryness. The residue (2.48 g beige crystals) was recrystallized from MeOH (15 mL) to give 2.26 g (88%) of pure **7**·HBr: white crystals; mp > 270 °C (dec); IR (KBr) 3473 (OH), 1725 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.42 (s, br, N*H*$^+$), 8.58 (s, br, O*H*), 7.36−7.16 (m, 5 arom H), 6.69 (d, *J* = 8.2, 1 arom H), 6.64 (d, *J* = 8.2, 1 arom H), 2.97 (d, *J* = 4.6, C*H*$_3$N$^+$), 1.52 (s, C*H*$_3$-C(5)); ^{13}C NMR (DMSO-d_6) δ 209.7, 142.9, 141.7, 139.9, 128.8, 128.3 (2C), 128.2 (2C), 125.8, 120.5 119.9, 118.0, 94.4, 75.4, 60.8, 59.4, 48.9, 46.7, 41.3, 33.1, 31.7, 30.7, 24.2, 23.5, 17.0; CI-MS *m*/*z* 434.3 (M$^+$ + 1). Anal. ($C_{27}H_{31}NO_4$·HBr) C, H, N.

4,5α-Epoxy-5β,17-dimethyl-14β-[(3-phenylpropyl)oxy]-3-[(prop-2-ynyl)oxy]morphinan-6-one Hydrochloride (8·HCl). A mixture of **7** (0.38 g, 0.88 mmol, the free base was obtained from the hydrobromide), K_2CO_3 (0.37 g, 2.68 mmol), propargyl bromide (0.32 g, 2.66 mmol), and acetone (25 mL) was refluxed for 7 h. The inorganic material was filtered off, and the filtrate was evaporated to give 0.46 g of an orange oil. Purification of this crude product by flash chromatography (silica gel, elution with CH_2Cl_2/MeOH/concentrated NH_4OH solution, 250:2:0.5) afforded 0.35 g of a yellow oil which was precipitated as hydrochloride from Et_2O to give 0.22 g (49%) of pure **8**·HCl: white powder; mp 130−133 °C; IR (KBr) 1727 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.38 (s, br, N*H*$^+$), 7.34−7.19 (m, 5 arom H), 6.92 (d, *J* = 8.4, 1 arom H), 6.78 (d, *J* = 8.4, 1 arom H), 4.81 (d, *J* = 1.0, HCCC*H*$_2$), 3.59 (t, *J* = 1.0, *H*CCCH$_2$), 2.94 (d, *J* = 3.8, C*H*$_3$N$^+$), 1.53 (s, C*H*$_3$-C(5)); ^{13}C NMR (DMSO-d_6) δ 209.4, 144.4, 141.8, 140.1, 129.3, 128.3 (2C), 128.2 (2C), 125.7, 124.0, 120.0, 117.2, 95.3, 79.1, 78.4, 75.2, 60.8, 59.1, 56.8, 48.9, 46.4, 41.3, 33.0, 31.8, 30.6, 24.3, 23.8, 23.5, 16.9; CI-MS *m*/*z* 472.3 (M$^+$ + 1). Anal. ($C_{30}H_{33}NO_4$·HCl·1.0H_2O) C, H, N.

4-Hydroxy-3-methoxy-5β,17-dimethyl-14β-[(3-phenylpropyl)oxy]morphinan-6-one Hydrochloride (9·HCl). To a refluxing mixture of **6** (3.70 g, 8.27 mmol), NH_4Cl (4.50 g, 84 mmol), and EtOH (50 mL) was added 5.50 g activated zinc powder (84 mmol) in five portions. After refluxing for 47 h, the inorganic material was filtered off, and the filtrate was evaporated to give 6.35 g of a white foam. This was dissolved in H_2O (200 mL) and alkalinized with concentrated NH_4OH solution, and the mixture was extracted with CH_2Cl_2 (1 × 100 mL, 4 × 50 mL). The combined organic layers were washed with H_2O (4 × 200 mL) and brine (200 mL), dried (Na_2SO_4), and evaporated to give 3.93 g of a yellow oil. Purification of this crude product by flash chromatography (silica gel, elution with CH_2Cl_2/MeOH/concentrated NH_4OH solution, 250:2:0.5) afforded 2.99 g (80%) of a yellow oil which was not crystallized for the next step. An analytical sample was precipitated as hydrochloride from Et_2O and recrystallized from Et_2O/*i*-PrOH: slightly yellow powder; mp > 210 °C (dec); IR (KBr) 3415 (OH), 1700 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.64 (s, br, N*H*$^+$), 8.88 (s, O*H*), 7.33−7.17 (m, 5 arom H), 6.91 (d, *J* = 8.2, 1 arom H), 6.70 (d, *J* = 8.2, 1 arom H), 3.78 (C*H*$_3$O), 2.80 (d, *J* = 3.4, C*H*$_3$N$^+$), 1.28 (d, *J* = 6.6, C*H*$_3$-C(5)); ^{13}C NMR (DMSO-d_6) δ 210.5, 146.7, 144.3, 142.2, 128.2 (2C), 128.1 (2C), 127.6, 125.9, 125.6, 118.2, 110.5, 74.6, 61.9, 60.4, 56.9, 56.0, 47.2, 46.8, 44.3, 32.7, 31.7, 30.6, 25.4, 25.2, 24.1, 13.5; CI-MS *m*/*z* 450.3 (M$^+$ + 1). Anal. ($C_{28}H_{35}NO_4$·HCl·1.1H_2O) C, H, N.

3,4-Dimethoxy-5β,17-dimethyl-14β-[(3-phenylpropyl)oxy]morphinan-6-one Hydrochloride (10·HCl). A mixture of **9** (1.55 g, 3.44 mmol), 40% tetrabutylammonium hydroxide solution (20 g), dimethyl sulfate (0.48 g, 3.80 mmol), and CH_2Cl_2 (15 mL) was vigorously stirred under N_2 at room temperature. After 12 h, H_2O (300 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with H_2O (6 × 200 mL, to remove the phasetransfer catalyst) and brine (200 mL), dried (Na_2SO_4), and evaporated to give 1.25 g of a yellow oil. Purification of this crude product by flash chromatography (silica gel, elution with CH_2Cl_2/MeOH/concentrated NH_4OH solution, 250:2:0.5) afforded 1.00 g of a colorless oil which was converted into the hydrochloride salt with Et_2O/HCl and recrystallized from Et_2O/*i*-PrOH to yield 0.86 g (50%) of pure **10**·HCl: slightly yellow powder; mp 135−138 °C (dec); IR (KBr) 1700 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.36 (s, br, N*H*$^+$), 7.31−7.18 (m, 5 arom H), 7.05 (d, *J* = 8.4, 1 arom H), 6.95 (d, *J* = 8.4, 1 arom H), 3.80 (C*H*$_3$O), 3.79 (C*H*$_3$O), 2.81 (d, *J* = 4.8, C*H*$_3$N$^+$), 1.53 (d, *J* = 7.0, C*H*$_3$-C(5)); ^{13}C NMR (DMSO-d_6) δ 209.6, 151.7, 147.2, 142.2, 134.9, 128.2 (2C), 128.1 (2C), 125.8, 125.6, 122.9, 112.4, 74.5, 60.5, 59.8, 56.7, 55.8, 48.2, 47.1, 44.7, 40.7, 32.6, 31.6, 30.4, 26.5, 25.3, 24.0, 12.6; CI-MS *m*/*z* 464.3 (M$^+$ + 1). Anal. ($C_{29}H_{37}NO_4$·HCl·1.0H_2O) C, H, N.

Opioid Receptor Binding Assays. Membranes were prepared from whole rat or guinea-pig brains as previously described.[24] Briefly, tissues were homogenized in 5 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4). After centrifugation at 40 000*g* for 20 min at 4 °C, the membrane pellets were resuspended in 30 volumes of 50 mM Tris-HCl buffer and incubated at 37 °C for 30 min. The centrifugation step described above was repeated, the final pellet was resuspended in 50 mM Tris-HCl buffer and stored at −70 °C until use. Binding experiments were performed in 50 mM Tris-HCl buffer in a final volume of 1 mL containing 0.3−0.5 mg protein as described.[24] Rat brain homogenates were incubated either with the μ-selective radioligand [^{3}H]DAMGO (0.5 nM, 45 min, 35 °C)[25] or with the δ-selective radioligand [^{3}H][Ile^{5,6}]deltorphin II (0.5 nM, 45 min, 35 °C).[26] Guinea pig brain homogenates were incubated with the κ-selective radioligand [^{3}H]U69,593 (1 nM, 30 min, 30 °C)[27] in the presence of increasing concentrations of the test compound. Reactions were terminated by rapid filtration through Whatman GF/B pretreated with 0.1% polyethyleneimine ([^{3}H]U69,593) or GF/C ([^{3}H]DAMGO and [^{3}H][Ile^{5,6}]deltorphin II) glass fiber filters using a Brandel Cell Harvester, followed by three washings with 5 mL of ice-cold 50 mM Tris-HCl buffer. The bound radioactivity was measured in Ultima Gold scintillation cocktail, using a Beckman LS1701 liquid scintillation counter. Nonspecific binding was defined in the presence of 10 μM unlabeled naloxone. Protein concentration was determined using bovine serum albumine as a standard.[28] All experiments were carried out in duplicate. The values presented are the mean ± SEM of 3−4 independent experiments. Competition inhibition constant (K_i) values were calculated with the GraphPad Prism (version 3.0, San Diego, CA) program.

In Vivo Assays. All animals received care according to the *Guide for the Care and Use of Laboratory Animals*, U.S. Department of Health and Human Services, 1985. The facilities are certified by the American Association for the Accreditation for Laboratory Animal Care. These studies were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

General Methods. ICR male mice (Harlan-Sprague−Dawley, Inc., Indianapolis, IN) weighing 20−30 g were used. Each animal was tested once only. All drugs were given by the subcutaneous (sc) route. At least three doses were tested, and 6−10 animals/dose were used. The drugs were dissolved in 5% hydroxypropyl-β-cyclodextrin in water or in dilute HCl (vehicles).

Hot Plate Test (HP). A modified method previously described was used.[29] A 1000 mL Pyrex beaker (bottom removed) was placed on the hot plate maintained at 56 °C. The test was initiated by placing a mouse in the specially designed beaker. This arrangement served to confine a mouse to a specific area of the hot plate. Each mouse was exposed to the hot plate for two trials spaced 5 min apart. Only mice that gave a control response latency in the range of 6−10 s on both trials served as subjects. Each subject received a dose of test drug and 30 min later was again tested on the hot plate. Activity was scored as positive if the mouse jumped, licked, or shook its paws at least 5 s beyond its average control

latency. Cutoff time was 15 s. Percent activity for each dose tested was calculated as (total number of mice scored as positive)/(total number of mice tested) × 100.

Tail-Flick Test (TF). The procedures[30,31] and their modifications were previously described.[32] Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photoelectric cell. Mice were injected with test drug or vehicle and tested 20 min later. Antinociception was calculated as % MPE (percent maximum possible effect) = (test latency − control latency)/(10 s − control latency) × 100 for each dose tested. Cutoff time was 10 s.

Paraphenylquinone Writhing Assay (PPQ). The procedure previously described[33] with modifications as indicated below was used. Six mice were injected per dose of test drug or vehicle and 10 min later received 2 mg/kg intraperitoneally (ip) of a freshly prepared paraphenylquinone solution. They were then placed in three cages in groups of two each. Then, the total number of stretches observed per group during each 1 min period was counted at 10 and 15 min. The total number of stretches for the three groups was determined. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles and extension of the hind limbs. The antinociceptive response was expressed as % inhibition of the paraphenylquinone-induced stretching response and was calculated as [1 − (total number of stretches in the medicated mice)/(total number of stretches in the control mice)] × 100.

Acknowledgment. We thank Tasmanian Alkaloids Pty., Ltd., Australia, for the generous gift of thebaine. We further thank Dr. D. Rakowitz for performing the mass spectra and Andrea Kaletsch for technical assistance. This work was supported by the Austrian Science Foundation (P12668). The pharmacological evaluation was carried out under the auspices of the Drug Evaluation Committee of the College on Problems of Drug Dependence of the U.S.A.

Note Added in Proof. While the manuscript was under review, additional work in the course of this project—concerning the stereochemistry of compounds **9** and **10** and the binding data of etorphine—was published.[35,36] Retention of the stereochemistry at C(5) (5β-methyl) upon cleavage of the 4−5-ring of compound **6** has been confirmed by X-ray analysis of the 14-hydroxy analog of compound **10**.[35] Supplementary, the binding data of etorphine have been measured to give the following K_i values (nM): 0.27 ± 0.02 for μ receptors (determined with [^{3}H]DAMGO and rat brain homogenates), 1.56 ± 0.29 for δ receptors (determined with [^{3}H][Ile^{5,6}]deltorphin II and rat brain homogenates), and 0.78 ± 0.07 for κ receptors (determined with [^{3}H]U69,593 and guinea pig brain homogenates).[36]

References

(1) Part 19 of this series: Schüllner, F.; Meditz, R.; Krassnig, R.; Morandell, G.; Kalinin, V. N.; Sandler, E.; Spetea, M.; White, A.; Schmidhammer, H.; Berzetei-Gurske, I. P. Synthesis and Biological Evaluation of 14-Alkoxymorphinans. 19. Effect of 14-*O*-Benzylation on the Opioid Receptor Affinity and Antagonist Potency of Naltrexone. *Helv. Chim. Acta* **2003**, in press.

(2) Pasternak, G. W. Incomplete Cross Tolerance and Multiple Mu Opioid Peptide Receptors. *Trends Pharmacol. Sci.* **2001**, *22*, 67−70.

(3) MacPherson R. D. The Pharmacological Basis of Contemporary Pain Management. *Pharmacol. Ther.* **2000**, *88*, 163−185.

(4) Fürst, S.; Búzás, B.; Friedmann, T.; Schmidhammer, H.; Borsodi, A. Highly Potent Novel Opioid Receptor Agonist in the 14-Alkoxymetopon Series. *Eur. J. Pharmacol.* **1993**, *236*, 209−215.

(5) Spetea, M.; Tóth, F.; Schütz, J.; Ötvös, F.; Tóth, G.; Benyhe, S.; Borsodi, A., Schmidhammer, H. Binding Characteristics of [^{3}H]-14-Methoxymetopon, a High Affinity μ-Opioid Receptor Agonist. *Eur. J. Neurosci.* **2003**, *18*, 290−295.

(6) Schmidhammer, H.; Schratz, A.; Mitterdorfer, J. Synthesis and Biological Evaluation of 14-Alkoxymorphinans. 8. 14-Methoxymetopon, an Extremely Potent Opioid Agonist. *Helv. Chim. Acta* **1990**, *73*, 1784−1787.

(7) Zernig, G.; Saria, A.; Krassnig, R.; Schmidhammer, H. Signal Transduction Efficacy of the Highly Potent Mu Opioid Agonist 14-Methoxymetopon. *Life Sci.* **2000**, *66*, 1871−1877.

(8) Urigüen, L.; Fernandez, B.; Romero, E. M.; De Pedro, N.; Delgado, M. J.; Guaza, C.; Schmidhammer, H.; Viveros, M. P. Effects of 14-Methoxymetopon, a Potent Opioid Agonist, on the Responses to the Tail Electric Stimulation Test and Plus-maze Activity in Male Rats: Neuroendocrine correlates. *Brain Res. Bull.* **2002**, *57*, 661−666.

(9) King, M. A.; Su, W.; Nielan, C. L.; Chang, A. H.; Schütz, J.; Schmidhammer, H.; Pasternak, G. W. 14-Methoxymetopon, a Very Potent μ-Opioid Analgesic with an Unusual Pharmacological Profile. *Eur. J. Pharmacol.* **2003**, *459*, 203−209.

(10) Freye, E.; Schmidhammer, H.; Latasch, L. 14-Methoxymetopon, a Potent Opioid, Induces no Respiratory Depression, Less Sedation, and Less Bradycardia than Sufentanil in the Dog. *Anesth. Analg.* **2000**, *90*, 1359−1364.

(11) Schmidhammer, H.; Aeppli, L.; Atwell, L.; Fritsch, F.; Jacobson, A. E.; Nebuchla, M.; Sperk, G. Synthesis and Biological Evaluation of 14-Alkoxymorphinans. 1. Highly Potent Opioid Agonists in the Series of (−)-14-Methoxy-*N*-methylmorphinan-6-ones. *J. Med. Chem.* **1984**, *27*, 1575−1579.

(12) Greiner, E.; Spetea, M.; Krassnig, R.; Schüllner, F.; Aceto, M.; Harris, L. S.; Traynor, J. R.; Woods, J. H.; Coop, A.; Schmidhammer, H. Synthesis and Biological Evaluation of 14-Alkoxymorphinans. 18. N-Substituted 14-Phenylpropyloxymorphinan-6-ones with Unanticipated Agonist Properties: Extending the Scope of Common Structure−Activity Relationships. *J. Med. Chem.* **2003**, *46*, 1758−1763.

(13) Portoghese, P. S.; Moe, S. T.; Takemori, A. E. A Selective δ_1 Opioid Receptor Agonist Derived from Oxymorphone. Evidence for Separate Recognition Sites for δ_1 Opioid Receptor Agonists and Antagonists. *J. Med. Chem.* **1993**, *36*, 2572−2574.

(14) Portoghese, P. S.; Sultana, M.; Moe, S. T.; Takemori, A. E. Synthesis of Naltrexone-Derived δ-Opioid Antagonists. Role of Conformation of the δ Address Moiety. *J. Med. Chem.* **1994**, *37*, 579−585.

(15) Schiller, P. W.; Nguyen, T. M.-D.; Weltrowska, G.; Wilkes, B. C.; Marsden, B. J.; Lemieux, C.; Chung, N. N. Differential Stereochemical Requirements of μ vs δ Opioid Receptors for Ligand Binding and Signal Transduction: Development of a Class of Potent and Highly δ-Selective Peptide Antagonists. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 11871−11875.

(16) Schmidt, R.; Vogel, D.; Mrestani-Klaus, C.; Brandt, W.; Neubert, K.; Chung, N. N.; Lemieux, C.; Schiller, P. W. Cyclic β-Casomorphin Analogues with Mixed μ Agonist/δ Antagonist Properties: Synthesis, Pharmacological Characterization, and Conformational Aspects. *J. Med. Chem.* **1994**, *37*, 1136−1144.

(17) Wilkes, B. C.; Schiller, P. W. Theoretical Conformational Analysis of the Opioid δ Antagonist H-Tyr-Tic-Phe-OH and the μ Agonist H-Tyr-D-Tic-Phe-NH_2. *Biopolymers* **1994**, *34*, 1213−1219.

(18) Wilkes, B. C.; Schiller, P. W. Comparative Analysis of Various Proposed Models of the Receptor-Bound Conformation of H-Tyr-Tic-Phe-OH Related δ-Opioid Antagonists. *Biopolymers* **1995**, *37*, 391−400.

(19) Brandt, W. A Uniform Molecular Model of δ Opioid Agonist and Antagonist Pharmacophore Conformations. *J. Comput.-Aided Mol. Des.* **1998**, *12*, 615−621.

(20) Husbands, S. M.; Sadd, J.; Broadbear, J. H.; Woods, J. H.; Martin, J.; Traynor, J. R.; Aceto, M. D.; Bowman, E. R.; Harris, L. S.; Lewis, J. W. 3-Alkyl Ethers of Clocinnamox: Delayed Long-Term μ-Antagonists with Variable μ Efficacy. *J. Med. Chem.* **1998**, *41*, 3493−3498.

(21) Schmidhammer, H.; Jacobson, A. E.; Brossi, A. Chemical and Biological Study of Aromatic Oxygenated 6-Ketomorphinans. *Med. Res. Rev.* **1983**, *3*, 1−19.

(22) Schmidhammer H. 14-Alkoxymorphinans − A Series of Highly Potent Opioid Agonists, Antagonists, and Partial Agonists. *Curr. Topics Med. Chem.* **1993**, *1*, 261−276.

(23) Schmidhammer, H.; Deeter, J. B.; Jones, N. D.; Leander, J. D.; Schoepp, D. D.; Swartzendruber, J. K. Synthesis, Structure Elucidation, and Pharmacological Evaluation of 5-Methyl-oxymorphone (= 4,5α-Epoxy-3,14-dihydroxy-5,17-dimethylmorphinan-6-one). *Helv. Chim. Acta* **1988**, *71*, 1801−1804.

(24) Spetea, M.; Erlandsson Harris, H.; Berzetei-Gurske, I.; Klareskog, L.; Schmidhammer, H. Binding, Pharmacological and Immunological Profiles of the δ-Selective Opioid Receptor Antagonist HS 378. *Life Sci.* **2001**, *69*, 1775−1782.

(25) Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. Analogues of β-LPH$_{61-64}$ Possessing Selective Agonist Activity at μ-Opiate Receptors. *Eur. J. Pharmacol.* **1981**, *70*, 531−540.

(26) Nevin, S. T.; Kabasakal, L.; Ötvös, F.; Tóth, G.; Borsodi, A. Binding Characteristics of the Novel Highly Selective δ Antagonist, [^{3}H]Ile^{5,6}deltorphin II. *Neuropeptides* **1994**, *268*, 261−265.
(27) Lahti, R. A.; Mickelson, M. M.; McCall, J. M.; Von Voigtlander, P. F. [^{3}H]U-69593, a Highly Selective Ligand for the Opioid κ Receptor. *Eur. J. Pharmacol.* **1985**, *109*, 281−284.
(28) Bradford, M. M. A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Proteine-Dye Binding. *Anal. Biochem.* **1976**, *15*, 248−254.
(29) Eddy, N. B.; Leimbach, D. Synthetic Analgesics. II. Dithienylbutenylamines and Dithienylbutylamines. *J. Pharmacol. Exp. Ther.* **1953**, *107*, 385−393.
(30) D'Amour, F. E.; Smith, D. L. A Method for Determinig Loss of Pain Sensation. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74−79.
(31) Dewey, W. L.; Harris, L. S. Antinociceptive Activity of the Narcotic Antagonist Analgesics and Antagonistic Activity of Narcotic Analgesics in Rodents. *J. Pharmacol. Exp. Ther.* **1971**, *179*, 652−659.
(32) May, E. L.; Aceto, M. D.; Bowman, E. R.; Bentley, C.; Martin, B. R.; Harris, L. S.; Medzihradsky, F.; Mattson, M. V.; Jacobson, A. E. Antipodal α-*N*-(Methyl through Decyl)-*N*-normetazocines (5,9α-Dimethyl-2′-hydroxy-6,7-benzomorphans): In Vitro and in Vivo Properties. *J. Med. Chem.* **1994**, *37*, 3408−3418.
(33) Aceto, M. D.; Harris, L. S.; Bowman, E. R. Etorphines: μ-Opioid Receptor-Selective Antinociception and Low Physical Dependence Capacity. *Eur. J. Pharmacol.* **1997**, *338*, 215−223.
(34) Niwa, M.; Al-Essa, L. Y.; Ohta, S.; Kohno, K.; Nozaki, M.; Tsurumi, K.; Iwamura, T.; Kataoka, T. Opioid Receptor Interaction and Adenyl Cyclase Inhibition of Dihydroetorphine: Direct Comparison With Etorphine. *Life Sci.* **1995**, *56*, 395−400.
(35) Schütz, J.; Schmidhammer, H.; Wurst, K. Crystal Structure of 14β-Hydroxy-3,4-dimethoxy-5β,17-dimethylmorphinan-6-one, $C_{20}H_{27}NO_4$. *Z. Kristallogr. NCS* **2003**, *218*, 181−183.
(36) Laus, G.; Schütz, J.; Schottenberger, H.; Andre, M.; Wurst, K.; Spetea, M.; Ongania, K.-H.; Müller, A. G.; Schmidhammer, H. Etorphine-Related Ferrocenyl Morphinan Alkaloids. *Helv. Chim. Acta* **2003**, in press.

JM030878B