Notes

Synthesis and δ-Opioid Receptor Antagonist Activity of a Naltrindole Analogue with a Regioisomeric Indole Moiety

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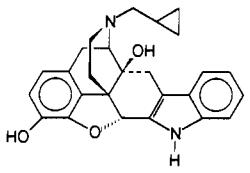
Indolomorphinans **2** and **3**, in which the indole moiety is fused to the 7,8-position of the morphinan system, have been synthesized from dihydropseudocodeinone **4** and evaluated for antagonist activity on the mouse vas deferens (MVD) and guinea pig ileum (GPI) preparations. Indolomorphinan **2** was found to be ~1/60th as potent as naltrindole **1** in the MVD and an agonist in the GPI preparation. A comparable difference in affinity between **1** and **2** was observed. The methyl analogue **3** was inactive in both preparations. The results of this study support the idea that the regio orientation of the indolic benzene moiety of **1** is optimal for δ-opioid receptor antagonist activity. It is proposed that the proper alignment of the benzene moiety with an address subsite on the δ receptor is critical for potent δ antagonist activity.

Naltrindole (**1**, NTI) is employed widely as a δ-opioid receptor antagonist.[1-3] As a naltrexone-derived product of the Fischer indole synthesis, its indole moiety is fused to the 6,7-positions of the C ring in the morphinan system.

It has been reported[2] that the indolic benzene moiety is largely responsible for the high affinity of NTI for δ-opioid receptors, and more recent studies[4,5] have revealed that the position of this moiety is highly favorable for δ antagonist activity. Here we report on the synthesis of indolomorphinans **2** and **3** having the indole ring fused to the 7,8-position in order to evaluate how it affects δ antagonist potency.



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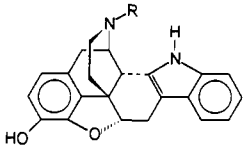
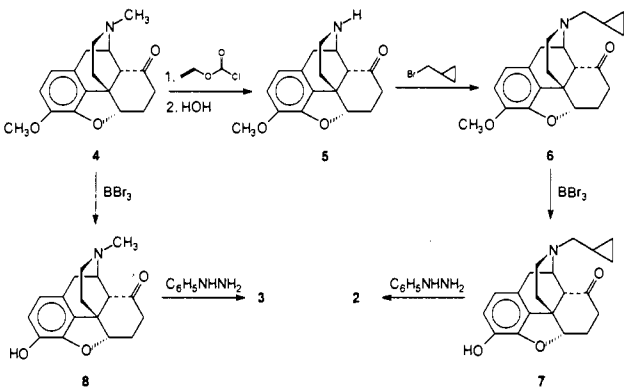


2, R = $CH_2CH(CH_2)_2$
3, R = CH_3

Chemistry

The target compounds **2** and **3** were synthesized as outlined in Scheme 1. Dihydropseudocodeinone[6] **4** was N-demethylated with vinyl chloroformate, and the resulting carbamate intermediate was hydrolyzed to afford the nor compound **5**. Reaction of **5** with cyclopropylmethyl bromide gave **6**, which was subsequently O-demethylated with BBr_3 to the corresponding phenol **7**. Target compound **2** was prepared in 85% yield by Fischer indole synthesis using intermediate **7** and phenylhydrazine hydrochloride. The methyl analogue **3** was synthesized in two steps from **4** by O-demethylation to give dihydroisomorphinone **8**, which was then subjected to Fischer indolization conditions.

Scheme 1



Pharmacology

Compounds **2** and **3** were tested on the electrically stimulated mouse vas deferens[7] (MVD) and guinea pig ileal longitudinal muscle[8] (GPI) preparations as described previously.[9] For evaluation of antagonist activity, **2** and **3** were incubated with the preparations 15 min prior to testing with either [D-Ala^2,D-Leu^5]enkephalin[10] (DADLE), morphine (M), or ethylketazocine (EK). These agonists are selective for δ-, μ-, and κ-opioid receptors, respectively. Concentration-response curves were obtained in the absence (control) and presence of the antagonist in the same preparation and were expressed as IC_{50} values. The IC_{50} ratio represents the IC_{50} in the presence of the antagonist divided by the control IC_{50} value. IC_{50} ratios for DADLE were obtained in the MVD and those for M and EK were determined in the GPI.

In the MVD, compound **2** antagonized the δ agonist, DADLE, with an IC_{50} ratio that is approximately 1/60th the potency of NTI (Table 1). At 1 μM **2** acted as a partial agonist having a 16% maximal effect. Evaluation of **2** in the GPI revealed it to be a full agonist with a potency 1.4 times that of morphine. No antagonist or agonist activity was observed in the testing of **3** in either of the smooth muscle preparations.

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Table 1. Evaluation of Indolomorphinans 2 and 3 in the MVD and GPI

	IC_{50} ratio[a]		
compd	DADLE[b] (δ)	M[c] (μ)	EK[c] (κ)
1 (NTI)	459 ± 104 (5)	11.2 ± 1.8 (4)	2.0 ± 0.1 (3)[d]
2	8.2 ± 1.9 (4)	e	e
3	1.0 ± 0.2 (3)	0.92 ± 0.17 (4)	0.99 ± 0.16 (3)

[a] IC_{50} of the agonist in the presence of the antagonist (100 nM) divided by the IC_{50} of the agonist. [b] MVD preparation. [c] GPI preparation. [d] U50488 was employed as the agonist. [e] Agonist potency of 2 in the GPI, 1.4 times that of morphine.

Table 2. Binding of Indolomorphinan 2

	K_i, nM[a]		
compd	δ	μ	κ
1[b] (NTI)	0.03	3.8	332
2	2.2	4.9	33

[a] Values are geometric means of three replicate experiments. [b] Data from ref 2.

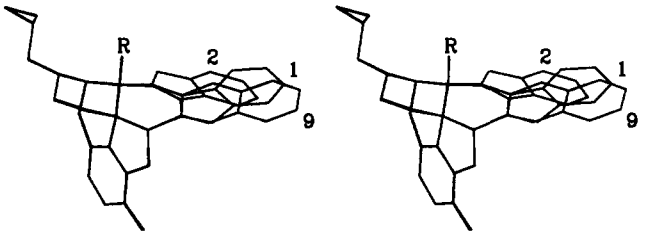


Figure 1. Superposition of NTI 1 (R = OH), 2 (R = H), and the quinoline analogue 9 (R = OH).

Binding

The binding of compound 2 was carried out on guinea pig brain membranes using a modification of the procedure of Werling et al.[11] Binding to δ, μ, and κ sites was determined in competition experiments using tritiated [D-Pen^2,D-Pen^5]enkephalin,[12] [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin,[13] and the benzeneacetamide, U69593,[14] respectively. The data (Table 2) reveal that 2 has substantially less affinity for δ sites than NTI, 1. Moreover, the affinity of 2 for δ and μ sites are comparable.

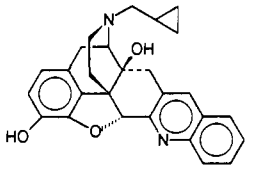
Discussion

Prior studies have revealed that the antagonist selectivity of NTI (1) is due to the indolic benzene moiety which enhances the potency at δ-opioid receptors while interfering with binding to non-δ-opioid sites.[2] In this context, the benzene moiety was proposed to interact with a unique "address"[15] subsite on the δ receptor.

In the present study we have synthesized analogue 2 which contains a regioisomeric indole moiety. The relatively low δ antagonist potency and absence of selectivity of 2 suggest that the recognition of its indolic benzene moiety by the putative address subsite on the δ receptor is greatly impaired. This is consistent with earlier structure–activity studies that have demonstrated the importance of the geometry of the spacer that is fused to the 6,7-position of the morphinan and the benzene moiety.[4] Thus, when the spacer was changed from a five- to six-membered ring (i.e., compound 9), the δ antagonist potency was 1/10th that of NTI. It was proposed that the different spacer geometry positions the benzene moiety so that it interacts less effectively with the δ address subsite. The superposed structures in Figure 1 corresponding to the indoles (1, 2) and quinoline analogue 9 illustrate how the positions of the indolic benzene moiety differ.

In addition to the improper alignment of the benzene moiety with the δ address subsite as an explanation for the low antagonist potency of 2, it is also possible that substitution at the 8-position in 2 may interfere with effective interaction.

While the coplanarity of the benzene moiety with ring C of the morphinan appears to contribute to the high δ antagonist potency of NTI, it appears unlikely to be essential in view of the fact that 7-spiroindanylnaltrexone 10, which contains an orthogonal benzene moiety, is a fairly potent δ antagonist (1/6th that of NTI).[5] Thus, the present results suggest that the regio requirements for recognition of the benzene moiety by the aromatic binding subsite on the δ receptor are more demanding than the conformational requirements.



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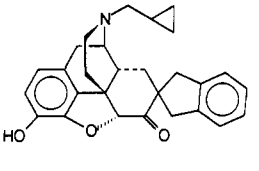

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It is noteworthy that 2 is a full agonist in the GPI, a preparation known to contain functional μ and κ receptors. This agonist activity is most likely mediated through μ receptors in view of the comparable affinity of 2 for δ and μ sites. The finding that 1 has 60-fold greater affinity than 2 for δ sites and that both ligands have equivalent affinity for μ sites is consistent with the proposal that a properly positioned benzene moiety confers selectivity in part through recognition by an aromatic binding subsite on the δ receptor.

Experimental Section

Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Analyses were performed by MHW Labs, Phoenix, AZ, and were within 0.4% of theoretical values. Nuclear magnetic resonance spectra were performed on an IBM-Bruker AC-300 spectrometer, and chemical shifts are reported as δ values (ppm) relative to Me_4Si or $CDCl_3$. IR spectra were recorded on a Nicolet 5DXC FT-IR spectrometer, and peak positions are expressed in cm^{-1}. Mass spectra were obtained on AEI MS 30, Finnigan 4000 CI, or VG 7070EHF instruments.

4,5α-Epoxy-3-methoxy-8-oxomorphinan (5). A solution of dihydropseudocodeinone[6] 4 (194 mg, 0.65 mmol) and vinyl chloroformate (2 mL, 23.5 mmol) in dichloroethane (5 mL) was refluxed for 48 h. The mixture was concentrated, and the residue was dissolved in ethanol (5 mL). To the solution was added 2 N HCl (5 mL), and the mixture was refluxed for 2 h. The solvent was removed, the residue was neutralized with 1 N NaOH, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. The solid residue was recrystallized from EtOAc to afford 5 (130 mg, 70%): mp 269–270 °C; FABMS m/z 286 (M + 1); ^{1}H NMR ($CDCl_3$) δ ppm 3.73 (3H, s, OCH_3), 4.98 (1H, s, H-5), 6.62 (1H, d, J = 8.4 Hz, H-1), 6.83 (1H, d, J = 8.4 Hz), 9.41 (1H, broad s, NH); IR (cm^{-1}, KBr) 3413, 1715, 1560.

17-(Cyclopropylmethyl)-4,5α-epoxy-3-methoxy-8-oxomorphinan (6). A mixture of 5 (759 mg, 2.7 mmol), cyclopropylmethyl bromide (1.3 mL), sodium bicarbonate (1.7 g), and ethanol (35 mL) was refluxed for 18 h. The mixture was concentrated, and water was added. The product was extracted with EtOAc. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel (EtOAc) to afford 6 (730 mg, 81%). The hydrochloride salt was prepared using ethanol/ether HCl: mp 175–177 °C; FABMS m/z 340 (M + 1); ^{1}H NMR (free base) δ ppm (DMSO-d_6) 0.16 (2H, m, H-20, H-21), 0.56 (2H, m, H-20, H-21), 0.88 (1H, m, H-19), 2.81 (1H, d, J =

17.7 Hz, H-10), 3.85 (3H, s, OMe), 4.14 (1H, m, H-9), 4.90 (1H, m, H-9), 4.90 (1H, m, H-5), 6.53 (1H, d, J = 7.5 Hz, H-1), 6.69 (1H, d, J = 7.5 Hz). IR (free base, neat, cm^{-1}) 1711, 1610.

17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxy-8-oxomorphinan (7). To a cooled (ice–salt bath) solution of 6 (600 mg, 1.6 mmol) in dichloromethane (40 mL) was added a 1 M solution of BBr_3 in dichloromethane (4 mL, 4 mmol). The mixture was stirred for 1 h at room temperature, and then 2 N HCl (12 mL) was added. The mixture was refluxed for 30 min and then neutralized with saturated sodium bicarbonate solution. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$. The insoluble solid was dissolved in a small amount of MeOH, and the solution was combined with the extract. The organic layer and the extract were combined, washed with water, dried, and evaporated. The residue was chromatographed on silica gel to afford 7 (417 mg, 80%). The hydrochloride salt was prepared using ethanol/ether HCl: mp 259–260 °C; FABMS m/z 326 (M + 1); ^1H NMR (free base) ($CDCl_3$) δ ppm 0.15 (2H, m, H-20, H-21), 0.56 (2H, m, H-20, H-21), 0.90 (1H, m, H-19), 2.80 (1H, d, J = 18.3 Hz, H-10), 4.21 (1H, m, H-9), 4.88 (1H, m, H-5), 6.35 (1H, s, OH), 6.47 (1H, d, J = 8.4 Hz, H-1), 6.65 (1H, d, J = 8.4 Hz, H-2); IR (free base, neat, cm^{-1}) 2570 (broad), 1710, 1610.

17-(Cyclopropylmethyl)-7,8-didehydro-4,5α-epoxy-3-hydroxyindolo[2′,3′:7,8]morphinan (2). A solution of 7·HCl (170 mg, 1.19 mmol) and phenylhydrazine hydrochloride (298 mg, 2.07 mmol) in ethanol (10 mL) and concentrated HCl (3 drops) was refluxed for 8 days. Sodium bicarbonate was added, and the mixture was stirred for 10 min at 60 °C. The residue was filtered and evaporated. The residue was chromatographed on silica gel to afford 2 (159 mg, 85%), mp >260 °C. The hydrochloride salt, mp >260 °C, was prepared using ethanol/ether HCl: FABMS m/z 399 (M + 1); ^1H NMR (free base) (DMSO-d_6) δ ppm 0.15 (2H, m, H-20, H-21), 0.52 (2H, m, H-20, H-21), 0.89 (1H, m, H-19), 3.04 (1H, dd, J = 17.1 and 7.5 Hz, D ring), 4.14 (1H, s, H-9), 5.07 (1H, d, J = 6.0 Hz, H-5), 6.20 (1H, d, J = 5.7 Hz, H-1), 6.29 (1H, d, J = 5.7Hz, H-2), 6.83 (1H, t, J = 7.2 Hz, indole ring), 6.90 (1H, t, J = 7.2 Hz, indole ring), 7.16 (1H, d, J = 8.4 Hz, indole ring), 7.24 (1H, d, J = 8.4 Hz, indole ring), 8.79 (1H, s, OH), 10.80 (1H, s, NH); IR (free base, neat, cm^{-1}) 2570 (broad), 1710, 1610. Anal. ($C_{26}H_{26}N_2O_2$·HCl·C_2H_5OH) H, N; C: calcd, 69.91; found, 70.37.

Dihydroisomorphinone (8). To a solution of 4·HCl (600 mg, 2.0 mmol) in CH_2Cl_2 (50 mL) was added 5 mL of a 1 M solution of BBr_3 in CH_2Cl_2, and the reaction mixture was stirred at 25 °C for 1 h. Dilute aqueous HCl (10%, 15 mL) was added dropwise, and the reaction mixture was stirred for 30 min at 50 °C (oil bath temperature). The reaction mixture was then cooled and made basic with solid $NaHCO_3$. The organic layer was separated and the aqueous layer washed with $CHCl_3$ (4 × 10 mL). The combined extract and washings were dried (anhydrous Na_2SO_4) and evaporated under vacuum. The resulting oil was chromatographed on a 1-mm Chromatotron plate (elution with 7% MeOH/$CHCl_3$/1% NH_3) to give a foam (210 mg, 36.7%) which was crystallized from EtOAc to afford 8: mp 199–200 °C dec (lit.[6] mp 198 °C); EIMS m/z 285 (M + 1).

17-Methyl-7,8-didehydro-4,5α-epoxy-3-hydroxyindolo[2′,3′:7,8]morphinan (3). A solution of 8·HCl (400 mg, 1.40 mmol) and phenylhydrazine hydrochloride (700 mg, 4.86 mmol) in methanol (20 mL) and concentrated HCl (5 drops) was refluxed at 60 °C for 8 days. The reaction mixture was treated with $NaHCO_3$ (1 g) and stirred for 10 min at 60 °C, filtered, and evaporated to dryness under vacuum. The residue was chromatographed on a 1- × 2-in. vacuum-flash column (gradient elution: 0% to 9% MeOH/$CHCl_3$/1% NH_3). Fractions containing the product were pooled, and the solvent was removed under reduced pressure. The resulting solid was washed with $CHCl_3$ (4 × 5 mL) to afford 3 (210 mg, 41.9%): mp >290 °C. The HCl salt was prepared from ethanolic etheral HCl: mp 268–270 °C dec; ^1H NMR (300 MHz, CDON (CD_3)$_2$) δ 10.75 (s, 1H, NH, D_2O exchange), 7.94 (s, 1H, OH, D_2O exchange), 7.20 (dd, 2H, H-4′ and H-7′), 6.82 (dt, 2H, H-5′ and H-6′), 6.25 (dd, 2H, H-1 and H-2), 5.06 (d, 1H, H-5), 3.91 (m, 1H, H-9), 3.45 (d, 1H, H-10a), 3.07 (dd, 1H, D ring), 2.36 (s, 3H, NCH_3); FABMS m/z 359 (M + 1). Anal. ($C_{23}H_{22}N_2O_2$·HCl·H_2O) C, H, N.

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