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Syntheses and receptor-binding studies of derivatives of the opioid antagonist naltrexone

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Abstract—Naltrexone (**1**), which is a member of the group of competitive opioid antagonists, shows a strong affinity for μ-receptors and its derivatives have been notable as novel receptor anatgonists. In this paper, the preparation of several naltrexone derivatives is described; these were used to investigate the role of the oxygenated functional groups in facilitating binding to a series of the opioid receptors. The derivatives showed affinity for opioid μ-receptors which was similar to that of naltrexone, but these compounds, which had masked hydroxyl functional groups, displayed a moderate activity. These results suggest that every oxygenated functional group in naltrexone (**1**) plays an important role in binding to the opioid receptor.

1. Introduction

It is generally accepted that there are at least three major types of homologous opioid receptors (μ, δ, and κ). A number of putative subtypes of the three major receptor types have been reported, but definitive evidence for their existence is lacking. The availability of selective opioid ligands has played an important role in the cloning and pharmacologic characterization of opioid receptors and in this regard, a variety of highly selective ligands have been developed as tools to determine the effects mediated by these receptors.

The opioid antagonist naltrexone (**1**) is useful as a parenteral adjunct for treating opioid addiction and alcoholism. Therefore the structure–activity relationships of compounds related to naltrexone (**1**) have been extensively studied and they have shown interesting characteristics in their actions at opioid receptors.[1]

β-Naltrexol (**2**) is reported to be a major metabolite of naltrexone (**1**) in mammals[2] (Scheme 1). The affinity of β-naltrexol (**2**) for the opioid receptors is similar to that of naltrexone (**1**).

Although the structure–activity relationships have been extensively investigated,[3] the systematic studies regarding the roles of the oxygenated functional groups, especially for the α-naltrexol (**3**) [the epimer of β-naltrexol (**2**)], have not been reported. This study reports on structure–activity relationships concerning the oxygenated functional groups of naltrexone (**1**).

2. Results

2.1. Chemistry

Chemical transformation of naltrexone (**1**) resulted in various naltrexone derivatives (Scheme 2). Methylation of naltrexone (**1**) with CH_2N_2 in Et_2O afforded 3-methoxynaltrexone (**4**)[4] and an unexpected epoxide (**5**) in 17% and 77% yield, respectively. The structure of **5**, $[\alpha]_D$ −144.9° (*c* 1.96, $CHCl_3$), was determined based on the spectral data. The mass spectrum of **5** exhibited a molecular ion peak $[M]^+$ at 369 which is consistent with the molecular formula $C_{22}H_{27}O_4N$. The ^1H and ^{13}C NMR resonances of **5** were unambiguously assigned by 2D HMQC and HMBC experiments. NOESY experiments determined that the stereochemistry of the C-6 position in **5** was *S*. Proof for the stereochemistry of **5** was provided by X-ray crystallography (Fig. 1).

Keywords: Naltrexone derivatives; Opioid; Receptor binding assay; Analgesia.
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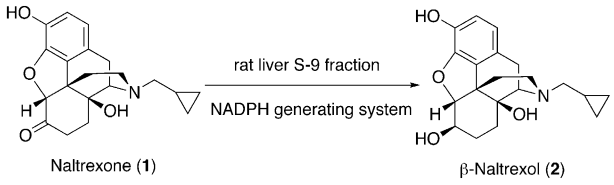
It was very interesting that epoxide (**5**) was obtained in a stereoselective manner as the conformation of newly formed epoxide is β. If the reaction mechanism followed the expected nucleophilic attack of the diazomethane on the carbonyl group to yield a diazonium betaine, it is difficult to explain the result, because the α-side of naltrexone (**1**) is sterically hindered compared to β-side. Our accepted hypothesis of the reaction mechanism is a concerted cycloaddition that forms a 1,3,4-oxadiazoline intermediate (Scheme 3).[5] The formation of the 1,3,4-oxadiazoline intermediate is stereochemically determined (diazomethane access from the β-side). Thus the stereoselective step of the reaction might occur during the concerted elimination of N_2 from the oxadiazoline. The carbonyl ylide is not favored due to electrostatic repulsion between the oxygen atom of the carbonyl ylide and the oxygen at the 5-position. Thus the internal rotation of the carbonyl ylide allows formation of a more stable intermediate, which leads to formation of the epoxide ring.

Reduction of the C-6 keto group of naltrexone (**1**) with $NaBH_4$ gave α-naltrexol (**3**),[6] the epimer of the major metabolite. Methylation of α-naltrexol (**3**) was performed with CH_3I. Base, in the form of K_2CO_3 was used to methylate the aromatic OH at the C3 position, giving 3-methyl-α-naltrexol (**6**)[7] in 99% yield. Methylation of the OH at both C3 and the aliphatic C6 position with KH gave 3,6-dimethyl-α-naltrexol (**7**) in 66% yield.



Scheme 1. Metabolic reduction of naltrexone (**1**) with rat liver S-9 fraction.

2.2. Opioid receptor binding

Opioid receptor binding data for compounds **1** and **3**–**7** were obtained with mouse brain or guinea pig cerebellum using the procedure of Chang et al.[8] Binding was evaluated by competition between the following selective radio-ligands: [^{3}H]DAMGO (μ) [^{3}H]deltorphin II, (δ) or [^{3}H]U-69,593 (κ) and the test compounds chosen.

Table 1 describes the results. Among these compounds, the K_i values for the known α-naltrexol (**3**) showed the

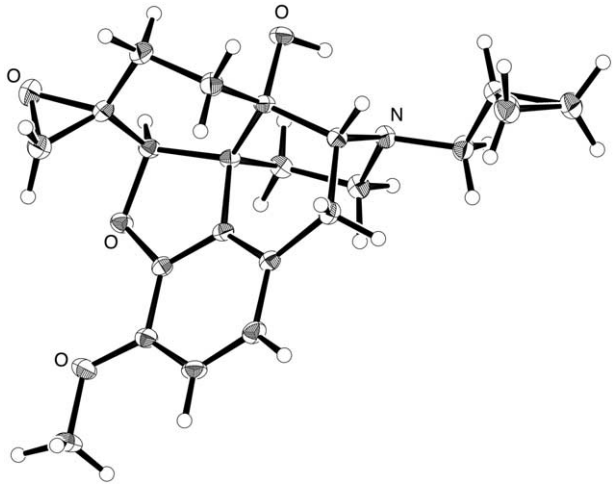
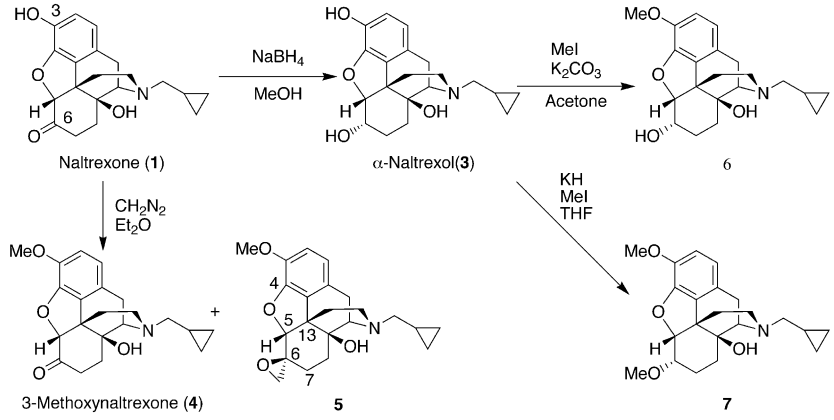
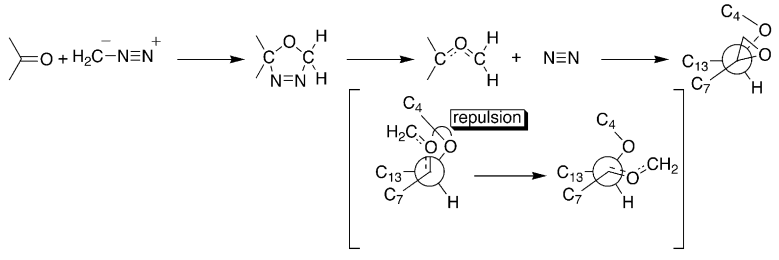


Figure 1. ORTEP drawing of compound (**5**). Thermal ellipsoids are drawn at a 30% probability level.



Scheme 2. Syntheses of naltrexone derivatives.



Scheme 3. The supposed mechanism of formation of epoxide (**5**) from naltrexone (**1**) by diazomethane.

Table 1. Receptor binding assays of naltrexone (**1**) derivatives

	μ		δ		κ	
	IC_{50} (nM)[a]	Ki (nM)	IC_{50} (nM)[a]	Ki (nM)	IC_{50} (nM)[a]	Ki (nM)
1	2.57±0.48	1.55±0.29	196.7±64.9	7.84±25.9	1.78±0.36	0.711±0.144
3	2.55±0.24	0.959±0.087	213.3±6.7	8.22±5.5	3.30±0.10	1.32±0.040
4	295.0±35.5	110.2±13.2	>10000	ND	4.20±70.9	167.4±28.3
5	580.0±82.9	219.1±31.3	>10000	ND	463.3±63.8	191.9±15.0
6	>1000	ND	>10000	ND	882.5±129.9	351.7±51.8
7	966.7±167.1	361.2±62.4	>10000	ND	463.3±63.9	184.7±25.4
DAMGO	3.42±0.52	1.32±0.20	ND	ND	ND	ND
Deltorphin II	ND	ND	8.33±2.37	3.45±0.98	ND	ND
U-69,593	ND	ND	ND	ND	9.13±100	3.17±0.35

[a] Concentration that gives half-maximal effect. Data are given as the mean±SEM ($n=3$).

highest binding affinity to the μ-receptors, at the relatively low concentration of ~1.0 nM, which is of a similar order of magnitude to that of the parent compound, naltrexone (**1**). The remaining ligands showed moderate activities since their hydroxyl group(s) were masked with methyl groups or were transformed to the epoxide; however, all six compounds were μ-selective.

3. Conclusion

In the present study, naltrexone analogues **3**–**7** were synthesized. Their potencies in inhibiting the specific binding of [^{3}H]DAMGO, a μ subunit-selective opioid agonist, to opioid receptors in the mouse brain, were evaluated. In addition, their ability to inhibit binding of [^{3}H]deltorphin II, a δ subunit-selective opioid agonist, to opioid receptors in the mouse brain, and [^{3}H]U-69,593, a κ subunit-selective opioid agonist, to opioid receptors in the guinea pig cerebellum were assessed. Our findings demonstrate that the C-3 and C-6 hydroxyls or the carbonyl moieties are critical for receptor binding. Moreover, although there are *O*-containing groups (e.g., methoxyl or epoxide) at the C-3 and/or C-6 positions, 'naked' *O*-functional groups (hydroxyl or ketone) are essential for activity.

Despite the fact that the newly synthesized compounds (**5**, **6** and **7**) did not show the strong activity that we had anticipated, we expect that some of them could possibly reveal a potent specific affinity to the putative subtypes of the three major receptor types (e.g., 3-methoxynaltrexone (**4**)). If so, they would be useful tools for the characterization of new opioid receptors.

Further studies are currently underway in our laboratory to identify more effective analogues.

4. Experimental

4.1. Chemistry

All reactions were conducted in oven-dried glassware under a nitrogen atmosphere. All solvents were solvent grade. Wako gel C-200 (70-150 μm, Wako pure chemicals) was used for column chromatography. Precoated Kieselgel 60F-254plate (0.25 mm, Merck) was used for TLC analysis and the spots were detected by the absorbance of UV light at 254 nm spraying with Dragendorff's reagent. The optical rotation was recorded on a JASCO DIP-360 polarimeter. ^{1}H NMR spectra were measured on JEOL JNM EX270 (270 MHz), JEOL JNM 400 (400 MHz), and JEOL JNM 600 (600 MHz) spectrometers. Chemical shifts (δ) are reported as ppm downfield from tetramethylsilane (TMS), and coupling constants are given in Hz. Multiplicity is indicated as follows: s (singlet); d (doublet); dd (doublet of doublets); ddd (doublet of double doublets); t (triplet); m (multiplet); etc. Mass spectra were recorded on JEOL JMS DX-303 and JMA-DA 5000 spectrometers.

4.1.1. (5α,6α)-17-(Cyclopropylmethyl)-4,5-epoxymorphinan-3,6,14-triol (6α-naltrexol (3)). $NaBH_4$ (68.4 mg, 1.80 mmol) was added to a solution of naltrexone (**1**) HCl salt (454 mg, 1.20 mmol) in MeOH (12 mL) at −20 °C. After 30 min, acetone was added to the reaction mixture, which was then concentrated in vacuo. The residue was diluted with Et_2O and then successively washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Purification by column chromatography on silica gel ($CHCl_3$–MeOH, 97:3 v/v) gave the title compound **3** (385 mg, 94%) as a colorless oil: All spectral data are consistent with previously reported data.[6]

4.1.2. (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-14-hydroxy-3-methoxymorphinan-6-one (*O*3-Methyl-(−)-naltrexone (4)), (5α)-17-(Cyclopropylmethyl)-4,5-epoxy-14-hydroxy-3-methoxy-6-methylenemorphinan 6β-oxide (5). 0.2 M CH_2N_2 in Et_2O (25 mL) was added to a solution of naltrexone (**1**) HCl salt (917 mg, 2.4 mmol) at 0 °C. After 1 h, the reaction mixture was evaporated. The residue was dissolved with $CHCl_3$ and successively washed with a 10% aqueous solution of NH_4OH and brine, dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography on silica gel ($CHCl_3$–MeOH, 99:1 v/v and 97:3 v/v) gave *O*3-Methyl-(−)-naltrexone (**4**) (148 mg, 17%) as a colorless oil, and compound **5** (677 mg, 77%) as colorless needles. Compound **4**: All spectral data are consistent with previously

reported data[4]. Compound **5**: $[\alpha]_D$ −144.9° (*c* 1.96, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ: 6.72 (d, 1H, *J* = 8.1 Hz, C-2H), 6.63 (d, 1H, *J* = 8.1 Hz, C-1H), 4.65 (s, 1H, C-5β H), 3.86 (s, 3H, –OCH_3), 3.12 (d, 1H, *J* = 5.5 Hz, C-9H), 3.05 (d, 1H, *J* = 18.0 Hz, C-10β H), 2.91 (d, 1H, *J* = 5.7 Hz, epoxyl OCH_2 H), 2,66 (dd, 1H, *J* = 12.1, 5.1 Hz, C-16H), 2.61 (dd, 1H, *J* = 18.0, 5.5 Hz, C-10α H), 2.50 (dt, 1H, *J* = 2.2, 14.0 Hz, C-7β H), 2.41–2.22 (m, 3H, C-15H, C-17 2H), 2.28 (d, 1H, *J* = 5.7 Hz, epoxyl OCH_2 H), 2.11 (ddd, 1H, *J* = 12.1, 12.1, 4.0 Hz, C-16H), 1.70 (dt, 1H, *J* = 13.2, 3.3 Hz, C-8α H), 1.56-1.44 (m, 2H, C-8β H, C-15H), 1.13 (dt, 1H, *J* = 14.0, 3.3 Hz, C-7α H), 0.89-0.77 (m, 1H, C-18H), 0.61-0.46 (m, 2H, C-19 2H), 0.19–0.07 (m, 2H, C-19 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 144.22 (s, C-4), 143.04 (s, C-3), 131.77 (s, C-12), 125.39 (s, C-11), 118.93 (d, C-1), 114.13 (d, C-2), 88.66 (d, C-5), 70.23 (s, C-14), 62.33 (d, C-9), 59.71 (s, C-6), 59.71 (t, C-17), 59.12 (q, –OMe), 52.93 (t, epoxide), 48.68 (s, C-13), 43.89 (t, C-16), 30.81 (t, C-15), 30.38 (t, C-8), 26.71 (t, C-7), 22.57 (t, C-10), 9.40 (d, C-18), 3.87 (t, C-19), 3.77 (t, C-19). MS *m*/*z*: 369 (M^+, 100%), 328, 314, 55. High-resolution MS calcd for $C_{22}H_{27}NO_4$ (M^+): 369.1940. Found: 369.1935.

Crystal data for compound (**5**): $C_{22}H_{27}NO_4$, Colorless Plate, Crystal Size = 0.40×0.35×0.08 mm, *a* = 14.688(5), *b* = 9.089(3), *c* = 13.790(5) Å, β = 93.402(5)°, *V* = 1837(1)Å^3, Space group = Monoclinic *C*2, (No. 4), Z = 4, *D*calc = 1.335 g/cm^3, T = 173 K, Rigaku CCD diffractometer using Mo-K_α radiation, measured reflections = 8100 ($2\theta < 55°$), independent reflections = 4189, final *R* = 0.0346, *R*w = 0.036 for 3024 observed reflections [$Io > 3\sigma$ (*I*o)], GOF = 0.98. All calculations were performed using the TeXsan program (Crystal Structure Analysis Package, Molecular Structure Corporation, 1985&1999).

Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 219210 in CIF format. Copies of the data can be obtained, free of charge, upon request to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, (fax:44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

4.1.3. (5α,6α)-17-(Cyclopropylmethyl)-4,5-epoxy-3-methoxymorphinan-6,14-diol (6). A mixture of α-naltrexol (**3**), (104 mg, 0.31 mmol), MeI (190 μL, 3.05 mmol), K_2CO_3 (421 mg, 3.05 mmol) in acetone (3 mL) was refluxed while stirring for 5.5 h. The reaction mixture was then concentrated in vacuo. Purification by column chromatography on silica gel ($CHCl_3$–MeOH, 49:1 v/v) gave the title compound **6**, (108 mg, 99%) as a colorless oil. All spectral data are consistent with previously reported data.[7]

4.1.4. (5α,6α)-17-(Cyclopropylmethyl)-4,5-epoxy-3, 6-dimethoxymorphinan-14-ol (7). A solution of α-naltrexol (**3**) (110 mg, 0.32 mmol) in THF (2mL) was added, dropwise, to a suspension of KH (54.4 mg, 1.36 mmol) in THF (1 mL) at 0 °C and stirred for 15 min. Then MeI (100 μL, 1.61 mmol) was added. After 7.5 h, the reaction mixture was dissolved in $CHCl_3$ and successively washed with a 1% aqueous solution of HCl, a saturated solution of aqueous $NaHCO_3$, and brine, dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography on silica gel ($CHCl_3$) gave the title compound **7** (81.0 mg, 68%) as a colorless oil: $[\alpha]_D$ −158.2° (*c* 7.9, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ: 6.70 (d, 1H, *J* = 8.4 Hz, C-2H), 6.55 (d, 1H, *J* = 8.4 Hz, C-1H), 4.76 (dd, 1H, *J* = 4.4, 1.1 Hz, C-5β H), 3.85 (s, 3H, C-3–OCH_3), 3.81 (dt, 1H, *J* = 11.4, 4.4 Hz, C-6α H), 3.45 (s, 3H, C-6–OCH_3), 3.08 (d, 1H, *J* = 6.2 Hz, C-9H), 3.04 (d, 1H, *J* = 18.7 Hz, C-10β H), 2.66–2.60 (m, 1H, C-16H), 2.60 (dd, 1H, *J* = 18.7, 7.0 Hz, C-10α H), 2.39–2.32 (m, 1H, C-17H), 2.27–2.20 (m, 2H, C-15β H, C-16H), 1.75–1.71 (m, 1H, C-7β H), 1.64 (dt, 1H, *J* = 14.7, 8.1 Hz, C-8β H), 1.58–1.55 (m, 1H, C-15α H), 1.49–1.45 (m, 1H, C-8α H), 1.24–1.20 (m, 1H, C-7α H), 0.87–0.82 (m, 1H, C-18H), 0.56–0.51 (m, 2H, C-19 2H), 0.14–0.11 (m, 2H, C-19 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 147.30 (s, C-4), 141.66 (s, C-3), 131.15 (s, C-12), 125.86 (s, C-11), 118.07 (d, C-1), 113.84 (d, C-2), 88.39 (d, C-5), 75.72 (d, C-6), 69.93 (s, C-14), 62.14 (d, C-9), 59.49 (t, C-17), 57.05(q, C-3-OMe), 56.41 (q, C-6-OMe), 47.20 (s, C-13), 43.23 (t, C-16), 33.58 (t, C-15), 28.66 (t, C-8), 22.73 (t, C-7), 20.30 (t, C-10), 9.34 (d, C-18), 3.91 (t, C-19), 3.72 (t, C-19). MS *m*/*z*: 371 (M^+, 100%), 356, 330, 110, 55. High-resolution MS calcd for $C_{22}H_{29}NO_4$ (M^+): 371.2096. Found: 371.2096.

4.2. Binding assays

Synaptosomal fractions were prepared from mice spinal cord and guinea pig cerebellum according to the method of Cheng et al.[8] Briefly, spinal cord or cerebella were homogenized in a 0.32 M sucrose solution at 0 °C and centrifuged at 6000*g* for 15 min. The supernatant was centrifuged at 40,000*g* for 30 min, and the pellets were homogenized in 5 mM Tris–HCl buffer (pH 7.4) at 0 °C. The resulting suspension was centrifuged at 6000*g* for 15 min. The supernatant was then centrifuged twice at 40000*g* for 30 min each. After the second centrifugation, the membranes were resuspended in 50 mM Tris–HCl buffer (pH 7.4).

[^{3}H]DAMGO, [^{3}H]deltorphin-II, and [^{3}H]U-69593 were used to determine the relative affinities for μ-, δ-, and κ-receptors, respectively. Binding assays were conducted by incubating an aliquot of the membrane fraction (250 μg for [^{3}H]DAMGO, 350 μg for [^{3}H]deltorphin-II, and 180 μg for [^{3}H]U-69593) containing protease inhibitors and the labeled ligand (3 nM for [^{3}H]DAMGO or [^{3}H]deltorphin-II, and 2 nM for [^{3}H]U-69593) in 50 mM Tris–HCl buffer (pH 7.4). After 60 min at 25 °C, the reaction mixture was filtrered through a Whatman GF/B filter, which was soaked with 0.1% polythyleneimine and was washed twice with cold Tris–HCl buffer. Filters were counted in a liquid scintillation counter after extracting overnight with liquid scintillation fluid (3 mL). Specific binding was determined as the difference between total binding and that in the presence of excess (10 μM) unlabeled ligand. K_d and B_{max} values were obtained using Scatchard analysis.

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