δ Opioid Antagonist Activity and Binding Studies of Regioisomeric Isothiocyanate Derivatives of Naltrindole: Evidence for δ Receptor Subtypes

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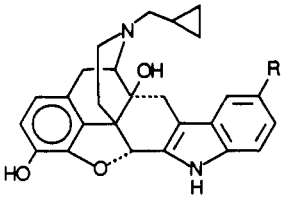
The isothiocyanate group was attached to the 4′-, 5′-, 6′-, or 7′-position of naltrindole in an effort to determine the importance of the position of this electrophilic group on the selectivity for subtypes of δ opioid receptors. All of the ligands were δ-selective when tested against standard agonists in smooth muscle preparations. However, the rank-order δ antagonism of antinociception in mice did not parallel the in vitro pharmacologic data. The 5′-isothiocyanate **2** was the most potent and selective antagonist in vivo, causing a 52-fold increase of the ED_{50} for [D-Ser^2,D-Leu^5]enkephalin-Thr^6 (DSLET) and no increase for [D-Pen^2,D-Pen^5]enkephalin (DPDPE). The effect of each of the ligands on the binding of [^{3}H]DSLET and [^{3}H]DPDPE to guinea pig brain membranes clearly differentiated between the binding sites that recognize these radioligands. These studies provide additional evidence for the presence of two subtypes of δ opioid receptors.

Introduction

There are at least three major types of opioid receptors that are located in the central nervous system and in peripheral tissues.[1] One of these receptor types, designated as δ, selectively binds the opioid peptides, leucine- and methionine-enkephalin.

In a recent study,[2] we have attached an isothiocyanate group to the 5′-position of naltrindole[3] (NTI) (**1**), a highly selective and potent δ opioid receptor antagonist, to obtain a nonequilibrium δ-selective antagonist. This compound, naltrindole 5′-isothiocyanate (5′-NTII) (**2**), produces a



1, R = H

2, R = NCS

time-dependent blockage of δ receptors in the mouse vas deferens preparation (MVD) and prolonged antagonism of the δ-selective agonist, [D-Ser^2,D-Leu^5]enkephalin-Thr^6 [4] (DSLET), in vivo. Presently, only **2** and [D-Ala^2,Leu^5]-enkephalin-Cys^6 [5] (DALCE) are known to produce non-equilibrium δ antagonism.[3,5] In this connection, it has been found[6] that **2** selectively antagonizes the δ agonist, DSLET, while DALCE selectively antagonizes the agonist effect of [D-Pen^2,D-Pen^5]enkephalin[7] (DPDPE). These and related studies[8-14] suggest the existence of δ receptor subtypes, δ_1 and δ_2, that are selectively blocked by DALCE and **2**, respectively.

In the present study we describe the pharmacologic and binding properties of isothiocyanate regioisomers of naltrindole in an effort to determine the importance of the position of the isothiocyanate group on δ subtype selectivity.

Rationale

The rationale for preparing the isothiocyanate regioisomers **2-5** was based on the supposition that different

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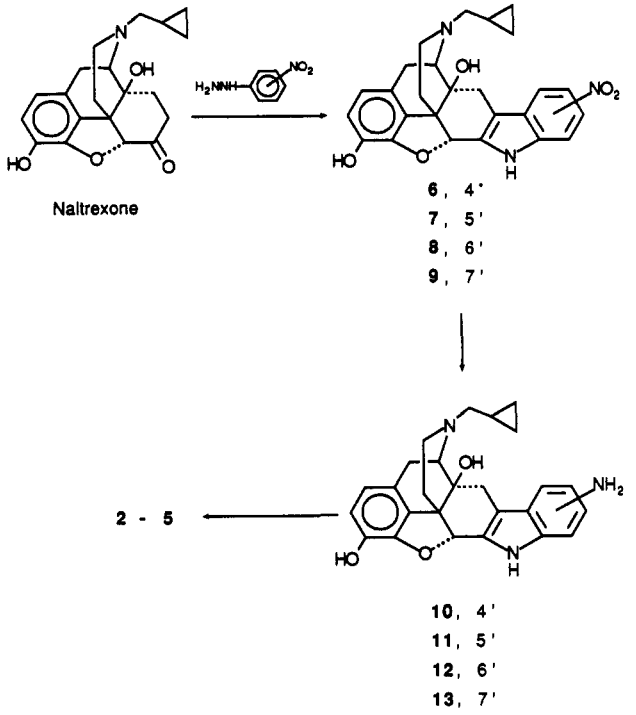
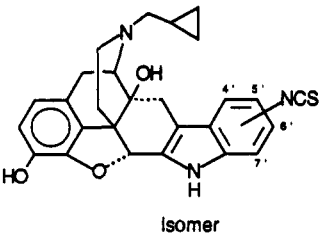
Scheme I





subtypes of δ opioid receptors may contain different distributions of nucleophiles that are proximal to, or a part of, the recognition sites. If one of the sites contains a receptor-based nucleophile in the vicinity of the isothiocyanato group of the reversibly bound ligand, covalent binding would be the result of a second recognition step that involves the juxtaposition of a pair of reactive groups. In principle, it should be possible to distinguish between closely related receptor subtypes because this interaction involves a double recognition process that results in recognition amplification.[15] This differentiation has been displayed by the 5′-isomer **2** in that it antagonizes antinociception of DSLET but not DPDPE, despite the fact that both of these enkephalin analogues are δ-selective agonists.

Chemistry

The isothiocyanate regioisomers **2–5** were synthesized from naltrexone. Treatment of naltrexone with 2-, 3-, or 4-nitrophenylhydrazine under conditions of the Fischer indole synthesis[16] afforded the desired isomeric nitroindoles **6–9** (Scheme I). When (3-nitrophenyl)hydrazine was employed, the 4′- and 6′-nitro products (**6**, **8**) were obtained in a ratio of 1:4, the regiochemistry being assigned on the basis of the distinctive NMR spectra of the isomeric amines obtained by catalytic reduction. Hydrogenation of the nitro groups in **6–9** using palladium or Raney nickel catalysts afforded the corresponding amines **10–13**. Treatment of the amines with thiophosgene in the presence of base afforded the desired isothiocyanato target compounds **2–5**.

Biological Results

Smooth Muscle Preparations. The target compounds were tested on the electrically stimulated guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD) preparations as described previously (Tables I and II).[17] The ligands (100 nM) were incubated with the preparations 30 min prior to testing. Morphine, ethylketazocine (EK), and [D-Ala^2-D-Leu^5]enkephalin^4 (DADLE) were employed as μ, κ, and δ selective agonists, respectively. Morphine and EK were employed in the GPI, and DADLE was used in the MVD.

If the agonist concentration–response curve was shifted significantly in the presence of ligand, the preparation was washed extensively (postwash) and the IC_{50} of the standard agonist was redetermined on this treated preparation. The data are expressed as IC_{50} ratios: the IC_{50} of the antagonist-treated preparation divided by the IC_{50} of the control preparation.

Compounds **2–5** exhibited nonequilibrium antagonism of DADLE in the MVD. Because the 7′-isomer **5** appeared to be exceptionally potent, its IC_{50} ratio was determined at 1 nM, rather than 100 nM, in order to measure the agonist response. The order of postwash δ antagonist potencies was **5** > **4** > **2** > **3**. With the exception of **5**, all of the compounds were inactive as antagonists of morphine or EK in the GPI. It is noteworthy that the post-wash antagonist response of **5** was substantially greater than its prewash value. One explanation for this change is that washing more easily removes an agonist pharmacologic effect of **5**, as no change was observed in the washed control preparation.

The agonist activity of the antagonists was evaluated at 1 μM (Table II). In the GPI the agonism ranged from 1 to 12% of a typical full agonist (100%). In the MVD, with the exception of **4**, the agonist response generally was higher (19–32%). Thus, it appears that the antagonists possess very weak partial agonist activity.

Antagonist Selectivity Studies in Vivo. The isothiocyanates **2–5** (10 nmol icv) were administered to mice 24 h prior to the determination of the ED_{50} values of the δ agonists (icv), DPDPE (δ_1) and DSLET (δ_2), morphine (μ) (sc), or the κ agonist, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide[18] (U50488) (sc). The antinociceptive activites of the selective agonists in the antagonist-treated and untreated (control) mice were determined using the abdominal stretch assay.[19] The activity of each of the antagonists is expressed as a ratio of ED_{50} values (treated/control) in Table III.

The 4′-isomer **3** weakly antagonized the antinociceptive effect of DPDPE and did not alter the ED_{50} values of

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Table I. Antagonist IC_{50} Ratios of Agonists upon Treatment of Smooth Muscle Preparations with Naltrindole Isothiocyanate Isomers

	GPI				
	morphine		EK	MVD: DADLE	
no.	prewash[a]	postwash[b]	prewash	prewash	postwash
2	0.90 ± 0.04 (3)		0.93 ± 0.04 (3)	6.68 ± 1.61 (6)	7.71 ± 7.17 (6)
3	0.77 ± 0.13 (3)[c]		0.51 ± 0.12 (3)	4.59 ± 1.07 (6)	3.71 ± 0.67 (6)
4	1.44 ± 0.20 (3)		1.04 ± 0.39 (3)	155 ± 44 (4)	185 ± 40 (4)
5	15.0 ± 2.9 (3)	53.0 ± 9.8 (3)	0.81 ± 0.18 (3)	7.99 ± 1.76 (5)[d]	16.1 ± 5.4 (5)[d]

[a] Prewash IC_{50} ratios were obtained by dividing the IC_{50} of the agonist in the presence of the antagonist (100 nM, 30-min incubation) by the IC_{50} of the agonist alone in the same tissue preparation. [b] Postwash IC_{50} values were obtained by thoroughly washing the preparation that was incubated with the antagonist (incubation for 5 min with naltrexone, 500 nM, followed by >40 washes) and then redetermining the agonist IC_{50}. Postwash IC_{50} ratios were caclulated by dividing the postwash IC_{50} by the control IC_{50}. [c] Values are expressed as the mean ± standard error of the mean with the number of experiments in parentheses. [d] Antagonist concentration was 1 nM.

Table II. Agonist Activities of Naltrindole Isothiocyanate Isomers in Smooth Muscle Preparations

	agonist (% inhibition at 1 μM)[a]	
no.	GPI	MVD
2	−2.4 ± 4.5 (3)[b]	18.8 ± 5.3 (3)
3	1.0 ± 1.0 (3)	24.4 ± 20.6 (3)
4	12.4 ± 7.4 (3)	0 ± 0 (3)
5	5.9 ± 10.9 (3)	31.8 ± 2.3 (3)

[a] Values are expressed as the mean ± the standard error of the mean with the number of experiments in parentheses. [b] A negative value indicates the twitch height increased by the percent given.

DSLET, morphine, or U50488. The differences between the ED_{50} ratios of DPDPE and that of DSLET and other agonists are sufficiently small to be of questionable significance. The 6′-isomer 4 antagonized both DPDPE and DSLET to a moderate degree, but it was ineffective against morphine and U50488. The 7′-isomer 5 exhibited a profile similar to that of 4, but it was less potent. In contrast to compounds 3–5, the antagonist activity of the 5′-isomer 2, which was reported[2] in an earlier preliminary communication, produced a 52-fold increase in the ED_{50} of DSLET-induced antinociception. It is approximately 7.5- and 17.5-fold more potent than 4 and 5, respectively. In addition, the 5′-isomer 2 did not antagonize the effect of DPDPE.

Opioid Receptor Binding

Guinea pig brain membranes were incubated with 4 nM of compounds 2–5 for 1 h, and the membranes were then washed three times. The opioid receptor binding assay was performed using a modification of the procedure of Werling.[20] Because the in vivo data indicated that 2 antagonizes DSLET but not DPDPE, the effect of 2–5 on the binding of both [^{3}H]DPDPE and [^{3}H]DSLET was examined. When [^{3}H]DSLET was used, the μ sites were protected with 100 nM of the unlabeled μ ligand [D-Ala^2,-MePhe^4,Gly-ol^5]enkephalin[21] (DAMGO).

The isothiocyanates 2–4 produced significant decreases in the B_{max} values of [^{3}H]DPDPE binding without affecting the K_d values (Table IV). Since the 7′-isomer 5 at 4 nM caused total inhibition of binding, a lower concentration (0.25 nM) was employed. This change resulted in a 50% decrease in the K_d and a decrease in the B_{max} similar to those observed with the other isomers. The effects of the isothiocyanates on the binding of [^{3}H]DSLET were very different from those on [^{3}H]DPDPE binding, in that large increases (9- to 29-fold) of B_{max} and K_d (3.5- to 14.5-fold) values were observed for [^{3}H]DSLET sites.

Discussion

The in vitro pharmacological data indicated that all of the isothiocyanates 2–5 were δ-selective. While the 7′-isomer 5 was the most potent δ antagonist of the series, it was considerably less selective than the other isomers (Tabel I). However, the in vivo δ antagonist data (Table III) revealed that the rank-order potencies and selectivities differ substantially from those obtained in the smooth muscle assays. In this regard, the 5′-isomer 2 displayed by far the most potent and selective antagonism of the antinociceptive activity of the δ_2 agonist DSLET, while exhibiting no antagonism of the δ_1 agonist DPDPE. Interestingly, antagonists 4 and 5 that are more potent in vitro showed little, if any, ability to distinguish between δ_1 and δ_2 receptor agonists. However, it appears that 4 is marginally more potent at δ_1 than at δ_2 sites.

The absence of correlation between the in vitro and in vivo pharmacological results may be explained by differences between subtypes of δ receptors in the MVD and in the mouse CNS and/or by differences in the pathways that involve enkephalinergic receptors. The former possibility is not unreasonable in view of the reported[14] difference between MVD and rat-brain δ receptors.

Although the binding data show no correlation with the in vivo pharmacology, they do reveal a clear difference between the sites that are selective for [^{3}H]DPDPE and those that are selective for [^{3}H]DSLET (Table IV). The observed decrease in the B_{max} value of [^{3}H]DPDPE binding is expected with nonequilibrium blockage of δ sites. With the exception of the 7′-isomer 5, this decrease in available sites occurred without a significant change of the K_d of [^{3}H]DPDPE. The effects of the isothiocyanate isomers on the sites that bind [^{3}H]DSLET were very different from those involved in the binding of [^{3}H]DPDPE, in that both K_d and B_{max} values of [^{3}H]DSLET showed large increases. One possible explanation for the unusual binding behavior of [^{3}H]DSLET binding sites after exposure to the isomeric isothiocyanates is that there are a small number of high-affinity sites and a larger pool of lower affinity sites.

The increase in K_d values of [^{3}H]DSLET upon exposure to 2–5 appears to correlate better with the rank order IC_{50} ratios (Table I) obtained in the MVD than with the in vivo data (Table III). Whether or not this reflects a similarity between DSLET-selective δ opioid sites in guinea pig brain membranes and those in the MVD remains to be clarified. In any case, the binding data strongly suggest that there is a difference between the recognition sites that bind [^{3}H]-DSLET and those that bind [^{3}H]DPDPE. These binding

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Table III. Antagonism of Opioid Agonist-Induced Antinociception by Naltrindole Isothiocyanate Isomers in Mice

antagonist[b]	ED_{50} ratio (95% confidence limits)[a]			
	DPDPE[c]	DSLET[c]	Morphine[d]	U-50,488H[d]
2[e]	0.6 (0.2–3.1)	52.1 (27.3–106.4)*	1.3 (0.6–3.9)	3.2 (1.5–13.4)*
3	2.6 (1.3–5.9)*	1.6 (0.9–3.0)	1.2 (0.5–3.4)	1.6 (0.9–3.3)
4	9.2 (4.9–22.1)*	6.8 (4.2–11.6)*	1.4 (0.6–1.6)	0.9 (0.7–2.0)
5	3.7 (1.5–13.1)*	3.0 (1.6–5.6)*	2.0 (0.6–7.0)	0.8 (0.6–1.2)

[a] ED_{50} ratio = ED_{50} + NTII isomer + control ED_{50}; ratios significantly greater than 1 are designated by asterisks. [b] All isothiocyanate derivatives of naltrindole were administered icv at a dose of 10 nmol/mouse 24 h before antinociceptive testing with the various agonists. [c] DPDPE and DSLET were administered icv. [d] Morphine and U50,488H were administered sc. [e] Results with 2 were reported earlier[2] and are included here for comparison.

Table IV. Effect of Naltrindole Isothiocyanate Isomers on the Receptor Binding of [^{3}H]DPDPE and [^{3}H]DSLET

NTII derivative[a]	concn (nM)	[^{3}H]DPDPE binding (δ_1)		[^{3}H]DSLET binding (δ_2)	
		K_d ± SE (nM)	B_{max} ± SE (fmol/mg of protein)	K_d ± SE (nM)	B_{max} ± SE (fmol/mg of protein)
control		4.6 ± 0.4	17.3 ± 2.6 (3)	4.2 ± 1.7	12.9 ± 1.9
2	4	5.9 ± 1.0	8.6 ± 1.8* (3)	37.2 ± 9.1*	191 ± 25* (3)
3	4	4.3 ± 0.7	7.0 ± 0.9* (3)	14.7 ± 1.9*	112 ± 9* (4)
4	4	5.6 ± 1.0	8.3 ± 2.0* (3)	39.8 ± 8.6*	380 ± 85* (4)
5	4	total inhibition of binding		60.8 ± 13.2*	361 ± 105* (6)
	0.25	2.3 ± 0.5*	5.6 ± 1.2* (4)	15.7 ± 2.1*	208 ± 31* (4)

[a] Brain membranes were incubated with 4 nM of the isothiocyanate for 1 h and washed three times. Asterisks signify values are significantly different from the control. Statistical significance (*) was assessed with the Student *t* test.

data provide additional evidence for the existence of at least two δ opioid receptor subtypes: δ_1, which is selective for DPDPE, and δ_2, which is selective for DSLET. Very recently we have reported[22] on additional support for δ_1 and δ_2 receptor subtypes with the highly selective δ_1 opioid receptor antagonist 7-benzylidenenaltrexone (BNTX).

Experimental Section

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1610 FTIR. NMR spectra were recorded on a Varian VXR-300 or a Bruker AC-300 spectrometer. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. Mass spectra were obtained on a VG-7070, VG-70SEQ, Finnigan 4000, or AEI MS-30 instrument. Optical rotations were measured on a JASCO-DIP-4 digital polarimeter. Analytical thin-layer chromatography (TLC) was performed on Analtech silica gel HLF glass plates. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh). Reagents and solvents were reagent grade and used without purification. Raney nickel catalyst (50% slurry in H_2O, pH > 9) was purchased from Aldrich Chemical Co. Naltrexone hydrochloride was supplied by Mallinckrodt Inc. and the National Institute on Drug Abuse. Microanalyses were performed by Desert Analytics, Tucson, AZ, or MHW Laboratories, Phoenix, AZ. Where indicated by the symbols of the elements, analyses were within ±0.4% of theoretical values.

4′-Nitronaltrindole (6) and 6′-Nitronaltrindole (8). A mixture of naltrexone hydrochloride (5.0 g, 12.7 mmol) and (3-nitrophenyl)hydrazine hydrochloride (2.58 g, 13.3 mmol) in methanol (50 mL) was stirred for 24 h. After evaporation of the solvent, the residue was treated with concentrated (37%) aqueous hydrochloric acid (25 mL) and then heated at 115 °C for 90 min. After cooling to ambient temperature, the mixture was carefully poured into a mixture of $NaHCO_3$ (30 g) and water (300 mL). After foaming had subsided, the mixture was extracted with 25% ethanol–CH_2Cl_2 (5 × 100 mL). The combined extracts were dried (Na_2SO_4) and the solvents were evaporated. The residue was purified by flash chromatography (130 g of silica gel) eluting with 4% triethylamine–3% methanol–CH_2Cl_2 (6 L) followed by 4% triethylamine–5% methanol–CH_2Cl_2 (1 L) to give, after triturating with ether, nitroindole 8 (4.74 g, 81%): 141–152 °C dec; $[\alpha]_D$ −187.5° (*c* = 0.30, CH_2Cl_2). Further elution with 4% triethylamine–7% methanol–CH_2Cl_2 (2 L) gave nitroindole 6 (1.11 g, 19%): dec above 220 °C; $[\alpha]_D$ −141.5° (*c* = 0.30, CH_2Cl_2).

6: ^{1}H NMR ($CDCl_3$) δ 0.1–0.25 (m, 2 H, cyclopropyl CH_2), 0.5–0.7 (m, 2 H, cyclopropyl CH), 0.75–0.95 (m, 1 H, cyclopropyl CH), 1.65–1.75 (m, 1 H, C-15 H), 2.2–2.5 (m, 4 H, C-15 H, C-16 H, and NCH_2-cyclopropyl), 2.6–2.95 (m, 3 H, C-8 H, C-10α H, and C-16 H), 3.0–3.15 (m, 2 H, C-8 H and C-10β H), 3.36 (d, *J* = 5.3 Hz, 1 H, C-9 H), 5.2 (br s, 2 H, movable, OH), 5.54 (s, 1 H, C-5 H), 6.53 (d, *J* = 8.0 Hz, 1 H, C-2 H), 6.62 (d, *J* = 7.9 Hz, 1 H, C-1 H), 6.65–6.8 (m, 2 H, C-6′ H and C-7′ H), 7.4–7.55 (m, 1 H, C-5′ H), 9.4 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 3.67, 4.26, 9.28, 22.95, 31.38, 31.43, 43.55, 47.11, 59.31, 61.64, 72.36, 84.35, 109.21, 116.99, 117.12, 117.44, 118.49, 119.37, 120.42, 125.25, 130.35, 133.14, 138.55, 138.66, 141.81, 142.62; FTIR (KBr) 3700–2800 (OH and NH), 1618, 1515, 1458, 1321 cm^{-1}; HREIMS calcd for $C_{26}H_{25}N_3O_5$ 459.1794, found 459.1796.

8: ^{1}H NMR ($CDCl_3$) δ 0.1–0.25 (m, 2 H, cyclopropyl CH_2), 0.45–0.65 (m, 2 H, cyclopropyl CH_2), 0.75–0.95 (m, 1 H, cyclopropyl CH), 1.50–1.65 (m, 1 H, C-15 H), 2.15–2.6 (m, 6 H, C-8 2H, C-15 H, C-16 H, and NCH_2-cyclopropyl), 2.6–2.7 (m, 1 H, C-16 H), 2.95 (dd, *J* = 6.0 and 17.6 Hz, 1 H, C-10α H), 3.12 (d, *J* = 18.9 Hz, 1 H, C-10β H), 3.32 (d, *J* = 5.9 Hz, 1 H, C-9 H), 5.49 (s, 1 H, C-5 H), 6.3–6.4 (m, 1 H, C-4′ H), 6.55–6.7 (m, 2 H, C-2 H and C-7′ H), 6.6 (br s, 1 H, movable, OH), 7.31 (d, *J* = 8.7 Hz, 1 H, C-5′ H), 9.3 (br s, 1 H, movable, NH); ^{13}C NMR ($CDCl_3$) δ 3.72, 4.16, 9.30, 22.91, 27.88, 31.16, 43.53, 47.76, 59.31, 61.81, 72.51, 83.71, 107.26, 110.49, 113.68, 117.54, 118.27, 119.74, 125.45, 129.84, 130.48, 134.32, 135.03, 139.03, 141.94, 143.61; FTIR (KBr) 3700–2800 (OH and NH), 1592, 1508, 1471, 1379, 1323 cm^{-1}; HREIMS calcd for $C_{26}H_{25}N_3O_5$ 459.1794, found 459.1796.

7′-Nitronaltrindole (9). Naltrexone hydrochloride (377 mg, 1.0 mmol) and (2-nitrophenyl)hydrazine (153 mg 1.0 mmol) were dissolved in 1:1 mixture of hydrochloric acid and glacial acetic acid (10 mL). The resulting solution was heated at 85 °C for 6 h, cooled, basified with $NaHCO_3$, and extracted with ethyl acetate (3 × 100 mL). The combined organic extract was dried over Na_2SO_4 and after removal of solvent gave solid residue. The 7′-nitroindole 9 was purified by flash chromatography (silica gel) using gradient elution ($CHCl_3$–MeOH, 90:10 to 80:20) to give nitroindole 9 (232 mg, 50%): mp 200 °C dec; R_f 0.54 (silica gel, $CHCl_3$–MeOH–NH_4OH, 9:1:0.5); ^{1}H NMR (DMSO-d_6) δ 9.0 (brs, 1 H, >NH), 8.33 (s, 1 H, OH), 8.12 (d, 1 H, H-6′), 8.08 (d, 1 H, H-4′), 7.17 (m, 1 H, H-5′), 5.60 (s, 1 H, H-5); EIMS *m*/*z* 459 (M^+). The free base was converted to its hydrochloride salt, mp 230 °C dec. Anal. ($C_{26}H_{25}N_3O_5$·HCl·3H_2O) C, H, N.

4′-Aminonaltrindole (10). A mixture of nitroindole 6 (400 mg, 0.87 mmol) and 10% palladium on carbon (40 mg) in methanol (35 mL) was treated with hydrogen gas at 50 psi on a Parr

(22) Portoghese, P. S.; Sultana, M.; Nagase, H. S.; Takemori, A. E. A Highly Selective δ_1 Opioid Receptor Antagonist: 7-Benzylidenenaltrexone (BNTX). *Eur. J. Pharmacol.* **1992**, *218*, 195–196.

apparatus for 17 h and then filtered through a 1-in. pad of Celite under suction and under a stream of argon. The solids were rinsed with methanol (50 mL), and the combined filtrates were evaporated to give **10** (380 mg, 100%) as a white powder: dec above 230 °C; $[\alpha]_D$ −57.0° (*c* = 0.30, methanol); ^{1}H NMR (methanol-d_4) δ 0.3–0.4 (m, 2 H, cyclopropyl CH_2), 0.6–0.75 (m, 2 H, cyclopropyl CH_2), 0.95–1.05 (m, 1 H, cyclopropyl CH), 1.7–1.85 (m, 1 H, C-15 H), 2.45–3.35 (m, 9 H, C-8 2 H, C-10 2 H, C-15 H, C-16 2 H, and NCH_2-cyclopropyl), 3.79 (d, *J* = 5.5 Hz, 1 H, C-9 H), 5.61 (s, 1 H, C-5 H), 6.27 (d, *J* = 6.6 Hz, 1 H, C-5′ H), 6.60 (s, 2 H, C-1 H and C-2 H), 6.75 (d, *J* = 7.8 Hz, 1 H, C-7′ H), 6.83 (t, *J* = 7.8 Hz, 1 H, C-6′ H); ^{13}C NMR (methanol-d_4) δ 6.02, 7.91, 10.68, 26.88, 33.31, 34.59, 48.67, 50.19, 61.76, 65.78, 87.85, 106.16, 108.51, 111.72, 119.85, 121.06, 122.35, 126.38, 130.85, 133.43, 142.30, 143.69, 144.19, 146.93; FTIR (KBr) 3700–2600 (NH and OH), 1618, 1508, 1459 cm^{-1}; HREIMS calcd for $C_{26}H_{27}N_3O_3$ 429.2052, found 429.2060.

5′-Aminonaltrindole (11). To a stirred solution of 5′-nitronaltrindole[23] (**7**) (459 mg, 1.0 mmol) and Raney nickel (1.0 g) in ethanol (25 mL) at 25 °C was added hydrazine hydrate (0.27 g, 6.0 mmol) dropwise under argon. After stirring for 30 min, the solution was filtered carefully. The filtrate was then evaporated to give a residue which was taken into water (20 mL) and extracted with ethyl acetate (3 × 25 mL). The combined organic extract was dried over Na_2SO_4 and evaporated to give a residue which was triturated in ether (5 mL) and filtered to afford an amorphous solid; the solid was crystallized from ethanol to give the free base **11**, which was converted to its hydrochloride salt (300 mg, 70%): mp 250 °C dec; R_f 0.38 (silica gel, $CHCl_3$–MeOH–NH_4OH, 9:1:0.1); ^{1}H NMR (DMSO-d_6) δ 7.05 (d, 1 H, H-7′), 6.7 (d, 1 H, H-6′), 6.6 (s, 1 H, H-4′), 6.5 (dd, 2 H, H1 and H2) 5.5 (s, 1 H, H-5); FABMS *m/z* 430 ($M^+ + H^+$). Anal. ($C_{26}H_{27}N_3O_3$·HCl·H_2O) C, H, N.

6′-Aminonaltrindole (12). A mixture of nitroindole **8** (710 mg, 1.55 mmol) and 10% palladium on carbon (70 mg) in a 10% aqueous acetic acid (50 mL) was treated with hydrogen gas at 50 psi on a Parr apparatus for 30 min and then filtered through a 1-in. pad of Celite under suction. The solids were rinsed with 5% aqueous acetic acid solution (100 mL). The combined filtrates were poured carefully over $NaHCO_3$ (15 g), and then the mixture was extracted with 25% ethanol–CH_2Cl_2 (4 × 50 mL). The combined extracts were dried ($MgSO_4$) and then filtered through a 1.5-in. column of silica gel under suction. The solids were rinsed with ethanol (250 mL), and then the combined filtrates were evaporated to give aminoindole **12** (500 mg, 75%) as a white powder: dec, above 220 °C; $[\alpha]_D$ −99.0° (*c* = 0.30, 50% ethanol-CH_2Cl_2); ^{1}H NMR (methanol-d_4) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.45–0.6 (m, 2 H, cyclopropyl CH_2), 0.8–0.95 (m, 1 H, cyclopropyl CH), 1.6–1.7 (m, 1 H, C-15 H), 2.15–2.85 (m, 8 H, C-8 2 H, C-10α H, C-15 H, C-16 2 H, and NCH_2-cyclopropyl) 3.10 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.33 (d, *J* = 6.1 Hz, 1 H, C-9 H), 5.53 (s, 1 H, C-5 H), 6.49 (dd, *J* = 1.9 and 8.3 Hz, 1 H, C-5′ H), 6.51 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.55 (d, *J* = 8.1 Hz, 1 H, C-2 H), 6.66 (d, *J* = 1.7 Hz, 1 H, C-7′ H), 7.12 (d, *J* = 8.3 Hz, 1 H, C-4′ H); ^{13}C NMR (methanol-d_4) δ 4.16, 4.65, 10.16, 24.07, 29.87, 32.59, 44.86, 60.27, 63.48, 74.38, 86.34, 98.50, 111.23, 111.37, 118.10, 119.46, 119.84, 121.74, 125.84, 128.50, 132.14, 139.92, 140.89, 143.68, 144.55; FTIR (KBr) 3600–2600 (NH and OH), 1636, 1508, 1458 cm^{-1}; HREIMS calcd for $C_{26}H_{27}N_3O_3$ 429.2052, found 429.2060.

7′-Aminonaltrindole (13). A suspension of Raney nickel (1.0 g) in EtOH (200 mL) and **9** (459 mg, 1.0 mmol) was treated dropwise with an excess of hydrazine (200 mg, 6.0 mmol) and with stirring at room temperature. The reaction mixture was monitored by TLC, and when no starting material was left (1–1.5 h), the solvent was decanted and Raney nickel was washed several times with hot ethanol. The combined extracts were evaporated to give a residue which was taken into water and extracted with ethyl acetate (3 × 100 mL). The organic layer was concentrated in vacuo, and the solid was removed and converted to its hydrochloride salt (180 mg, 42%): mp 280 °C dec; R_f 0.27 (silica gel, $CHCl_3$–MeOH–NH_4OH, 9:1:0.5); NMR (DMSO-d_6) δ 6.34 (d, 1 H, H-6′), 6.55 (dd, 2 H, H-1 and H-2), 6.62 (m, 2 H, H-5′ and H-4′), 5.45 (s, 1 H, H-5); FABMS *m/z* 430 ($M^+ + H^+$). Anal. ($C_{26}H_{27}N_3O_3$·2HCl·H_2O) C, H, N.

5′-Isothiocyanatonaltrindole (2). To a stirred solution of 5′-aminonaltrindole (**11**) (429 mg, 1.0 mmol) in $CHCl_3$ (10 mL), water (10 mL), and $NaHCO_3$ (504 mg, 6.0 mmol) at 5 °C under argon was added freshly distilled thiophosgene (126 mg, 1.2 mmol) dropwise. The reaction mixture was allowed to return to room temperature and was stirred vigorously. When all the starting material had disappeared (1.5–2 h), the $CHCl_3$ layer was removed and washed successively with water (10 mL), $NaHCO_3$ solution (25%, 2 × 10 mL), and brine (10 mL). The $CHCl_3$ was then evaporated to give **11**, which was converted to its hydrochloride salt (180 mg, 38%): mp 230 °C dec; R_f 0.54 (silica gel, $CHCl_3$–MeOH–NH_4OH, 9:1:0.01); ^{1}H NMR (DMSO-d_6) δ 7.5 (s, 1 H, H-6′), 7.40 (d, 1 H, H-4′), 7.10 (d, 1 H, H-7′), 6.5 (dd, 2 H, H1 and H2), 5.5 (s, 1 H, H-5); FABMS *m/z* 471 (M^+). Anal. ($C_{27}H_{25}N_3O_3S$·HCl·3.5H_2O) C, H, N.

4′-Isothiocyanatonaltrindole (3). To a vigorously stirred mixture of aminoindole **10** (220 mg, 0.51 mmol) and $NaHCO_3$ (90 mg, 1.08 mmol) in CH_2Cl_2 (5.0 mL) was added thiophosgene (41 μL, 0.54 mmol) dropwise. After stirring vigorously for 6 h with an oil bubbler attached, the mixture was treated with half-saturated aqueous $NaHCO_3$ solution (20 mL) and then extracted with 25% EtOH–CH_2Cl_2 (3 × 25 mL). The combined extracts were dried ($MgSO_4$) and the volatiles were evaporated. The residue was triturated with ether (2 × 20 mL) to give isothiocyanate **3** (80 mg, 31%), which was converted into its hydrochloride salt (2-propanol–ether): dec > 240 °C; $[\alpha]_D$ −41.5° (*c* = 0.30, CH_2Cl_2); ^{1}H NMR of free amine ($CDCl_3$) δ 0.1–0.2 (m, 2 H, cyclopropyl CH_2), 0.55–0.65 (m, 2 H, cyclopropyl CH_2), 1.7–1.8 (m, 1 H, cyclopropyl CH), 2.2–2.5 (m, 4 H, C-15 H, C-16 H, and NCH_2-cyclopropyl), 2.68 (d, *J* = 16.4 Hz, 1 H, C-8 H), 2.7–2.8 (m, 1 H, C-16 H), 2.86 (dd, *J* = 6.6 and 18.7 Hz, 1 H, C-10α H), 3.13 (d, *J* = 16.2 Hz, 1 H, C-8 H), 3.15 (d, *J* = 18.6 Hz, 1 H, C-10β H), 3.37 (d, *J* = 6.2 Hz, 1 H, C-9 H), 5.61 (s, 1 H, C-5 H), 6.60 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.65 (d, *J* = 8.2 Hz, 1 H, C-2 H), 6.76 (d, *J* = 7.8 Hz, 1 H, C-7′ H), 6.83 (d, *J* = 6.8 Hz, 1 H, C-5′ H), 6.93 (t, *J* = 7.8 Hz, 1 H, C-6′ H), 8.40 (br s, 1 H, NH); ^{13}C NMR of free amine (acetone-d_6) δ 10.40, 10.51, 16.32, 30.05, 36.88, 38.54, 50.41, 54.55, 66.21, 69.72, 79.10, 90.88, 116.41, 118.53, 124.17, 124.23, 125.63, 128.58, 128.85, 129.15, 131.82, 137.98, 138.73, 139.74, 145.20, 146.85, 150.27; FTIR of free amine (neat) 3600–2800 (OH and NH), 2118 (NCS), 1618, 1502 cm^{-1}; HREIMS calcd for $C_{27}H_{25}N_3O_3S$ 471.1616, found 471.1610. Anal. ($C_{27}H_{25}N_3O_3S$·HCl·1.5H_2O) C, H, N.

6′-Isothiocyanatonaltrindole (4). To a vigorously stirred mixture of aminoindole **12** (930 mg, 2.17 mmol) and $NaHCO_3$ (400 mg, 4.8 mmol) in CH_2Cl_2 (15 mL), cooled in an ice bath, was added thiophosgene (180 μL, 2.4 mmol) dropwise. After 5 min the cooling bath was removed and the mixture was stirred vigorously for 18 h with an oil bubbler attached. The mixture was treated with half-saturated aqueous $NaHCO_3$ solution (50 mL) and then extracted with 25% EtOH–CH_2Cl_2 (2 × 25 mL). The combined extracts were washed with water (50 mL) and dried ($MgSO_4$), and the volatiles were evaporated to give **4**, which was converted to its hydrochloride salt (390 mg, 36%) (recryst ethanol–ether): dec above 220 °C; $[\alpha]_D$ −150.0° (*c* = 0.30, CH_2Cl_2); ^{1}H NMR of free amine (acetone-d_6) δ 0.1–0.3 (m, 2 H, cyclopropyl CH_2), 0.45–0.65 (m, 2 H, cyclopropyl CH_2), 0.85–1.0 (m, 1 H, cyclopropyl CH), 1.6–1.75 (m, 1 H, C-15 H), 2.2–2.45 (m, 2 H, C-15 H and C-16 H), 2.45 (d, *J* = 6.3 Hz, 2 H, NCH_2-cyclopropyl), 2.57 (d, *J* = 15.7 Hz, 1 H, C-8 H), 2.7–2.9 (m, 3 H, C-8 H, C-10α H, and C-16 H), 3.16 (d, *J* = 18.5 Hz, 1 H, C-10β H), 3.37 (d, *J* = 6.3 Hz, 1 H, C-9 H), 5.57 (s, 1 H, C-5 H), 6.53 (d, *J* = 8.2 Hz, 1 H, C-1 H), 6.60 (d, *J* = 8.1 Hz, 1 H, C-2 H), 6.90 (dd, *J* = 1.5 and 8.3 Hz, 1 H, C-5′ H), 7.24 (s,1 H, C-7′ H), 7.34 (d, *J* = 8.3 Hz,.1 H, C-4′ H), 10.43 (br s, 1 H, NH); ^{13}C NMR of free amine (acetone-d_6) δ 10.38, 10.56, 16.30, 29.93, 35.66, 38.55, 50.53, 54.87, 66.13, 69.40, 79.32, 91.19, 115.53, 118.32, 123.78, 124.09, 125.55, 126.47, 131.15, 131.86, 133.12, 138.01, 139.35, 143.52, 146.77, 150.33; FTIR of HCl salt (KBr) 3600–2500 (OH and NH), 2115 (NCS), 1624, 1501 cm^{-1}; HREIMS calcd for $C_{27}H_{25}N_3O_3S$ 471.1616, found 471.1617. Anal. ($C_{27}H_{25}N_3O_3S$·HCl), C, H, N.

7′-Isothiocyanatonaltrindole (5). A solution of **13** (214 mg, 0.5 mmol), in water (5 mL), $CHCl_3$ (15 mL) and $NaHCO_3$ (252

(23) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of Peptidomimetic δ Opioid Receptor Antagonists Using the Message–Address Concept. *J. Med. Chem.* **1990**, *33*, 1714–1720.

mg, 3.0 mmol) was stirred for 5 min and then treated with redistilled thiophosgene (0.063 mg, 0.6 mmol). After 1 h the $CHCl_3$ layer was removed and washed successively with dilute aqueous NH_4OH (2 × 10 mL) and water (2 × 15 mL), dried, and evaporated to give a residue which was purified on preparative TLC (silica gel, $CHCl_3$–MeOH–NH_4OH 90:10:0.1) to afford **5** (70 mg, 29%): mp 230 °C dec; R_f 0.51 (silica gel, $CHCl_3$–MeOH–NH_4OH 9:1:0.1 v/v); ^1H NMR (DMSO-d_6) δ 9.03 (s, 1 H, >NH), 8.32 (s, 1 H, OH), 7.43 (d, 1 H, H-6′), 7.42 (d, 1 H, H-4′), 6.94 (t, 1 H, H-5′), 6.52 (dd, 2 H, H1 and H2), 5.51 (s, 1 H, H-5); FABMS *m/z* 471. The free base was converted to its hydrochloride salt, mp 280 °C dec. Anal. ($C_{27}H_{25}N_3O_3S$·HCl·$3H_2O$), C, H, N.

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Registry No. **2**, 126876-64-0; **2**·HCl, 143680-17-5; **3**, 143619-50-5; **3**·HCl, 143680-18-6; **4**, 143619-51-6; **4**·HCl, 143680-19-7; **5**, 143619-52-7; **5**·HCl, 143680-20-0; **6**, 143619-53-8; **7**, 122431-09-8; **8**, 143619-54-9; **9**, 136349-13-8; **9**·HCl, 143680-21-1; **10**, 143619-55-0; **11**, 126898-43-9; **11**·HCl, 143680-22-2; **12**, 143619-56-1; **13**, 143619-57-2; **13**·HCl, 143680-23-3; $H_2NNHC_6H_4$-3-NO_2·HCl, 51516-96-2; $H_2NNHC_6H_4$-2-NO_2, 3034-19-3; naltrexone hydrochloride, 16676-29-2.