Electrophilic *N*-Benzylnaltrindoles as δ Opioid Receptor-Selective Antagonists

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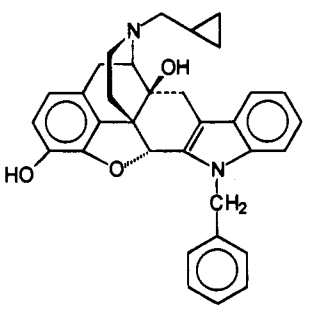
The *N*-benzyl group of *N*-benzylnaltrindole (**1**, BNTI), a potent and selective δ_2 opioid receptor antagonist, was employed as a scaffold to hold electrophilic moieties (isothiocyanate and haloacetamide) in an effort to obtain selective affinity labels (**2**–**4** and **8**–**11**). The corresponding acetamide derivatives (**5**–**7**) also were synthesized to serve as nonelectrophilic controls. The *o*- and *p*-isothiocyanates (**2** and **4**) and the haloamides (**8**–**11**) were selective δ opioid receptor antagonists in the mouse vas deferens (MVD) preparations, while the *meta* isomer **3** was a δ-selective full agonist (IC_{50} = 5 nM). The fact that the effect of **2** and **4** was found to increase as a function of time in MVD suggests a covalent mechanism for the wash resistant component. The *m*-isothiocyanate **3** was found to be a δ-selective and irreversible agonist in the MVD, and it is suggested that it may be covalently binding to an agonist recognition site. In the mouse abdominal stretch antinociceptive assay, compounds **2**–**4** and **9** were δ-selective antagonists but exhibited δ_2/δ_1 selectivity ratios lower than that of BNTI.

Introduction

With the recent cloning and sequencing of opioid receptors, it is now well established that there are at least three types of G protein-coupled receptors (μ, δ, and κ) that possess high sequence homology.[1] Electrophilic affinity labels have played a prominent role in the pharmacologic characterization of opioid receptors.[2] Among the affinity labels having nonequilibrium δ antagonist activity, naltrindole 5′-isothiocyanate (5′-NTII) and [D-Ala^2,Leu^5]enkephalin-Cys^6 (DALCE) have been used to investigate the putative δ_2 and δ_1 receptor subtypes.[3,4]

In an effort to expand the armamentarium of subtype-selective δ antagonists, and on the basis of the high δ_2 antagonist selectivity of *N*-benzylnaltrindole[5] (BNTI, **1**), we have synthesized a series of analogues that contain isothiocyanate (**2**–**4**) and haloacetamide (**8**–**11**) substituents on the *ortho*, *meta*, or *para* position of the benzyl group. Here we report on the pharmacologic selectivity of compounds in this series and compare the activities with the nonelectrophilic analogues **5**–**7**.



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Design Rationale and Chemistry

BNTI (**1**) was recently reported to be a potent and selective δ opioid receptor antagonist which displays δ_2 opioid receptor antagonism *in vivo*.[5] We have employed BNTI as a lead compound in order to develop affinity labels with δ opioid receptor selectivity. In order to cover a range of reactivity and chemical selectivity, the isothiocyanate and haloacetamide groups were considered for attachment to the benzyl group at the *ortho*, *meta*, or *para* position. The rationale for preparing regioisomers was based on the hypothesis that different receptor subtypes may possess different reactivity with the electrophilic group of the affinity labels.[6] This could occur if only one of the subtypes contains a receptor-based nucleophile in close proximity to the electrophilic group of a reversibly bound regioisomer. Such differentiation may be the basis for the δ_2 selectivity of 5′-NTII.[7]

The choice of electrophilic groups was based on their chemical reactivity. Isothiocyanates react readily with the amino and thiol groups of amino acid residues, while bromoacetamides and iodoacetamides react with a broader spectrum of nucleophiles that include amino, thiol, hydroxyl, and carboxylate. The acetamides **5**–**7** were synthesized to serve as the nonelectrophilic analogues of the haloacetamides **8**–**11**.

Chemistry

Target compounds **2**–**11** were synthesized from the previously reported[5] *o*-, *m*-, and *p*-aminobenzyl derivatives **12**–**14** (Scheme 1). Isothiocyanates **2**–**4** were prepared from compounds **12**–**14** using thiophosgene in the presence of sodium bicarbonate in a chloroform–water mixture at room temperature. Purification of the reaction mixture using dry column chromatography worked well on a small scale (0.25 mmol) but led to the formation of impurities when done on a larger scale because of the longer time required to conduct the chromatographic purification (4–5 h).

The acetamides **5**–**7** were prepared by reacting the amines **12**–**14** with acetic anhydride. Any diacetylated products, **15**, which were formed were converted to the desired acetamides by methanolysis. Bromoacetamides **8**–**10** were obtained by acylation of **12**–**14** with bromoacetyl bromide. An earlier attempt to synthesize the

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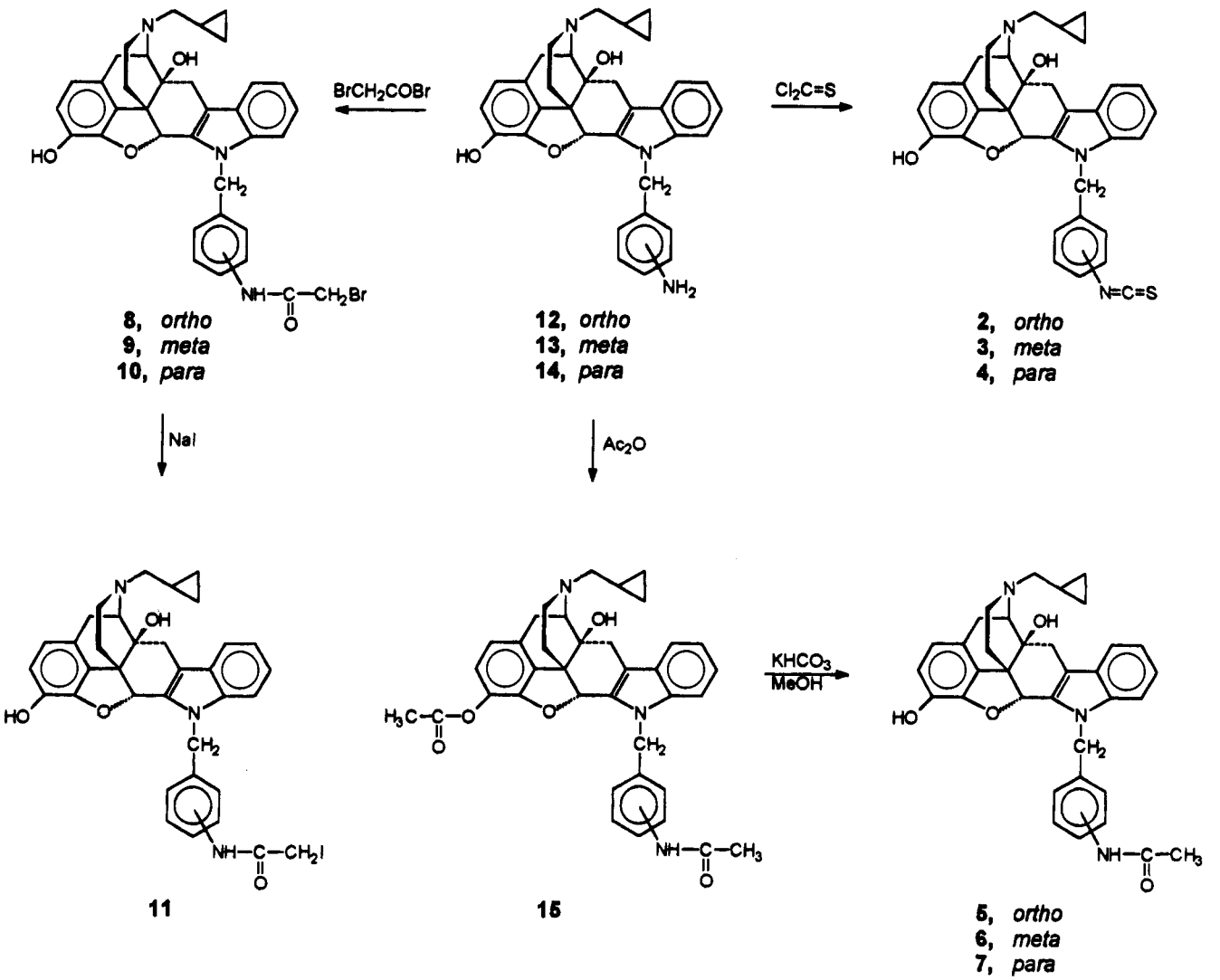
Scheme 1



p-bromoacetamide **10** from the amine **14** and bromoacetic acid in the presence of dicyclohexylcarbodiimide and 1-hydroxy-1*H*-benzotriazole (HOBt) resulted in the formation of **16** and the HOBt conjugate **17**. Compound **16** may have been formed by the reaction of **17** with the parent amine **14**. Alternatively, **16** could have been formed from nucleophilic attack by amine **14** on bromoacetamide **10**. The *p*-iodoacetamide **11** was synthesized from **10** using excess sodium iodide in THF.

Pharmacological Results

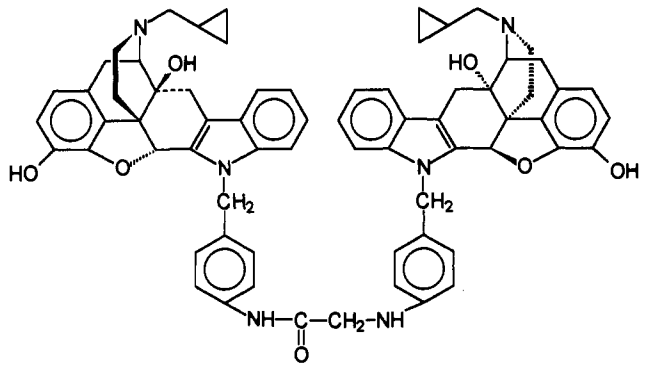
Smooth Muscle Experiments. The opioid agonist and antagonist potencies of the target compounds and their precursors were determined on the electrically stimulated guinea pig ileum[8] (GPI) and mouse vas deferens[9] (MVD) preparations (Table 1). The antagonist potencies were determined by employing morphine (M), ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin[10] (DADLE) as μ-, κ- and δ-selective agonists, respectively. Opioid antagonism is expressed as an IC_{50} ratio, which is the IC_{50} of agonist in presence of antagonist divided by the control IC_{50}. Agonist IC_{50} values were obtained either directly after incubation with target compound without washing or after five washes with buffer. The incubation times of the target compounds ranged from 10 to 60 min.

All target compounds (100 nM), with the exception of the isothiocyanate **3**, were inactive as agonists in these tissue preparations. However, at 1 μM some of the compounds displayed weak partial agonist activity, with up to 50% agonism in the MVD. Compounds **2** and **4** exhibited relatively good δ opioid antagonism, with little or no activity at μ and κ receptors (Table 1).

Time-dependent studies involving incubation of the ligands with the MVD were carried out in an effort to distinguish between possible entrapment and covalent binding to the receptor. If indeed the ligand was involved in a covalent bond formation with the receptor, a time-dependent increase in IC_{50} ratio would be expected. However, if the ligand were to bind reversibly, there should be little change in the IC_{50} ratio after 15 min, as equilibrium is attained within that period.

It was found that the IC_{50} ratios of both the *o*- and *p*-isothiocyanates **2** and **4** exhibited a time-dependent increase in the postwash IC_{50} ratio, suggesting that the isothiocyanate moiety may have reacted covalently with a receptor nucleophile. The postwash IC_{50} ratio of **2** in the MVD is shown in Figure 1.

The *m*-isothiocyanate **3** was unique in that it was found to be a full agaonist at δ opioid receptors with a potency one-tenth that of DADLE (Figure 2). The agonism of **3** was not reversed by washing with either



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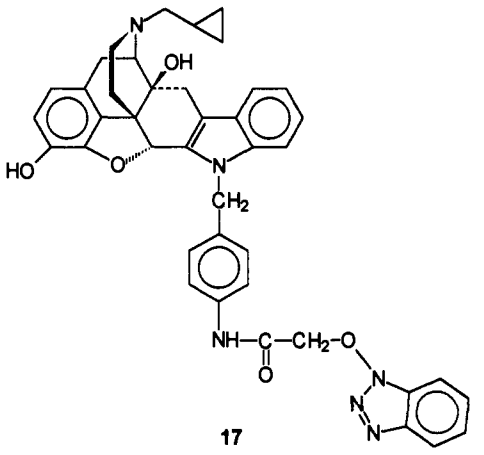

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naltrexone (500 nM) or Naltrindole[11] (NTI) (500 nM). However, when the agonist effect curve of **3** was determined in the presence of NTI (20 nM), the agonist dose–response curve was shifted by a factor of 40 to higher concentration. Consequently, it is likely that **3** acts at the same receptor as NTI. In the GPI compound, **3** showed no agonism or antagonism at 1 μM.

Among the haloacetamide derivatives **8**–**11**, the *m*-bromoacetamide **9** was the most potent δ antagonist, with an IC_{50} ratio (511) at least 10-fold greater than other members in this group. However, it was found that a substantial fraction of the antagonism of the bromoacetamides **8**–**10** either could be removed upon washing or exhibited no apparent time dependence. This suggests that the antagonist effect did not involve a significant amount of covalent association with the receptor within the time frame of the experiment. The *o*-iodoacetamide **11** exhibited wash resistant antagonism, but it did not appear to be time-dependent. This is consistent with a noncovalent localization of **11** in the membrane, as no time-dependent increase of the antagonist effect was observed.

Nonelectrophilic analogues **5**–**7** corresponding to the haloacetamides **8**–**11** were evaluated for comparison purposes. Like the bromoacetamides, washing the treated preparation afforded lower IC_{50} ratios and no substantial wash resistant antagonism. In this connection, it appeared that the antagonism produced by *m*-acetamide **6** was not removed by washing as easily as **5** or **7**. It appears likely that residual **6** may give rise to this persistent antagonism.

***In Vivo* Studies.** The isothiocyanate regioisomers **2**–**4** and the *m*-bromoacetamide **9** were evaluated for δ antagonist activity in mice using the abdominal stretch assay as described previously.[11] The antinociception produced by the δ_1 agonist [D-Pen^2,D-Pen^5]enkephalin[13,14] (DPDPE) and the δ_2 agonist [D-Ser^2,Leu^5] enkephalin-Thr^{6,10,14} (DSLET) was determined 1.5 and 24 h after the mice were pretreated (5 nmol icv) with the test compounds. The results were expressed as ED_{50} ratios, which represent the ED_{50} of the pretreated divided by the ED_{50} of the control (Table 2).

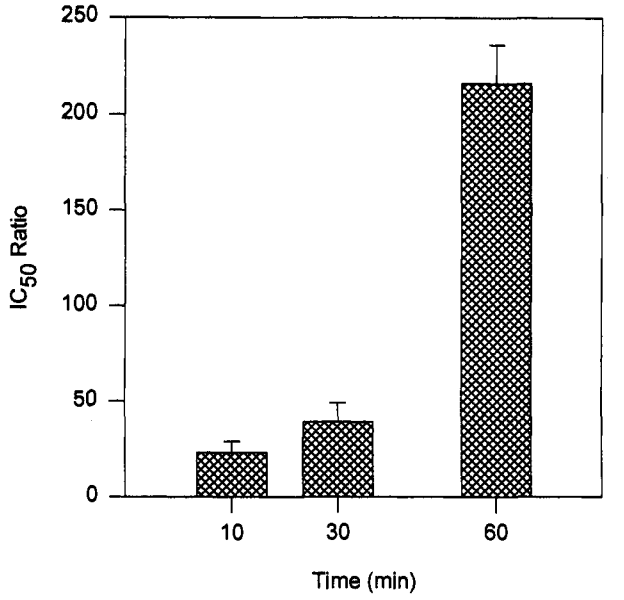


Figure 1. Time course of antagonism of DADLE by *o*-isothiocyanate **2** (100 nM) in the MVD.

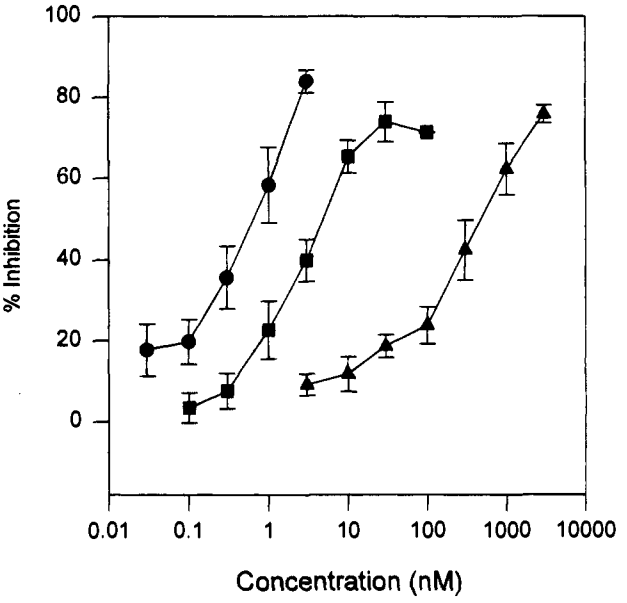
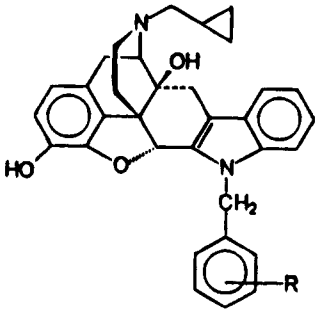


Figure 2. Dose–response curves of DADLE control (●), *m*-isothiocyanate **3** (■), and **3** in presence of NTI (▲) in the MVD. The preparation was incubated with NTI (20 nM) for 15 min prior to testing of **3**.

All of the compounds were more potent as antagonists 24 h after pretreatment compared to 1.5 h. This was particularly evident for the antagonism of DSLET which generally exhibited substantially higher ED_{50} ratios relative to those of DPDPE. The *o*-thiocyanate **2** possessed the highest DSLET ED_{50} ratio (8.8 at 24 h) which was comparable to that of BNTI (**1**) (**11.1**). However, unlike BNTI, **2** appeared not to differentiate between the δ receptor subtypes as reflected by the low δ_2/δ_1

Table 1. Opioid Antagonist Potencies of *N*-Benzyl-NTI Analogues in the MVD and GPI Preparations



		IC_{50} ratio[a]		selectivity ratio[b]			
compd	R	DADLE (δ)	incubation time (min)	M (μ)	EK (κ)	δ/μ	δ/κ
1 (BNTI)	H	208 ± 28	30[c]	1.6 ± 0.76	0.4 ± 0.08	131	208
2	o-NCS	57 ± 16	30[c]	0.6 ± 0.13	1.3 ± 0.3	57	45
		23 ± 5.9	10[d]				
		39 ± 10	30[d]				
		216 ± 20	60[d]				
3	*m*-NCS	agonist[e]		0.8 ± 0.2	1.2 ± 0.6		
4	*p*-NCS	18 ± 3.7	30[c]	1.3 ± 0.4	0.6 ± 0.2	25	18
		6.7 ± 1.0	10[d]				
		20 ± 5.6	30[f]				
5	o-$NHCOCH_3$	54 ± 14.2	30[c]	1.9 ± 0.6	1.3 ± 0.3	29	42
		10 ± 1.9[d]	30[d]				
6	*m*-$NHCOCH_3$	33 ± 8.1	30[c]	5.3 ± 1.5	0.9 ± 0.2	6.2	33
		16 ± 6.7	10[d]				
		18 ± 6.4	30[d]				
7	*p*-$NHCOCH_3$	31 ± 6.1	30[c]	0.9 ± 0.2	0.2 ± 0.05	31	31
		1.6 ± 0.92	10[d]				
		3.1 ± 0.94	30[d]				
		7.1 ± 2.25	60[d]				
8	o-$NHCOCH_2Br$	35 ± 9.4	30[c]	1.7 ± 0.3	1.1 ± 0.3	21	33
		13 ± 2.4	30[d]				
9	*m*-$NHCOCH_2Br$	511 ± 115	30[c]	3.8 ± 0.9	0.9 ± 0.4	135	511
		44 ± 13.4	10[d]				
		51 ± 19.0	30[d]				
10	*p*-$NHCOCH_2Br$	30 ± 6.8	30[c]	0.7 ± 0.1	0.9 ± 0.1	30	30
		3.6 ± 0.57	10[d]				
		12 ± 5.1	30[d]				
		1.4 ± 0.16	60[d]				
11	o-$NHCOCH_2I$	50.7 ± 9.0	30[c]	0.7 ± 0.3	0.5 ± 0.2	51	51
		51.2 ± 12.6	10[d]				
		25.5 ± 6.6[d]	30[d]				

[a] IC_{50} ratios were determined in the presence of 100 nM antagonist after a 30 min incubation. [b] Selectivity ratios were calculated using the value 1 when IC_{50} ratios were <1. [c] IC_{50} ratio determined without washing (prewash). [d] Preparation was washed (5×) with buffer after incubation with antagonist. [e] Showed δ agonism with about 0.1 times the potency of DADLE (Figure 2). [f] Postwash (40×) after incubation with naltrexone (500 nM, 5 min).

selectivity ratio. This also was the case for other members of the series. It is noteworthy that the *m*-isothiocyanate **3**, which was a potent agonist in the MVD, exhibited δ antagonism and failed to show any agonistic effect.

Binding Studies. Opioid receptor binding of selected compounds was determined by a radioligand competition assay in guinea pig brain membranes using a modification of the method of Werling et al.[16] Binding to κ receptors was evaluated with [^{3}H]U69593,[17] to μ receptors with [^{3}H]DAMGO, and δ receptors with [^{3}H]-DPDPE (δ_1) or [^{3}H]DSLET (δ_2) in the presence of 100 nM [D-Ala^2,MePhe^4,Gly-ol^6]enkephalin[17] (DAMGO). The binding data are expressed as K_i values (Table 3).

Receptor binding qualitatively was in agreement with the prewash δ selectivities of ligands in the smooth muscle preparations (Table 1). While all of the ligands were δ-selective, there were little or no differences in binding between δ_1 and δ_2 receptor subtypes. Bromoacetamide **9**, which was the most potent and selective compound in the smooth muscle preparations, showed the highest affinity for δ_1 and δ_2 opioid receptor subtypes. Its binding affinity was comparable to that of NTI.[11] The isothiocyanate **3**, which was found to be an agonist in the smooth muscle preparations, also showed good binding affinity for δ opioid receptors.

Table 2. Antagonism by *N*-Benzyl-NTI Derivatives of the Antinociceptive Effect of icv-Administered DSLET and DPDPE in Mice

	time	ED_{50} ratio		δ_2/δ_1 selectivity
antagonist	(h)	DSLET	DPDPE	ratio[a]
1[b] (BNTI)	1.5	3.2 (1.5–8.6)	1.0 (0.5–2.0)	3.2
	24	11.1 (5.9–18.2)	1.5 (0.9–3.1)	7.4
2	1.5	1.4 (0.7–4.4)	1.1 (0.6–4.4)	1.3
	24	8.8 (5.6–14.1)	19.4 (10–43)	0.5
3	1.5	1.9 (1.5–2.4)	1.1 (0.7–2.1)	1.7
	24	4.1 (1.7–9.9)	3.5 (2.1–6.2)	1.2
4	1.5	3.8 (2.0–8.3)	1.1 (0.5–2.8)	3.5
	24	4.2 (3.0–6.0)	2.5 (0.9–11)	1.7
9	1.5	2.4 (1.9–3.2)	1.0 (0.6–2.0)	2.4
	24	6.9 (5.5–8.7)	2.8 (1.3–9.8)	2.5

[a] DSLET ED_{50} ratio divided by DPDPE ED_{50} ratio. [b] Data from ref 5.

Table 3. Opioid Receptor Binding of *N*-Benzyl-NTI Analogues

	K_i[a] (nM)				selectivity ratio	
compd	[^{3}H]DPDPE (δ_1)	[^{3}H]DSLET (δ_2)	[^{3}H]DAMGO (μ)	[^{3}H]U69593 (κ)	μ/δ_1	κ/δ_1
2	2.0	3.6	151	615	76	308
3	4.2	7.0	66.2	104	16	5
4	5.3	5.0	289	245	55	46
9	0.019	0.038	15.3	8.8	805	463

[a] K_i values represent the geometric mean of at least three replicate determinations.

Discussion

On the basis of the reported high δ antagonist potency and selectivity of BNTI[5] (**1**) *in vitro* and *in vivo*, we have synthesized a series of compounds (**2**–**4** and **8**–**11**) containing electrophilic substituents on the benzyl group. All but one (**3**) of the ligands in this series exhibited δ-selective antagonist activity in smooth muscle preparation. This one exception was discovered to be a potent δ-selective agonist.

It was found that the *in vitro* activities of the isothiocyanates **2**–**4** were retained after washing the MVD preparation. Moreover, the time-dependent, wash resistant blockage of **2** and **4** in the MVD preparation suggested that the postwash antagonism may be due to covalently bound δ receptor.

Since the *in vitro* agonistic effect of **3** was blocked by pretreatment with naltrindole and could not be reversed on washing with a solution of naltrexone, it appears that covalent binding to δ receptors may have been involved in the agonism. Conceivably, the *m*-isothiocyanate **3** might have reacted with a receptor-based nucleophile at an agonist recognition site that is inaccessible to its regioisomers. Accordingly, the *ortho* and *para* isomers (**2** and **4**) might have convalently bound to an antagonist recognition site. The presence of discrete agonist and antagonist recognition sites have been suggested from studies with mutant δ opioid receptors.[19]

Similar *in vitro* testing of the bromoacetamides **8**–**10** revealed that they also were selective δ antagonists, but this effect was largely reversible. These data suggest that none of the bromoacetamides were involved in covalent binding to δ receptors. The fact that their washout characteristics were somewhat similar to those of the nonelectrophilic acetamides **5**–**7** is consistent with this suggestion. The iodoacetamide **11** differed from the isothiocyanates in that it was wash resistant. However, the absence of a clear time-dependent blockage of δ receptors is consistent with either rapid alkylation of receptors or slow removal on washing.

The *in vivo* pharmacologic profiles of the *o*- and *m*-isothiocyanates **2** and **3** and the bromoacetamide **9** were not unlike that of BNTI (**1**), in that they antagonized DSLET-induced antinociception more effectively at 24 h than at 90 min after icv administration. However, the 24 h δ_2/δ_1 selectivity ratios were substantially lower than that of BNTI. Interestingly, the agonistic effect observed for the *meta* isomer **3** in the MVD preparation was not seen *in vivo*. One possible explanation for this discrepancy may be different δ receptor subtypes. A similar relationship has been reported for other NTI-related ligands.[20] However, the question remains as to why an apparently minor modification such as moving an *o*- or *p*-isothiocyanate group to the *meta* position should produce a profound change from antagonism to agonism *in vitro*.

Experimental Section

General. Melting points were determined in open capillary tubes with a Thomas Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of the theoretical values. ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on an IBM Bruker, AC-300, AC-200, or GE-300 spectrometer, and the chemical shifts are recorded as δ values (ppm) relative to TMS. Mass spectra were obtained on a Finnigan 4000, VG 7070-HF, or AEI MS-30 instrument. IR spectra were obtained from samples in KBr pellets from a Nicolet 5DXC FT-IR spectrometer, and peak positions are expressed as cm^{-1}. Column chromatography was carried out on silica gel 60 (230–400 mesh) from E. Merck. HPLC separations were performed on a Beckman model 110-A system using a 10 μm (Rsil, Alltech) semipreparative (10 mm × 250 mm) silica gel column or a 5 μm (Ultrasphere, Beckman) analytical (4.6 mm × 250 mm) silica gel column. All TLC data were determined using plastic-backed sheets with silica gel, Machery Nagel, and the eluent $CHCl_3$–MeOH–NH_4OH (CMA). All reagents and solvents were reagent grade and used without further purification. All chemicals were obtained from Aldrich Chemical Co. Naltrexone hydrochloride was a generous gift from Mallinckrodt Chemical Works.

Hydrochloride salts were prepared by precipitating from the solutions of the free bases in ethyl acetate/methanol mixtures by the addition of ethyl acetate–HCl or methanolic HCl followed by filtering and drying under high vacuum. Methanesulfonate salts were prepared in an analogous fashion by addition of methanesulfonic acid diluted with ethyl acetate.

Chemistry. General Procedure: 17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*o*-, *m*-, or *p*-isothiocyanatobenzyl)indolo[6,7:2′,3′]morphinan (2, 3, or 4). A solution of compound **12**, **13**, or **14** (130 mg, 0.25 mmol) and sodium bicarbonate (126 mg, 1.5 mmol) in $CHCl_3$ (7.5 mL) and water (2.5 mL) was stirred for 10 min at room temperature and then treated with redistilled thiophosgene (0.02 mL, 0.3 mmol). The progress of the reaction, which was followed by TLC, was essentially complete in 3 h. Chloroform was removed from the aqueous layer and washed successively with 10% aqueous NH_4OH (2 × 10 mL) and water (2 × 15 mL). The chloroform phase was dried over sodium sulfate and evaporated to give a solid product. This solid contained trace impurities which were removed by dry column chromatography (CA, 100:1).

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*o*-isothiocyanatobenzyl)indolo[6,7:2′,3′]morphinan (2): yield 120.6 mg, 86%; mp (HCl) >230 °C dec; TLC R_f 0.76 (CMA, 99:1:1); ^{1}H NMR (DMSO-d_6) δ 7.566 (d, J = 7.9 Hz, 1 H, Ar), 7.451 (d, J = 7.53 Hz, 1 H, Ar), 7.33 (t, J = 7.3 Hz, 1 H, Ar), 7.27 (d, J = 8.2 Hz, 1 H, Ar), 7.16–6.99 (m, 3 H, Ar), 6.53 (s, 2 H, H_1 & H_2), 6.445 (d, J = 7.8 Hz, 1 H, Ar), 5.65 (dd, J = 17.6 Hz, 2 H, benzylic protons), 5.61 (s, 1 H, H_5); IR (cm^{-1}) 2088 (NCS group); FAB MS *m/z* 562 $(MH)^+$, 560 $(M - H)^-$. Anal. ($C_{34}H_{31}N_3O_3S$·HCl) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*m*-isothiocyanatobenzyl)indolo[6,7:2′,3′]morphinan (3): yield 103 mg, 73.5%; mp >225 °C dec; TLC R_f 0.68 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 9.04 (s, 1 H, phenolic OH), 7.42 (d, J = 7.7 Hz, 1 H, Ar), 7.23–7.33 (m, 5 H, Ar), 7.09 (t, J = 7.5 Hz, 1 H, Ar), 6.99 (t, J = 7.3 Hz, 1 H, Ar), 6.62–6.56 (m, 2 H, H_1 & H_2), 5.74 (s, 1 H, H_5), 5.55 (dd, J = 16.9 Hz, 2 H, benzylic protons); IR (cm^{-1}) 2113 (NCS group); FAB MS *m/z* 562 $(MH)^+$, 560 $(M - H)^-$. Anal. ($C_{34}H_{31}N_3O_3S$·HCl) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*p*-isothiocyanatobenzyl)indolo[6,7:2′,3′]morphinan (4): yield 105.3 mg, 75%; mp >225 °C dec; TLC R_f 0.51 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 8.94 (s, 1 H, phenolic OH), 7.4 (d, J = 8.1 Hz, 1 H, Ar), 7.28–7.34 (m, 4 H, Ar), 7.22 (d, J = 8.0 Hz, 1 H, Ar), 7.06 (t, J = 7.3 Hz, 1 H, Ar), 6.97 (t, J = 7.0 Hz, 1 H, Ar), 6.50–6.56 (m, 2 H, H_1 & H_2), 5.66 (s, 1 H, H_5), 5.53 (dd, J = 17.4 Hz, 2 H, benzylic protons); IR (cm^{-1}) 2073, 2178 (isothiocyanate); FAB MS *m/z* 560 $(M - H)^-$. Anal. ($C_{34}H_{31}N_3O_3S$·HCl) C, H, N.

General Procedure: 17-(Cyclopropylmethyl)-6,7-didehydro-4,5-α-epoxy-3,14-dihydroxy-1′-(*o*-, *m*-, or *p*-acetamidobenzyl)indolo[6,7:2′,3′]morphinan (5, 6, or 7). The amine **12**, **13**, or **14** (130 mg, 0.25 mmol) was dissolved in $CHCl_3$ (2 mL) and stirred under nitrogen for 5 min at 23 °C followed by the addition of acetic anhydride (51 mg, 0.50 mmol). Disappearance of the starting amine was monitored by TLC. The reaction was completed in 1.5 h, and the products consisted of the desired acetamides **5**, **6**, or **7** and the diacetylated product **15**. The latter was converted to the corresponding acetamide by dissolving it in methanol containing potassium bicarbonate (6 equiv) and stirring overnight at room temperature. The pure product **5**, **6**, or **7** was obtained by performing dry column chromatography (CMA, 98:2:1).

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*o*-acetamidobenzyl)indolo[6,7:2′,3′]morphinan (5): yield 128 mg, 91.5%; mp (HCl) >240 °C dec; TLC R_f 0.18 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 9.0 (s, 1 H, phenolic OH), 7.432 (d, J = 7.7 Hz, 1 H, Ar), 7.333 (d, J = 7.9 Hz, 1 H, Ar), 7.21 (t, J = 7.5 Hz, 1 H, Ar), 7.1–6.97 (m, 4 H, Ar), 6.6–6.5 (m, 2 H, H_1 & H_2), 6.37 (d, J = 7.7 Hz, 1 H, Ar), 5.51 (s, 1 H, H_5), 5.44 (dd, J = 17.4 Hz, 2 H, benzylic protons), 2.17 (s, 3 H, acetamide methyl protons); IR (cm^{-1}) 1667 (amide carbonyl); FAB MS m/z 562 $(MH)^+$, 560 $(M - H)^-$. Anal. ($C_{35}H_{35}N_3O_4$·HCl·1.75H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*m*-acetamidobenzyl)indolo[6,7:2′,3′]morphinan (6): yield 136 mg, 97.0%; mp >200 °C dec; TLC R_f 0.26 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 8.98 (s, 1 H, phenolic OH), 7.53 (s, 2 H, Ar), 7.41 (d, J = 7.62 Hz, 1 H, Ar), 7.33 (d, J = 8.27 Hz, 1 H, Ar), 7.20 (t, J = 8.0 Hz, 1 H, Ar), 7.10 (t, J = 7.6 Hz, 1 H, Ar), 6.98 (t, J = 7.4 Hz, 1 H, Ar), 6.57–6.51 (m, 2 H, H_1 & H_2), 5.58 (s, 1 H, H_5), 5.50 (dd, J = 16.6 Hz, 2 H, benzylic protons), 2.03 (s, 3 H, acetamide methyl protons); IR (cm^{-1}) 1669 (amide carbonyl); FAB MS m/z 562 $(MH)^+$, 560 $(M - H)^-$. Anal. ($C_{35}H_{35}N_3O_4$·HCl·1.25 H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*p*-acetamidobenzyl)indolo[6,7:2′,3′]morphinan (7): yield 130 mg, 92.5%; mp >200 °C dec; TLC R_f 0.25 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 9.89 (s, 1 H, acetamide NH), 9.02 (s, 1 H, phenolic OH), 7.48 (d, J = 8.3 Hz, 2 H, Ar), 7.38 (d, J = 7.8 Hz, 1 H, Ar), 7.26 (d, J = 8.2 Hz, 1 H, Ar), 7.22 (d, J = 8.4 Hz, 2 H, Ar), 7.04 (t, J = 7.4 Hz, 1 H, Ar), 6.94 (t, J = 7.4 Hz, 1 H, Ar), 6.57–6.48 (m, 2 H, H_1 & H_2), 5.62 (s, 1 H, H_5), 5.42 (dd, J = 16.3 Hz, 2 H, benzylic protons), 2.0 (s, 3 H, acetamide methyl protons); IR (cm^{-1}) 1671 (amide carbonyl); EIMS m/z 561 (M^+). Anal. ($C_{35}H_{35}N_3O_4$) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*o*-, *m*-, or *p*-bromoacetamido)indolo-[6,7:2′,3′]morphinan (8, 9, or 10). A mixture of compound **14**, **15**, or **16** (130 mg, 0.25 mmol) and sodium bicarbonate (63 mg, 0.75 mmol) was suspended in freshly distilled THF (2 mL) and stirred at 0 °C under nitrogen for 5 min. Bromoacetyl bromide (100.5 mg, 0.5 mmol), diluted with THF (2 mL), was added dropwise over a period of 1 min, and the reaction mixture was stirred for 15 min. The pure products **8**, **9**, or **10** was obtained by dry column chromatography (CMA, 96:4:1).

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-13,14-dihydroxy-1′-(*o*-bromoacetamido)indolo[6,7:2′,3′]-morphinan (8): yield 138 mg, 86%; mp (HCl) > 230 °C dec; TLC R_f 0.51 (CMA, 96:4:1); ^{1}H NMR (DMSO-$_{d6}$) δ 8.96 (s, 1 H, phenolic OH), 7.42 (d, J = 8.0 Hz, 1 H, Ar), 7.35 (d, J = 71.8 Hz, 1 H, Ar), 7.23 (t, J = 7.1 Hz, 1 H, Ar), 7.18 (d, J = 8.0 Hz, 1 H, Ar), 7.1–6.9 (m, 3 H, Ar), 6.7–6.6 (m, 2 H, H_1 & H_2), 6.41 (d, J = 7.8 Hz, 1 H, Ar), 5.71 (s, 1 H, H_5), 5.47 (dd, J = 17.6 Hz, 2 H, benzylic protons), 4.13 (s, 2 H, methylene protons); IR (cm^{-1}) 1673 (amide carbonyl); FAB MS m/z 640, 642 (MH^+) and $(MH + 2)^+$. Anal. ($C_{35}H_{34}N_3O_4Br$·HCl·1.5H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*m*-bromoacetamido)indolo[6,7:2′,3′]-morphinan (9): yield 132 mg, 82.5%; mp (HCl) >225 °C dec; TLC R_f 0.50 (CMA, 97:3:1); ^{1}H NMR (DMSO-d_6) δ 9.02 (s, 1 H, phenolic OH), 7.65–7.5 (m, 2 H, Ar), 7.415 (d, J = 7.8 Hz, 1 H, Ar), 7.325 (d, J = 8.4 Hz, 1 H, Ar), 7.24 (t, J = 8.0 Hz, 1 H, Ar), 7.11 (t, J = 7.4 Hz, 1 H, Ar), 6.99 (t, J = 7.5 Hz, 1 H, Ar), 6.87 (d, J = 7.7 Hz, 1 H, Ar), 6.57 (s, 2 H, H_1 & H_2), 5.64 (s, 1 H, H_5), 5.53 (dd, J = 16 Hz, 2 H, benzylic protons), 4.04 (s, 2 H, methylene protons); IR (cm^{-1}) 1677 (amide carbonyl); FAB MS m/z 640, 642 $(MH)^+$ and $(MH + 2)^+$, 638, 640 $(M - H)^-$ and $(M - H - 2)^-$. Anal. ($C_{35}H_{34}N_3O_4Br$·HCl·2.25H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*p*-bromoacetamido)indolo[6,7:2′,3′]morphinan (10): yield 104.5 mg, 65.4%; mp >250 °C dec; TLC R_f 0.29 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 10.4 (s, 1 H, bromoacetamide NH), 9.07 (s, 1 H, phenolic OH), 7.54 (d, J = 8.4 Hz, 2 H, Ar), 7.42 (d, J = 7.5 Hz, 1 H, Ar), 7.33–7.28 (m, 3 H, Ar), 7.08 (t, J = 7.4 Hz, 1 H, Ar), 6.98 (t, J = 7.3 Hz, 1 H, Ar), 6.57 (s, 2 H, H_1 & H_2), 5.68 (s, 1 H, H_5), 5.48 (dd, J = 16.3 Hz, 2 H, benzylic protons), 4.04 (s, 2 H, methylene protons); IR (cm^{-1}) 1697, 1541, 1514 (amide carbonyl); FAB MS m/z 640, 642 $(MH)^+$ and $(MH + 2)^+$. Anal. ($C_{35}H_{34}N_3O_4Br$·H_2O) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-1′-(*o*-iodoacetamido)indolo[6,7:2′,3′]morphinanyl Methanesulfonate (11). To a suspension of the bromoacetamide **8** (160 mg, 0.25 mmol) in THF was added sodium iodide (150 mg, 1.0 mmol), and the mixture was stirred at room temperature for 4.5 h under nitrogen. The reaction mixture was filtered, and the filtrate was subjected to column chromatography (CM, 96:4) to obtain pure 11 which was converted to its methanesulfonate salt: yield 70.4 mg, 41%; mp >250 °C dec; TLC R_f 0.26 (CM, 96:4); FAB MS m/z 688 $(MH)^+$, 686 $(M - H)^-$. Anal. ($C_{35}H_{34}N_3O_4I$·CH_3SO_3H) C, H, N.

Formation of 16 and 17 in the Coupling of 14 with Bromoacetic Acid. Bromoacetic acid (69.5 mg, 0.5 mmol), dicyclohexylcarbodiimide (DCC) (105 mg, 0.51 mmol), and HOBt (69 mg, 0.51 mmol) were dissolved in dry dichloromethane (3 mL) at 0 °C under nitrogen and stirred for 4 h at room temperature. The amine **14** (260 mg, 0.5 mmol) was then added, and the reaction was worked up after 3 h. Dry column chromatography (CMA, 98:2:1) followed by preparative HPLC using gradient elution ($CHCl_3$:MeOH:EtOAc:NH_4OH, 80:0:20:1–80:2:18:1) over a 45 min period afforded **16** and **17**. **16**: TLC R_f 0.18 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 9.9 (s, 1 H, acetamide NH), 9.0 (s, 2 H, phenolic OH), 5.63 (s, 1 H, H_5), 5.60 (s, 1 H, H_5'), 5.4 (dd, 2 H, 16.3 Hz, benzylic protons), 5.3 (dd, 2 H, J = 15.8 Hz, benzylic protons), 3.8 (d, 2 H, J = 1.9 Hz, methylene protons); FAB MS m/z 1079 $(MH)^+$, 1077 $(M - H)^-$. Anal. ($C_{68}H_{66}N_6O_7$) C, H, N.

17: TLC R_f 0.31 (CMA, 98:2:1); ^{1}H NMR (DMSO-d_6) δ 10.33 (s, 1 H, amide NH), 9.01 (s, 1 H, phenolic OH), 8.05 (d, J = 8.4 Hz, 1 H, Ar), 7.95 (d, J = 6.0 Hz, 1 H, Ar), 7.91 (s, 1 H, Ar), 7.62 (t, J = 8.1 Hz, 1 H, Ar), 7.38–7.50 (m, 4 H, Ar), 7.27 (d, J = 7.6 Hz, 3 H, Ar), 7.05 (t, J = 8.0 Hz, 1 H, Ar), 6.96 (t, J = 7.4 Hz, 1 H, Ar), 6.57 (d, J = 11 Hz, 1 H, H_1), 6.52 (d, J = 8.1 Hz, 1 H, H_2, H_1 and H_2 appear as a quartet), 5.65 (s, 1 H, H_5), 5.45 (dd, J = 16.2 Hz, 2 H, benzylic protons), 5.29 (s, 2 H, methylene protons); IR (cm^{-1}) 1663 (amide carbonyl); FAB MS m/z 695 $(MH)^+$, 693 $(M - H)^-$.

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