(Aminoalkyl)indole Isothiocyanates as Potential Electrophilic Affinity Ligands for the Brain Cannabinoid Receptor

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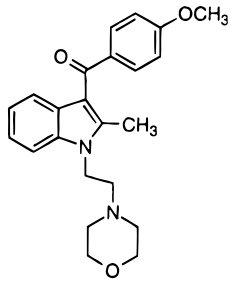
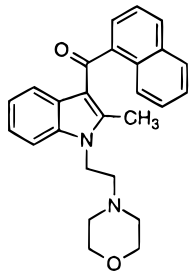
Received December 21, 1995[⊗]

A series of (aminoalkyl)indole compounds, naphthalene analogs of pravadoline (**1**), has been shown to exhibit cannabinoid agonist activities such as antinociception in animals, inhibition of adenylate cyclase in brain membranes, and binding to the cannabinoid receptor. These pravadoline analogs were selected for the preparation of potential electrophilic affinity ligands based on the synthesis of isothiocyanate derivatives. One isothiocyanatonaphthalene derivative (**8**) displaced [^{3}H]CP-55940 binding to a rat brain P2 membrane preparation with an IC_{50} of 690 nM, which was 10-fold less potent than the parent molecule (IC_{50} = 73 nM). Isothiocyanate substitution at various positions on the naphthalene moiety of the desmethyl analog **10** gave compounds that displaced [^{3}H]CP-55940 with IC_{50} values between 400 and 1000 nM, compared with 46 nM for the parent compound **10**. However, 6-isothiocyanato substitution on the indole ring of the desmethyl analog provided isothiocyanate **12** that displaced [^{3}H]CP-55940 binding with an IC_{50} of 160 nM. After pretreatment of brain membranes with this high-affinity isothiocyanato ligand followed by washing out the ligand, the membranes were depleted of 90% of the cannabinoid receptor binding capacity. Loss of receptor binding capacity was half-maximal at 300 nM of the derivative under standard assay conditions. As a control, pretreatment with the parent compound at concentrations that were 20 times the K_d failed to alter subsequent binding activity. This study demonstrates that an isothiocyanato (aminoalkyl)-indole (**12**) can behave as an affinity ligand which binds irreversibly to the cannabinoid receptor in brain and which precludes subsequent binding of the cannabinoid ligand [^{3}H]CP-55940.

Research conducted at Sterling Winthrop Research Institute demonstrated that a series of analogs of pravadoline, termed (aminoalkyl)indoles, act as cannabinoid receptor agonists.[1−3] Pravadoline (**1**), the first compound in this series, exhibited potent antinociceptive activity and inhibited brain cyclooxygenase as expected for a nonsteroidal antiinflammatory agent. However, pravadoline's spectrum of activities deviated from classical nonsteroidal antiinflammatory agents in that it exhibited no gastrointestinal cytotoxicity and was not antiinflammatory.[4] The (aminoalkyl)indole analogs of pravadoline inhibited the electrically evoked contraction in a mouse vas deferens smooth muscle preparation in a parallel fashion to their ability to behave as antinociceptive agents.[4−7] Replacing the monocyclic 4-methoxybenzoyl group of **1** with a naphthoyl moiety to provide **2** increased the potency by nearly 80-fold in the mouse vas deferens and 10-fold in the antinociception determination.[2,7]



Pravadoline (**1**)



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The antinociceptive action of the (aminoalkyl)indole compounds can be attributed to an interaction with the cannabinoid receptor in brain. Specific binding of [^{3}H]-WIN-55212-2 in brain membranes was displaced not only by (aminoalkyl)indole analogs but also by Δ^9-THC and other central nervous system (CNS)-active cannabinoid compounds.[8] (Aminoalkyl)indole analogs were able to displace [^{3}H]CP-55940 binding to the cannabinoid receptor in brain membranes[6] but failed to displace radioligands selective for at least 20 other neuroreceptors.[2] Potency ratios for both classes of compounds were consistent with their cannabimimetic activities reported in animals and the mouse vas deferens assay. (Aminoalkyl)indole agonists inhibited ade-

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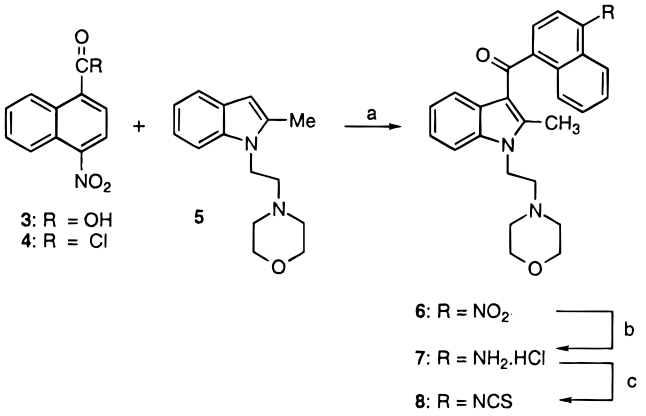
⊗ Abstract published in *Advance ACS Abstracts,* April 1, 1996.

S0022-2623(95)00932-0 CCC: $12.00

Scheme 1[a]



[a] Reagents: (a) $AlCl_3$;[1] (b) Fe, HCl−aqueous EtOH; (c) $CSCl_2$, AcOEt, aqueous $NaHCO_3$.

nylate cyclase in rat striatal or cerebellar membranes[7] and reduced Ca^{2+} conductance of an N-type Ca^{2+} channel in a neuronal cell,[9] both responses being attributed to a receptor−G protein mechanism.

The cannabinoid receptor subtype CB1 was cloned and demonstrated to have an amino acid sequence consistent with a tertiary structure typical of the seven-transmembrane-spanning proteins that are coupled to G proteins.[10,11] CB1 mRNA is found in abundance in brain tissue. The CB2 subtype has been demonstrated in spleen tissue; however, CB2 mRNA could not be found in brain tissue by either Northern analysis or *in situ* hybridization studies.[12] Several recent reviews have described the pharmacology, biochemistry, and CNS distribution of the CB1 receptor in brain.[13−15] The present report describes electrophilic affinity ligands that have been developed from a potent (aminoalkyl)-indole agonist. One ligand from this series exhibits a high affinity for the cannabinoid receptor and binds irreversibly to the receptor at a position in the protein that precludes subsequent interaction with the cannabinoid ligand [^{3}H]CP-55940.

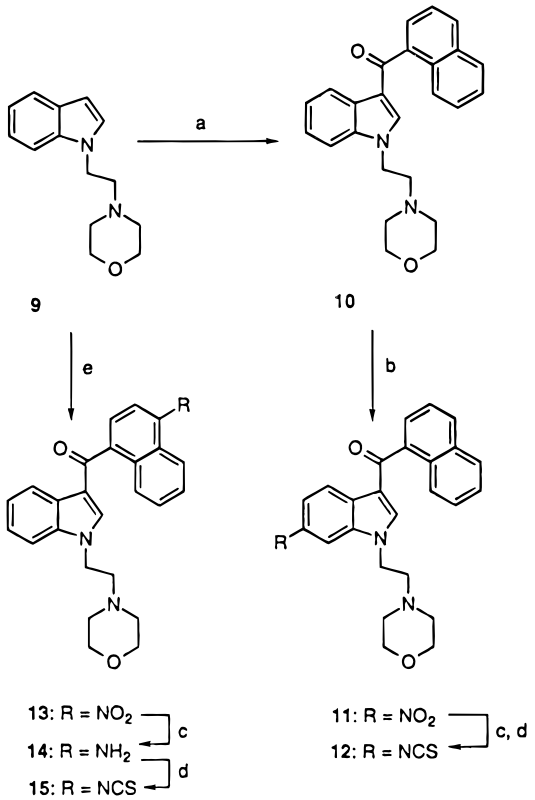
Chemistry

Since there was no *a priori* way of determining where an isothiocyanate moiety should be located in an (aminoalkyl)indole to enable its interaction with a bionucleophile such as amino or sulfhydryl in the receptor, compounds were prepared in which the moiety was situated in different spatial areas. Five potential electrophilic affinity ligands were prepared, as shown in Schemes 1−3.

Treatment of 4-nitro-1-naphthoic acid (**3**)[16] with thionyl chloride gave acid chloride **4** which was reacted with indole **5**[1] under previously described conditions[1] to give **6**, the 4-nitronaphthyl derivative of pravadoline (**1**). Reduction of the 4-nitro group of **6** gave amine **7** which was then converted to the isothiocyanate **8** as shown in Scheme 1.

Indole **9**[1] was used to prepare the other four isothiocyanates, **12**, **15**, **22**, and **24**, where the isothiocyanate group was placed at various positions on the naphthyl subunit for compounds **15**, **22**, and **24** (at C-4, C-6, and C-3, respectively) or on the indole nucleus for **12** (at C-6) (Schemes 2 and 3). Nitration of **10**[1] gave 6-nitroindole derivative **11** which was converted to isothiocyanate **12** by the usual procedure of reduction to the amine

Scheme 2[a]



[a] Reagents: (a) 1-naphthoyl chloride, $AlCl_3$;[1] (b) 70% HNO_3, Ac_2O; (c) Fe, HCl-aqueous EtOH; (d) $CSCl_2$, AcOEt, aqueous $NaHCO_3$; (e) $AlCl_3$,[1] 4-nitro-1-naphthoyl chloride.

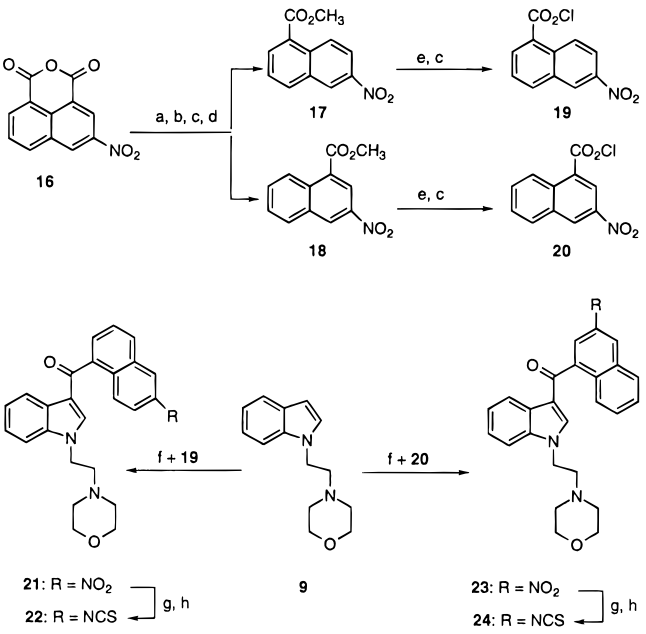
followed by treatment with thiophosgene. The structure of **12** was determined by single-crystal X-ray analysis, and the data are available as Supporting Information. Isothiocyanate **15** was similarly prepared from nitro compound **13**.

Using a modification of the published procedure, 3-nitro-1,8-naphthalic anhydride (**16**) was converted to a mixture of esters **17** and **18** which were separated by a combination of crystallization and chromatography (Scheme 3). Pure **17** and **18** were then converted to the corresponding acid chlorides **19** and **20** as indicated. Reaction of these compounds with indole **9** gave the corresponding nitro compounds **21** and **23** which were converted to the isothiocyanates **22** and **24** by the usual procedure. We were unsuccessful in our attempt to place the isothiocyanate at C-8 in the naphthyl nucleus. Previously synthesized compounds[1] **2** and **10**, the parent compounds of the isothiocyanates prepared in this study, were resynthesized for the binding affinity studies.

Pharmacology

Compound **2**, the 1-naphthoyl derivative of pravadoline, lacks the ability to inhibit cyclooxygenase but is >30-fold more potent than pravadoline in the mouse vas deferens smooth muscle assay.[1,5] This compound exhibited a K_i for the cannabinoid receptor of 18 nM (coefficient of variation 2.3%) (Figure 1). The slope factor was 1.1, consistent with single-site displacement of [^{3}H]CP-55940. The 4-isothiocyanato derivative **8** exhibited a 10-fold loss of potency for the receptor. Inasmuch as covalent binding would have taken place during the incubation, the reported IC_{50} would result

Scheme 3[a]



[a] Reagents: (a) NaOH, (b) $Hg(OAc)_2$/aqueous AcOH, reflux 4 days, HCl;[16] (c) $SOCl_2$; (d) MeOH; (e) aqueous NaOH, MeOH, reflux then HCl; (f) $AlCl_3$;[1] (g) Fe, HCl−aqueous EtOH; (h) $CSCl_2$, AcOEt/aqueous $NaHCO_3$.

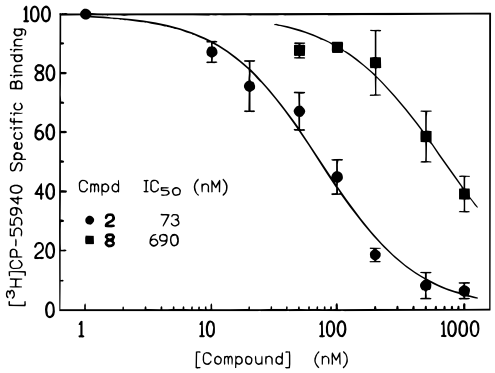


Figure 1. Affinity of 1-naphthyl[2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (**2**) (●) and (4-isothiocyanato-1-naphthyl)[2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (**8**) (■) for the cannabinoid receptor in brain membranes. [^{3}H]CP-55940 was present at 1.1 nM. Data points are the mean ± standard error from five (compound **2**) and three (compound **8**) individual experiments, each performed in triplicate.

in an underestimate of the K_i because the off rate would approach infinity for those covalently bound sites. Thus, a K_i cannot be calculated.

Compound **10** lacks the methyl group at the 2-position on the indole moiety of **2**. It also fails to inhibit cyclooxygenase but is about 2-fold more potent than compound **2** at inhibiting mouse vas deferens contractions.[1,7] The K_i for displacement of [^{3}H]CP-55940 from the cannabinoid receptor was 29 nM (coefficient of variation 3.3%), with a slope factor of 1.2 (Figure 2). Each of its isothiocyanato derivatives suffered a loss of affinity for the cannabinoid receptor in brain. Consistent with the observation for the methylated pair **2** and **8**, substitution on the naphthoyl moiety (compounds **15**, **22**, and **24**) resulted in a 10−20-fold loss of apparent affinity for the receptor. The isothiocyanato derivative extending from the indole (compound **12**) was more

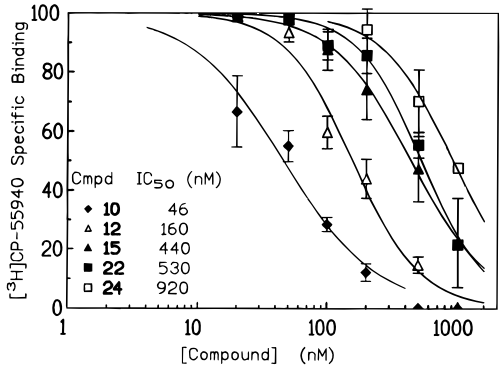


Figure 2. Affinity of 1-naphthyl[1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (**10**) and its isothiocyanato derivatives at the indole 6- (**12**) and naphthyl C-4- (**15**), C-6- (**22**), and C-3- (**24**) positions for the cannabinoid receptor in brain membranes. [^{3}H]CP-55940 was present at 0.25 nM. Data points are the mean ± standard error from four individual experiments, each performed in triplicate.

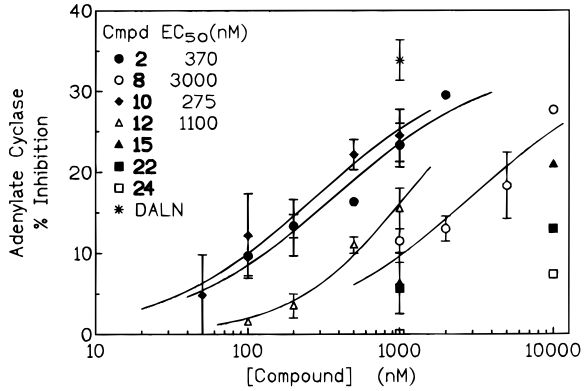


Figure 3. Agonist ability of isothiocyanato (aminoalkyl)-indoles to inhibit hormone-stimulated adenylate cyclase activity in N18TG2 membranes. Membranes were incubated as described in the text with 0.6 μM secretin to stimulate adenylate cyclase, 100 μM rolipram as a phosphodiesterase inhibitor and the indicated concentrations of compounds **2** (●), **8** (○), **10** (◆), **12** (△), **15** (▲), **22** (■), **24** (□), and desacetyllevonantradol (DALN). Data points are the mean ± standard error from three individual experiments, except for the 10 μM concentrations of compounds **15**, **22**, and **24** which are from a single experiment.

potent, exhibiting only a 3.5-fold loss of potency compared with the parent compound.

Each of the isothiocyanato (aminoalkyl)indole compounds behaved as agonists in inhibiting adenylate cyclase activity in the neuroblastoma model membrane system (Figure 3). Parent compounds **2** and **10** exhibited mean (95% confidence intervals) EC_{50} values of 370 (220−630) and 275 (175−440) nM, respectively. The most potent of the isothiocyanato derivatives was compound **12**, exhibiting an EC_{50} of 1.1 (0.67−1.8) μM, followed in potency by compound **8**, exhibiting an EC_{50} of 3.0 (1.1−7.9) μM. The other three analogs failed to attain an inhibition of adenylate cyclase equivalent to one-half that of the maximal attained by 1 μM desacetyllevonantradol (34% inhibition) at <10 μM concentrations. Therefore EC_{50} values were not determined because of excessive vehicle concentrations that would be required in the assay mixture. It is of interest that 3 μM compound **12** failed to inhibit adenylate cyclase activity when assayed in a reaction mixture containing 50 mM HEPES buffer, 1 mM EDTA, and 0.1 mg/mL bovine serum albumin, reagents which may provide a

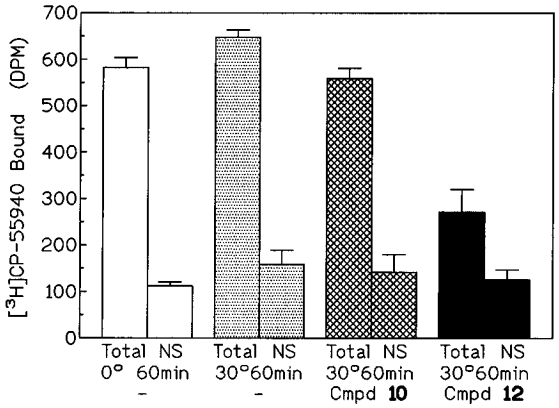


Figure 4. Irreversible decrease in specific binding of [^{3}H]CP-55940 after pretreatment of rat brain membranes with the isothiocyanate **12** but not **10**. Pretreatment of membranes with either 1 μM **12** (solid) or **10** (hatched) was performed at 30 °C for 60 min. Controls included parallel incubations of membranes with vehicle (0.05% β-cyclodextrin plus 0.01% dimethyl sulfoxide) at either 30 °C (stipled) or 0 °C (open). After washing away of the compounds by sedimentation, cannabinoid receptor binding was quantitated. Data bars are the mean ± standard error of the total binding and the nonspecific binding (not displaced by 0.1 μM desacetyllevonantradol) from three individual experiments, each performed in triplicate.

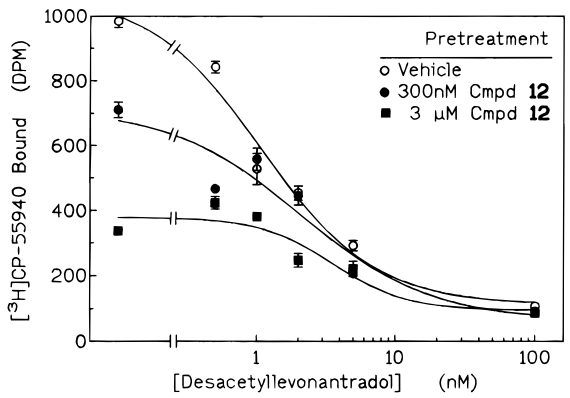


Figure 5. Dose dependency of the covalent binding of isothiocyanate **12** to the cannabinoid receptor. Rat brain membranes were incubated at 30 °C for 60 min with vehicle (○), or 300 nM (●) or 3 μM (■) compound **12**. After sedimentation and resuspension of the membranes, binding of 0.175 nM [^{3}H]CP-55940 to 50 μg of membrane protein was assessed. The data are the mean ± standard error of the triplicates within a single representative experiment.

source of nucleophiles in vast excess of the number of cannabinoid receptors present in the mixture.

Compound **12** was tested for its ability to bind irreversibly to the cannabinoid receptor in rat brain membranes (Figure 4). Membranes incubated with 1 μM compound **12**, a concentration that was 6-fold greater than the IC_{50} for displacing [^{3}H]CP-55940 under similar conditions in the ligand binding assay, resulted in a 70% loss of specific binding of [^{3}H]CP-55940 (significantly different at $p = 0.01$). No effect on nonspecific binding was noted. Preincubation of membranes at 0−4°C for 60 min showed no difference in the ability of [^{3}H]CP-55940 to bind specifically to the cannabinoid receptor, indicating that heat denaturation was not a factor in the experiment. There was a 15% difference (not significant at $p = 0.05$) in specific binding of [^{3}H]CP-55940 between control membranes and those preincubated with 1 μM compound **10**. This concentration was 30-fold the K_i and likely to interact with 97% of the receptors after equilibration. The small decrease in specific binding of [^{3}H]CP-55940 could indicate an incomplete removal of the ligand from the membranes prior to assay for cannabinoid receptors.

The depletion of cannabinoid receptor binding was dependent upon the concentration of compound **12** in the incubation mixture (Figure 5). Preincubation with 3 μM compound **12** resulted in the loss of 70% of the specific binding to cannabinoid receptors in the brain membranes. At 300 nM, a concentration that is only 2 times the IC_{50} for displacing [^{3}H]CP-55940, compound **12** eliminated 30% of the specific binding. No change in [^{3}H]CP-55940 binding was observed upon preincubation with 100 nM compound **12** (data not shown). No change in nonspecific binding was noted. The IC_{50} values for desacetyllevonantradol were 1.1, 2.1, and 3.5 nM, respectively, suggesting a trend toward incrementally decreasing affinities for agonist ligands by the remaining receptors. However, these differences failed to reach statistical significance at the 5% level.

Discussion

The active (aminoalkyl)indoles exhibit virtually the same spectrum of biological activity as do the classical cannabinoid compounds, including antinociceptive activity in rodents and inhibition of the electrically induced twitch response in the mouse vas deferens.[4,5,7,17] The potency order of several (aminoalkyl)indole analogs in the Martin multiparameter mouse model was consistent with that in the mouse vas deferens assay, with the exception of the test for hypothermia.[17] Active (aminoalkyl)indole analogs generalized to Δ^9-THC in drug discrimination studies in rats.[17] Competitive interactions occur in heterologous competition radioligand binding assays using either [^{3}H]CP-55940 or [^{3}H]-WIN-55212-2 for binding to the cannabinoid receptor in the brain (predominantly, if not entirely, CB1).[6,8] Thus, it is accepted that both cannabinoid and (aminoalkyl)indole compounds act at the same receptor in the brain to evoke their biological responses.

One question that is pertinent is whether these two classes of compounds interact with the same site(s) on the cannabinoid receptor. Structure−activity relationship studies have been extensive for cannabinoid ligands.[15,18−20] It has been concluded that a site for hydrophobic interaction is of major importance to the receptor interaction with bicyclic and classical cannabinoid structures.[21] In addition, functionalities exist on both bicyclic and classical cannabinoid structures for hydrogen-bonding interactions via the phenolic hydroxyl and a hydroxyl generated by metabolism of Δ^9-THC on the cyclohexenyl ring. An additional site for potential hydrogen-bonding interactions was elucidated within the series of AC-bicyclic and ACD-tricyclic cannabinoids.[22] It is not obvious that homologous ligand−receptor interactions occur with the (aminoalkyl)indole structures. The amino acids that interact with the cannabinoid hydrophobic or hydrogen-bonding sites may or may not be the same as those that interact with critical moieties on the (aminoalkyl)indole structures. Occlusion of access by the cannabinoid radioligand to its binding site(s) may result from occupancy by the (aminoalkyl)indole ligand of a region in space that includes one or more of the critical interaction sites. The irreversible depletion of binding sites for [^{3}H]CP-55940

by incubation with compound **12** observed in the present investigation is entirely consistent with this model.

One might hypothesize that the small cannabinoid and (aminoalkyl)indole ligands bind to the CB1 receptor within a pore region formed by the interaction of the seven transmembrane helixes, as do the amine neurotransmitter ligands.[23−24] It is expected that as compound **12** interacts with the receptor, the isothiocyanate group should be within close proximity of a target reactive substituent within the binding pocket such that covalent coupling is permitted. Nucleophilic amino groups on lysine, histidine, or arginine may form disubstituted thioureas by such a reaction. A lysine residue exists on the third transmembrane helix within the first or second turn adjacent to the extracellular surface of the CB1 receptor.[10] One could hypothesize that compound **12** is oriented within the binding pocket such that the indole substituent is able to gain access to the aminobutyl functionality of this target lysine. Although helix 3 lysine would appear to be an ideal candidate for covalent coupling to compound **12**, it should also be considered that potentially free sulfhydryl groups exist on cysteine residues located within helices 1, 4, 6, and 7.[10] Whether any of these are involved in transhelical disulfide interactions is unknown, in which case they would be unavailable to interact with compound **12** under the incubation conditions used in this study. It is also conceivable that compound **12** interacts with nucleophilic amino acids at the intracellular membrane−receptor interfaces, such as the histidines at helices 1 and 2, the arginine and lysine residues at helices 3 and 4, respectively, the lysine residues at helices 5 and 6, or the arginine at helix 7.

Interpretation of the present results to suggest that both cannabinoid and (aminoalkyl)indole ligands bind to mutually exclusive locations within the receptor may be complicated by the fact that both the covalently modifying ligand and the binding detection ligand are agonists. A scenario is possible by which the two ligand classes may occupy different regions of the receptor molecule but that appropriate interaction with either region is able to promote an effector response. If the irreversible occupancy of the (aminoalkyl)indole binding site by the agonist ligand were able to promote (and maintain) a state of dissociation of the G protein from the receptor, which would normally occur in the presence of GTP, then the receptor would remain in a low-affinity conformation for subsequent agonist ligand interactions at the alternative agonist binding site (i.e., for cannabinoid compounds). Using ligand binding concentrations of [^{3}H]CP-55940 below the high-affinity K_d, as was used in the present investigation, would not be suitable for the detection of populations of receptors in a low-affinity state. Although in the present study the brain membranes underwent three steps of washing by sedimentation, it is conceivable that sufficient guanine nucleotides were present to allow the availability of sufficient GTP to disrupt the receptor−G protein interaction. This problem could be resolved by utilization of an antagonist ligand for either covalent interaction or detection.

Experimental Section

Biological Methods. Determination of Affinity for the Cannabinoid Receptor. A P2 membrane fraction was prepared from rat forebrains as previously described[25] except that membranes were suspended in 20 mM potassium diethylmalonate, pH 7.5, plus 3 mM $MgCl_2$. An aliquot of 30 μg of membrane protein was incubated for 1 h at 30 °C with [^{3}H]-CP-55940 in 20 mM potassium diethylmalonate, pH 7.5, 2.25 mM $MgCl_2$, 0.025% β-cyclodextrin (Research Biochemicals Inc.), plus additional compounds as indicated. The test compounds were prepared as 10 mM stock solutions in dimethyl sulfoxide. The compounds were suspended in 5% β-cyclodextrin (100 μM) followed by further dilution in the above incubation buffer. The incubation was stopped by addition of 250 μL of 50 mg/mL bovine serum albumin and immediate filtration over glass fiber filters as previously described.[26] Specific binding was defined as that which could be displaced by 0.1 μM desacetyllevonantradol. Data from multiple experiments were averaged and analyzed by nonlinear regression of the homologous displacement data using the Graphpad Inplot computer program.

Adenylate Cyclase Activity. Plasma membranes from N18TG2 cells were prepared as previously described.[27] The adenylate cyclase assay was modified from the previous method as follows: The buffer was 20 mM diethylmalonate, pH 8.0, rather than HEPES, and EDTA and bovine serum albumin were eliminated from the reaction mixture. The desacetyllevonantradol and (aminoalkyl)indole derivatives were suspended in β-cyclodextrin and diluted as described above. Assays were conducted at 30 °C for 8 min.

Treatment of Membranes with (Aminoalkyl)indole Isothiocyanates. Washed P2 membranes (900 μg of protein) were sedimented and resuspended for incubation with the indicated compounds in 150 μL of 20 mM potassium diethylmalonate, pH 7.5, and 2.25 mM $MgCl_2$ buffer. The incubation was stopped after 60 min by addition of 15 mL of ice-cold TME buffer (20 mM Tris-Cl, pH 7.4, 3 mM $MgCl_2$, 1 mM EDTA) containing 1 mg/mL fatty acid-deficient bovine serum albumin followed by sedimentation at 39000*g* for 30 min. The membrane pellet was resuspended in TME buffer, and 50 μg of membrane protein was assayed for [^{3}H]CP-55940 binding in TME buffer containing a final concentration of 0.16 mg/mL fatty acid-deficient bovine serum albumin.

Chemistry. Melting points (uncorrected) were taken in a Thomas-Hoover capillary apparatus. IR, mass, and NMR spectra were consistent with the structures shown. IR spectra were obtained using a Beckman Acculab 8 instrument. Thin layer chromatography (TLC) was performed on 250 μm silica gel GHLF, Analtech uniplates and visualized with iodine vapor. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Compounds indicated by the molecular formula followed by the symbols for the elements (C, H, N) were found to be within 0.4% of theory. Chemical ionization (CIMS) and electron ionization (EIMS) mass spectra were obtained using a Finnigan 1050 mass spectrometer and a VG-Micro Mass 7070F mass spectrometer, respectively. ^{1}H-NMR spectra were obtained using a Varian XL-300 spectrometer and the solvents indicated. Silica gel 60 (60−240 mesh) with the solvent indicated was used for column chromatography.

1-Naphthyl[2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (2). This compound was prepared essentially by the previously described method[1] except that the acyl chloride−$AlCl_3$ complex was preformed in 1,2-dichloroethane prior to addition of a solution of **5**.[1] Purification of the crude material from 10 mmol of **5**[1] by chromatography (AcOEt−hexane, 2:1−10:1, v/v) afforded **2** (2.9 g, 73%) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 7H), 2.72 (t, 2H, J = 7.3 Hz), 3.71 (m, 4H), 4.28 (m, 2H), 7.03 (t, 1H, J = 8.2 Hz), 7.16−7.60 (m, 7H), 7.93 (d, 1H, J = 7.8 Hz), 8.12 (d, 1H, J = 8.3 Hz); CIMS (NH_3) m/e 399 (MH^+).

The free base **2** was converted into its oxalate and recrystallized from water−EtOH to afford colorless needles: mp 216−7 °C dec. Anal. ($C_{26}H_{26}O_2N_2 \cdot C_2H_2O_4 \cdot 0.25H_2O$) C, H, N.

4-Nitro-1-naphthoic Acid (3). Compound **3** was prepared in 74% yield as previously described,[16] giving yellow prisms (from AcOH): mp 217−9 °C dec (lit.[16] mp 225−6 °C).

(4-Nitro-1-naphthyl)[2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (6). As in the Friedel−Crafts preparation of **2**,[1] the indole **5**[1] (7.0 g, 28.7 mmol) was treated with a 1,2-dichloroethane (130 mL) solution of the complex

between $AlCl_3$ (11.6 g, 87 mmol) and acid chloride **4** (obtained from 4-nitro-1-naphthoic acid (**3**)[16] (7.0 g, 32.3 mmol) and thionyl chloride). The product was purified by chromatography ($CHCl_3$−MeOH, 80:1, v/v) and recrystallized from EtOH to give **6** (6.11 g, 52%) as yellow prisms: mp 156−60 °C; ^{1}H NMR ($CDCl_3$) δ 2.53 (m, 4H), 2.60 (s, 3H), 2.73 (t, 2H, J = 7 Hz), 3.71 (4H, m), 4.30 (2H, t, J = 7 Hz), 7.03 (d, 2H, J = 3.8 Hz), 7.18−7.26 (1H, m), 7.35 (d, 1H, J = 7.8 Hz), 7.54−7.62 (m, 2H), 7.76 (t, 1H, J = 8 Hz), 8.12 (d, 1H, J = 8.4 Hz), 8.27 (dd, 1H, J = 7.8, 1.8 Hz), 8.63 (d, 1H, J = 8.8 Hz); CIMS (NH_3) m/e 444 (MH^+).

(4-Amino-1-naphthyl)[2-methyl-1-[2-(4-morpholinyl)-ethyl]-1*H*-indol-3-yl]methanone (7). A mixture of **6** (4.10 g, 9.93 mmol), Fe powder (8 g), 6 N HCl (20 mL), and EtOH (90 mL) was refluxed for 1 h. The insoluble material was filtered by passing through Celite and washed with water. The filtrate and washings were combined, concentrated *in vacuo*, made alkaline with aqueous $NaHCO_3$, and extracted with AcOEt. The AcOEt extract was washed with water and brine and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by chromatography ($CHCl_3$−MeOH, 100:1−60:1, v/v) to afford a reddish brown foam, which was converted into its hydrochloride salt and recrystallized from EtOH−water to give **7**·HCl (3.44 g, 77%) as pale brown crystals: mp 215−20 °C dec; IR (KBr) 3440, 2600, 1615 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.54 (s, 3H), 6.80 (d, 1H, J = 8 Hz), 7.07 (t, 1H, J = 7.6 Hz), 7.23 (d, 1H, J = 8 Hz), 7.28 (d, 1H, J = 7.6 Hz), 7.47(d, 1H, J = 8.2 Hz), 7.52 (m, 2H), 7.78 (d, 1H, J = 8.2 Hz), 8.24 and 8.38 (m, 1H each); CIMS (NH_3) m/e 414 (MH^+).

(4-Isothiocyanato-1-naphthyl)[2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (8). To a stirred suspension of **7**·HCl (2.13 g,4.73 mmol), $NaHCO_3$ (3.0 g, 36 mmol), water (50 mL), and AcOEt (50 mL) in an ice−water bath was added thiophosgene (0.4 mL, 5.2 mmol). The mixture was stirred at room temperature for 20 min, and then the organic layer was washed with water and dried over anhydrous Na_2SO_4. After removal of solvent, the residue was purified by chromatography ($CHCl_3$−MeOH, 90:1, v/v) and recrystallized from EtOH−hexane to afford **8** (1.46 g, 68%) as pale yellow prisms: mp 147−8 °C; IR (KBr) 2120, 1640, 1610, 1415 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.58 and 2.57 (2s, 3H, $ArCH_3$ ratio 1 to 3), 2.52 (m, 4H), 2.72 (t, 2H, J = 6.8 Hz), 3.71 (m, 4H), 4.29 (t, 2H, J = 6.9 Hz), 7.03 (t, 1H, J = 7.9 Hz), 7.11 (d, 1H, J = 7.9 Hz), 7.21 (td, 1H, J = 8.2, 2 Hz), 7.34 (d, 1H, J = 8.2 Hz), 7.46−7.70 (m, 4H), 8.16 (d, 1H, J = 8.3 Hz), 8.23 (d, 1H, J = 8.3 Hz); CIMS (NH_3) m/e 456 (MH^+). Anal. ($C_{27}H_{25}O_2N_3S$) C, H, N, S.

1-Naphthyl[1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]-methanone (10). This material was prepared from **9**[1] analogous to the synthesis of **2**, purified by chromatography (AcOEt−hexane, 2:1, v/v), and recrystallized from ether to afford **10** (4.76 g, 48%) as pale yellow needles: mp 101−2 °C (lit.[1] mp 104−6 °C); CIMS (NH_3) m/e 385 (MH^+). Anal. ($C_{25}H_{24}N_2O_2$) C, H, N.

1-Naphthyl[1-[2-(4-morpholinyl)ethyl]-6-nitro-1*H*-indol-3-yl]methanone (11). To a stirred solution of **10** (3.73 g, 9.7 mmol) in Ac_2O (40 mL) was added 70% HNO_3 (8 mL) at 0 °C, and the reaction mixture was then stirred for 0.5 h. The reaction was quenched with ice−water (500 mL); then the mixture was treated with concentrated NH_4OH (100 mL) and stirred at room temperature for 30 min. The reaction mixture was extracted with AcOEt. The AcOEt extract was washed with water and brine and dried over anhydrous Na_2SO_4. After removal of solvent, the residue was purified by chromatography (AcOEt−hexane, 2:1−3:1, v/v) and recrystallized from AcOEt−hexane to afford **11** (1.07 g, 26%) as yellow prisms: mp 182−3 °C; IR (KBr) 1635, 1612, 1518, 1385, 1335 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.41 (m, 4H), 2.73 (t, 2H, J = 6 Hz), 3.54 (m, 4H), 4.24 (t, 2H, J = 6 Hz), 7.47−7.61 (m, 3H), 7.69 (dd, 1H, J = 7, 1.2 Hz), 7.73 (s, 1H), 7.74 (m, 1H), 8.02 (d, 1H, J = 8.3 Hz), 8.16 (m, 1H), 8.26 (dd, 1H, J=8.9, 2 Hz), 8.41 (d, 1H, J = 2Hz), 8.61 (d, 1H, J = 8.8 Hz); CIMS m/e 429 (M^+), 399, 329, 155, 127, 101 (base), 56. Anal. ($C_{25}H_{23}O_4N_3$) C, H, N.

1-Naphthyl[6-isothiocyanato-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (12). A mixture of **11** (1.07 g, 2.49 mmol), Fe powder (1.38 g, 25 mg-atom), 6 N HCl (4 mL), water (10 mL), and EtOH (50 mL) was refluxed for 30 min. The insoluble material was filtered and washed well with EtOH. The filtrate and washings were concentrated *in vacuo*, made basic with $NaHCO_3$, and extracted with AcOEt. The AcOEt extract was washed with water and concentrated to ca. 50 mL, and $NaHCO_3$ (4.0 g, 48 mmol) in water (30 mL) was added. Thiophosgene (0.4 mL, 5.2 mmol) was added at 5 °C and the reaction mixture then stirred at room temperature for 0.5 h. The AcOEt layer was washed with water and dried over anhydrous Na_2SO_4. After removal of solvent, the residue was purified by chromatography ($CHCl_3$−MeOH, 60:1, v/v) to afford an orange solid, which was recrystallized twice from AcOEt−hexane to give **12** (513 mg, 54.6%) as yellow prisms: mp 166−7 °C; IR (KBr) 2050, 1610, 1510, 1380 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.39 (m, 4H), 2.68 (t, 2H, J = 6.2 Hz), 3.55 (m, 4H), 4.11 (t, 2H, J = 6.2 Hz), 7.24−7.40 (m, 2H), 7.51 (s, 1H), 7.46−7.60 (m, 3H), 7.66 (dd, 1H, J = 7.0, 1.1 Hz), 7.92 (m, 1H), 7.99 (d, 1H, J = 8.2 Hz), 8.16 (d, 1H, J = 8.0 Hz), 8.48 (m, 1H); CIMS (NH_3) m/e 442 (MH^+). Anal. ($C_{26}H_{23}O_2N_3S$) C, H, N, S.

(4-Amino-1-naphthyl)[1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (14). The indole **9**[1] (10.5 g, 45.6 mmol) was treated analogous to the synthesis of **2** with acid chloride obtained from 4-nitro-1-naphthoic acid[16] (**3**) (12.4 g, 57.1 mmol) and thionyl chloride. The crude nitro compound **11** was reduced using the Fe-HCl method described for the preparation of **7**, and the product was purified by chromatography (AcOEt−hexane, 9:1, v/v) to afford **14** (1.95 g, 10% from **9**) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 2.44 (m, 4H), 2.73 (t, 2H, J = 6.4 Hz), 3.63 (m, 4H), 4.20 (t, 2H, J = 6.4 Hz), 4.45 (s, 2H), 6.77 (d, 1H, J = 7.7 Hz), 7.3−7.4 (m, 3H), 7.48−7.54 (m, 2H), 7.53 (s, 1H), 7.62 (d, 1H, J = 7.7 Hz), 7.84−7.90 (m, 1H), 8.42−8.50 (m, 2H); CIMS (NH_3) m/e 400 (MH^+).

(4-Isothiocyanato-1-naphthyl)[1-[2-(4-morpholinyl)-ethyl]-1*H*-indol-3-yl]methanone (15). The amine **14** (1.90 g, 4.76 mmol) was treated with thiophosgene (0.45 mL) as described in the preparation of **8**. The crude product was purified by chromatography ($CHCl_3$−MeOH, 50:1, v/v) and recrystallized from AcOEt−hexane to afford **15** (1.48 g, 71%) as colorless prisms: mp 153−4 °C. IR (KBr) 2080, 1660, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.40 (m, 4H), 2.71 (t, 2H, J = 6.3 Hz), 3.56 (m, 4H), 4.18 (t, 2H, J = 6.3 Hz),7.22−7.32 (m, 1H), 7.36−7.70 (m, 8H), 8.22 (d, 1H, J = 8.5 Hz), 8.45−8.52 (m, 1H); CIMS (NH_3) m/e 442 (MH^+). Anal. ($C_{26}H_{23}O_2N_3S$) C, H, N, S.

Methyl 6-Nitro-1-naphthoate (17) and Methyl 3-Nitro-1-naphthoate (18) from 3-Nitro-1,8-naphthoic Anhydride (16). These compounds were prepared by the following modification of the procedure for the ethyl esters. A solution of NaOH (13.3 g, 333 mmol) in water (500 mL) was stirred at room temperature for 0.5 h with 3-nitro-1,8-naphthalic anhydride[16] (**14**) (24.3 g, 100 mmol). To this mixture were added AcOH (30 mL) and $Hg(OAc)_2$ (39 g, 122 mmol) successively, and the mixture was refluxed for 4 days. The resulting precipitate was collected by filtration, washed with water, and then treated with 6 N HCl (400 mL) at reflux temperature for 1 h. After cooling, the precipitate was collected and dried over P_2O_5 to afford a pale yellow solid, which was treated with thionyl chloride (70 mL) and DMF (1 drop) in $CHCl_3$ (70 mL) at reflux temperature for 5 h. After removal of solvent and excess thionyl chloride, MeOH (500 mL) was added while cooling in an ice−water bath, and this was refluxed for 1 h. After cooling, the resulting precipitate was collected, washed well with cold MeOH, and dried *in vacuo*. The dark brown solid was dissolved in hot ethyl acetate and treated with activated charcoal, filtered while hot, and diluted with hexane to afford pure **18** (8.50 g, 39.2%) as pale yellow needles: mp 134−5 °C; IR (KBr) 1718, 1605, 1530, 1340, 1240, 1190 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.06 (s, 3H), 7.71(t, 1H, J = 7 Hz), 7.83 (m, 1H), 8.08 (d, 1H, J = 8 Hz), 8.94 (d, 1H, J = 2.4 Hz), 8.96 (d, 1H, J = 2.4 Hz), 9.04 (d, 1H, J = 9.0 Hz); CIMS (NH_3) m/e 231 (M^+).

All mother liquors and filtrates were combined and purified by chromatography (AcOEt−hexane, 1:9, v/v) to afford **17** (540 mg, 2.3%) as pale yellow needles: mp 137−8 °C; IR (KBr) 1735, 1605, 1510, 1355, 1285, 1250, 1205, 1140 cm^{-1}; ^{1}H NMR

($CDCl_3$) δ 4.04 (s, 3H), 7.69 (m, 1H), 8.22 (d, 1H, J = 8.2 Hz), 8.35 (dd, 1H, J = 9.6, 2.4 Hz), 8.41 (dd, 1H, J = 7.3, 1.2 Hz), 8.83 (d, 1H, J = 2.3 Hz), 9.15 (d, 1H, J = 9.5 Hz); CIMS (NH_3) m/e 231 (M^+).

(6-Nitro-1-naphthyl)[1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (21). A mixture of the nitro ester **17** (550 mg, 2.38 mmol), 10% NaOH (10 mL), and methanol (20 mL) was refluxed for 1 h. After removal of solvent, the residue was made acidic with 10% HCl and extracted with AcOEt. The AcOEt extract was washed with water, dried over Na_2SO_4, and evaporated to leave a yellow solid (0.52 g, quantitative), which was treated with thionyl chloride (5 mL) at reflux temperature for 1 h and then evaporated to give acid chloride **19** (0.50 g, quantitative). Reaction of **19** with indole **9**[1] as for the synthesis of **2**[1] gave crude material which was purified by chromatography ($CHCl_3$−MeOH, 50:1, v/v) and recrystallized from AcOEt−hexane to afford **21** (230 mg, 22%) as colorless prisms: mp 217−8 °C; IR (KBr) 1620, 1590, 1530, 1385, 1345 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.4 (m, 4H), 2.72 (t, 2H, J = 6.3 Hz), 3.5 (m, 4H), 4.19 (t, 2H, J = 6.3 Hz), 7.4 (m, 3H), 7.46 (s, 1H), 7.72 (dd, 1H, J = 8.2, 7.2 Hz), 7.89 (dd, 1H, J = 7.1, 1.2 Hz), 8.18 (d, 1H, J = 8.3 Hz), 8.22 (dd, 1H, J = 9.4, 2.3 Hz), 8.36 (d, 1H, J = 9.3 Hz), 8.48 (m, 1H), 8.87 (d, 1H, J = 2.2 Hz); CIMS (NH_3) m/e 430 (MH^+).

(6-Isothiocyanato-1-naphthyl)[1-[2-(4-morpholinyl)-ethyl]-1*H*-indol-3-yl]methanone (22). The nitroindole **21** was treated as in the conversion of **11** to **12**. After workup, the crude **22** was purified by chromatography (AcOEt−hexane, 2:1, v/v) and recrystallized from AcOEt−hexane to afford **22** (85 mg from 220 mg of **21**, 38%) as pale orange prisms: mp 160−1 °C; IR (KBr) 2030, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.41(m, 4H), 2.72 (m, 2H), 3.58 (m, 4H), 4.19 (m, 2H), 7.47 (s, 1H), 7.59 (m, 1H), 7.70 (m, 1H), 7.76 (d, 1H, J = 2.1 Hz), 7.92 (d, 1H, J = 8.4 Hz), 8.21 (d, 1H, J = 8.9 Hz), 8.48 (m, 1H); CIMS (NH_3) m/e 430 (MH^+). Anal. ($C_{26}H_{23}O_2N_3S$) C, H, N, S.

(3-Nitro-1-naphthyl)[1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (23). As in the synthesis of **21,** nitro ester **18** (8.5 g) was converted to acid chloride **20** which was reacted with indole **9** (6.10 g). After workup, the crude product was purified by chromatography ($CHCl_3$−MeOH, 50:1, v/v) and recrystallized from AcOEt−hexane to afford **23** (4.58 g, 40.3%) as yellow prisms: mp 180−2 °C; IR (KBr) 1630, 1530, 1400, 1350, 1335 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (m, 4H), 2.73 (t, 2H, J = 6.3 Hz), 3.52 (m, 4H), 4.20 (t, 2H, J = 6.3 Hz), 7.38−7.44 (m, 3H), 7.42 (d, 1H, J = 1.2 Hz), 7.66−7.74 (m, 2H), 8.10−8.16 (m, 1H), 8.24−8.30 (m, 1H), 8.43 (d, 1H, J = 2.2 Hz), 8.48−8.56 (m, 1H), 8.92 (d, 1H, J = 2.1 Hz); CIMS (NH_3) m/e 430 (MH^+).

(3-Isothiocyanato-1-naphthyl)[1-[2-(4-morpholinyl)-ethyl]-1*H*-indol-3-yl]methanone (24). As described in the preparation of isothiocyanate **22**, the nitro compound **23** was reduced by Fe-HCl in aqueous EtOH and treated with thiophosgene. The crude product was purified by chromatography ($CHCl_3$−MeOH, 50:1, v/v) and recrystallized twice from AcOEt−hexane to afford **24** (410 mg, 12%) as colorless prisms: mp 143−4 °C; IR (KBr) 2080, 1625, 1525, 1400, 1200 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.40 (m, 4H), 2.72 (t, 2H, J = 6.3 Hz), 3.54 (m, 4H), 4.19 (t, 2H, J = 6.3 Hz), 7.36−7.43 (m, 3H), 7.45−7.60 (m, 3H), 7.46 (s, 1H), 7.81 (d, 1H, J = 1.9 Hz), 7.85 (d, 1H, J = 8 Hz), 8.10 (d, 1H, J = 8.3 Hz), 8.46−8.53 (m, 1H); CIMS (NH_3) m/e 442 (MH^+). Anal. ($C_{26}H_{23}O_2N_3S$) C, H, N, S.

Acknowledgment. This work was supported in part by NIDA grants R01-DA06312 and K05-DA00182 to A.C.H. and Office of Naval Research and NIDA funding to J.L.F.-A. We thank Tracy Champion-Dorow, Catherine Cantrell, and Gerald Wilken for technical assistance with the biological determinations in this report.

Supporting Information Available: X-ray diffraction data for **12** including ORTEP drawing, crystal coordinates, bond distances, and bond angles (7 pages). Ordering information is given on any current masthead page. Atomic coordinates may also be obtained from the Cambridge Crystallographic Data Centre (Cambridge University Chemical Laboratory, Cambridge CB2 1EW, U.K.).

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JM950932R