Conformationally Restrained Analogues of Pravadoline: Nanomolar Potent, Enantioselective, (Aminoalkyl)indole Agonists of the Cannabinoid Receptor

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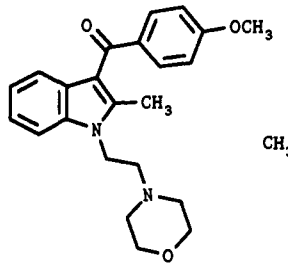
Pravadoline (1) is an (aminoalkyl)indole analgesic agent which is an inhibitor of cyclooxygenase and, in contrast to other NSAIDs, inhibits neuronally stimulated contractions in mouse vas deferens (MVD) preparations (IC_{50} = 0.45 μM). A number of conformationally restrained heterocyclic analogues of pravadoline were synthesized in which the morpholinoethyl side chain was tethered to the indole nucleus. Restraining the morpholine diminished the ability of these pravadoline analogues to inhibit prostaglandin synthesis in vitro. In contrast, mouse vas deferens inhibitory activity was enhanced in [2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-(4-methoxyphenyl)methanone (**20**). Only the *R* enantiomer of **20** was active (IC_{50} = 0.044 μM). An optimal orientation of the morpholine nitrogen for MVD inhibitory activity within the analogues studied was in the lower right quadrant, below the plane defined by the indole ring. A subseries of analogues of **20** and a radioligand of the most potent analogue, (*R*)-(+)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthalenyl)methanone (**21**) were prepared. Inhibition of radioligand binding in rat cerebellar membranes was observed to correlate with functional activity in mouse vas deferens preparations. Binding studies with this ligand (Win 55212-2) have helped demonstrate that the (aminoalkyl)indole binding site is functionally equivalent with the CP-55,940 cannabinoid binding site. These compounds represent a new class of cannabinoid receptor agonists.

Introduction

Pravadoline[1] (1) has demonstrated analgesic activity against postoperative pain in man.[2] Pravadoline and related (aminoalkyl)indoles had been designed as nonacidic analogues of non-steroidal anti-inflammatory drugs (NSAIDs) such as clometacin (2), which is itself related to the widely used drug indomethacin (3).[3] Thus based on their measured pK_a's in the range of 4.5–6.0, pravadoline and analogues should exist largely unprotonated at physiological pH.[4] It was hypothesized that this property might confer an advantage over the traditional carboxylic acid NSAIDs and spurred the initial development of this series.



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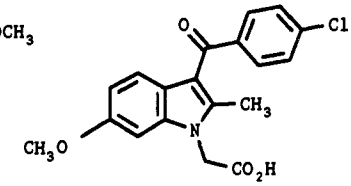

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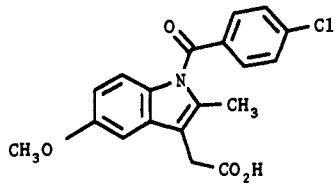

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Pravadoline's preclinical efficacy and safety profile have been reported.[5] The compound does not produce GI irritation following either acute or chronic administration.[5] Pravadoline also demonstrates greater antinociceptive efficacy than most NSAIDs in several animal models.[5] These observations of enhanced efficacy suggested that an additional factor or factors, in conjunction with NSAID-related cyclooxygenase inhibition, could be contributing to the profile of pravadoline.

Subsequent evaluations in isolated tissue preparations revealed that pravadoline may be acting by dual mechanisms. The compound inhibits prostaglandin (PG) synthesis in vitro, with an IC_{50} of 5 μM in mouse brain preparations.[5] Pravadoline also functionally inhibits neuronally stimulated contractions of guinea pig ileum and mouse vas deferens (MVD) preparations.[6] The inhibitory effect in these isolated tissue preparations (also referred to herein as MVD activity) has been demonstrated within

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(1) Pravadoline is the USAN-approved name for 1.
(2) Grieco, G.; DeAndrade, J.; Dorflinger, E.; Kantor, T.; Saelens, J.; Sunshine, A.; Wang, R.; Wideman, G.; Zellman, V. Pravadoline Maleate. A New Non-Opiod Analgesic, in the Treatment of Post Operative Pain. *Clin. Pharmacol. Ther. (St. Louis)* **1989**, *45*, 123.
(3) Bell, M. R.; D'Ambra, T. E.; Kumar, V.; Eissenstat, M. A.; Herrmann, J. L.; Wetzel, J. R.; Rosi, D.; Philion, R. E.; Daum, S. J.; Hlasta, D. J.; Kullnig, R. K.; Ackerman, J. H.; Haubrich, D. R.; Luttinger, D. A.; Baizman, E. R.; Miller, M. S.; Ward, S. J. Antinociceptive (Aminoalkyl)indoles. *J. Med. Chem.* **1991**, *34*, 1099–1110.
(4) The pK_a of pravadoline has been measured in separate determinations to have a value of 5.0 to 5.3 (25 °C) by potentiometric titration in CH_3OH/H_2O and extrapolation to 100% H_2O. Sieber, J. C.; Wiehler, W. R.; Kelly, C. A.; Wood, D., Department of Analytical Sciences, Sterling Research Group, Rensselaer, NY, private communication.
(5) Haubrich, D. R.; Ward, S. J.; Baizman, E.; Bell, M. R.; Bradford, J.; Ferrari, R.; Miller, M.; Perrone, M.; Pierson, A. K.; Saelens, J. K.; Luttinger, D. Pharmacology of Pravadoline: A New Analgesic Agent. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 511–522.
(6) Ward, S. J.; Arnold, R.; Casiano, F. Pravadoline: Profile in Isolated Tissue Preparations. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 1230–1239.

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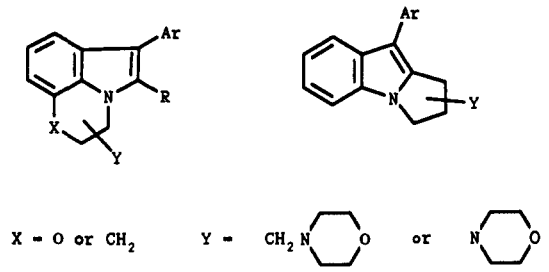


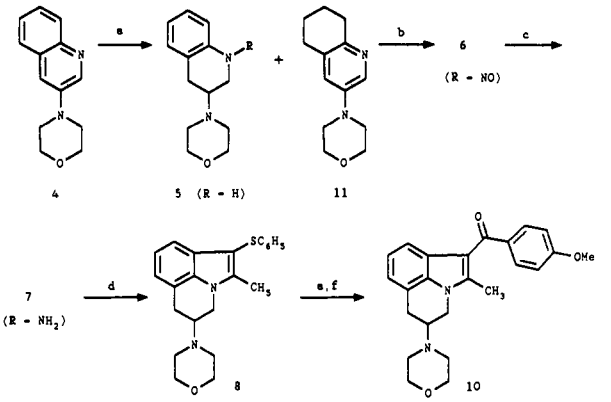
Figure 1.

the pravadoline series to correlate both with in vivo antinociceptive potency, independent of cyclooxygenase inhibition,[7] and with the inhibition of adenylate cyclase in cerebellar brain homogenates and primary cultures from rodents.[8]

This MVD functional activity of pravadoline is not shared by reference cyclooxygenase inhibitors (e.g., indomethacin, aspirin, ibuprofen, naproxen, acetaminophen, or ketorolac).[5] Further studies have identified close structural analogues of pravadoline which do not inhibit prostaglandin formation and possess potent MVD activity.[3,6,7,9] Extensive testing in isolated tissue preparations and in radioligand binding assays has ruled out the interaction of pravadoline with muscarinic, cholinergic, adrenergic, serotonin, opioid, purinergic, dopaminergic, histaminergic, glutaminergic, VIP, NPY, somatostatin, bombesin, GABAergic, neurokinin, bradykinin, and prostaglandin receptors.[6-8]

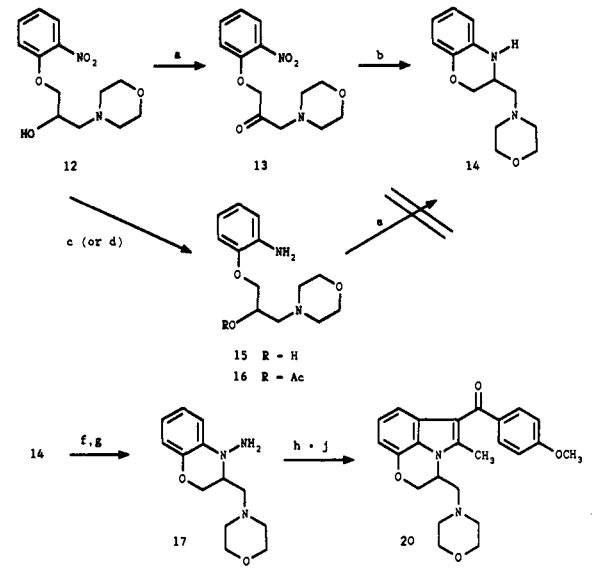
This report describes the synthesis and structure–activity relationships of side chain conformationally restrained analogues of pravadoline, as summarized in Figure 1. These compounds were studied to determine whether the activity of pravadoline was the result of location of the morpholine in a distinct three-dimensional position with respect to the rest of the molecule. Although not all possible orientations were explored, these studies have succeeded in defining a preferred positioning of the amine side chain for MVD activity, within the scope of the analogues presented. Representative nanomolar potent analogues have been resolved and enantioselectivity has been demonstrated, with eudismic ratios[10] greater than 20 000. The synthesis of a radioligand precursor from among these analogues is also described. The development and use of a binding assay, reported elsewhere,[11] with the radioligand derived from this precursor, has aided in the discovery that the second mechanism of action for pravadoline (MVD activity) is correlated to agonist interactions with the cannabinoid receptor.[11-13]

Scheme I



[a] (a) Na, *n*-BuOH, ↑↓; (b) $NaNO_2$, 2 N HCl; (c) $LiAlH_4$, THF; (d) phenylthioacetone, AcOH, ↑↓; (e) Ra-Ni, EtOH, ↑↓; (f) *p*-anisoyl chloride, $AlCl_3$, CH_2Cl_2.

Scheme II



[a] (a) (i) TFAA, DMSO, CH_2Cl_2, −78 °C; (ii) Et_3N; (b) H_2, Ra-Ni, EtOAc; (c) $SnCl_2$, HCl, EtOH; (d) (i) Ac_2O, C_5H_5N; (ii) H_2, Pd/C; (e) conc H_2SO_4 (ref 19); (f) $NaNO_2$, 2 N HCl; (g) $LiAlH_4$, THF; (h) phenylthioacetone, AcOH, ↑↓; (i) Ra-Ni, EtOH, ↑↓; (j) *p*-anisoyl chloride, $AlCl_3$, CH_2Cl_2.

(7) Ward, S. J.; Miller, M.; Luttinger, D.; Eissenstat, M. A.; Bell, M. Inhibitory Activity of the Analogs of Win 48098 in Isolated Tissue Preparations In Vitro is Reflective of a Mechanism of Antinociception. *Neurosci. Abstr.* **1988**, *14*, 324.

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Chemistry

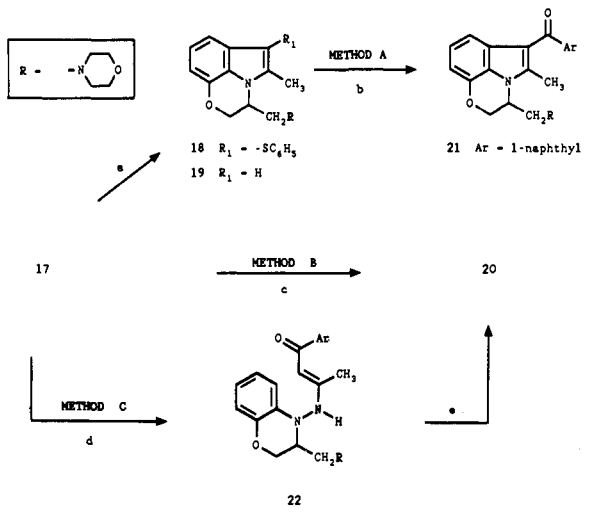
Preparations of the target compounds are summarized in Schemes I–VI The synthesis of the target **10** (Scheme I) was designed to parallel the preparation of somewhat related compounds by Stanton and Ackerman.[14] A key intermediate in the synthesis of **10** was the tetrahydroquinoline **5**. The quinoline precursor **4** was readily prepared by reaction of commercially available 3-aminoquinoline with 2-chloroethyl ether in 64% yield. Hydrogenation of **4** over PtO_2 in the presence of concentrated HCl,[14,15] however, afforded an inseparable 1:1 mixture of the tetrahydroquinoline derivative **5** and its tetrahydro isomer **11**. Derivatization of the mixture by treatment with $NaNO_2$/HCl and subsequent reagents, as in Scheme I, allowed easy separation of **8**, a transformation product of the desired isomer **5**, since **11** is inert to these conditions.

A more efficient synthesis of intermediate **8** was found when treatment of **4** with Na metal in refluxing *n*-BuOH[16] afforded **5** in 50% yield on a multigram scale, uncontaminated with any reduction byproducts.[17] Fischer indolization of **7** with phenylthioacetone in refluxing acetic acid afforded the indole **8** in 42% yield from the aniline **5**. Raney nickel desulfurization followed by Friedel–Crafts reaction provided the target **10**.

A similar indolization approach was utilized to prepare the pyrrolobenzoxazine **20** (Scheme II). Therefore, the key intermediate was the benzoxazine **14**. This compound has been reported to result from the treatment of **15** with concentrated H_2SO_4.[19] Perhaps because of the lack of available experimental detail, we have been unable to effect this cyclization on either **15** or its acetate derivative **16**.

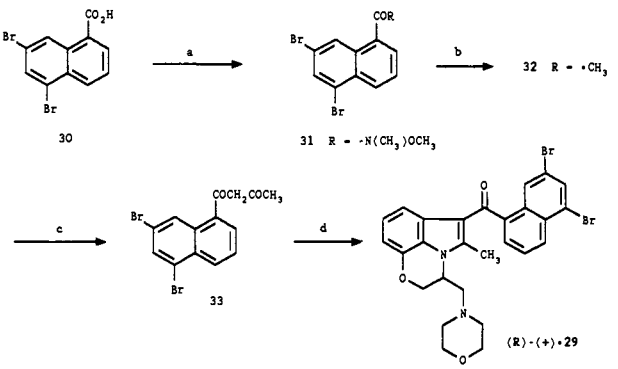
In contrast, Swern oxidation[20] of **12** afforded the very sensitive α-amino ketone **13** (81%).[21] Reductive cyclization of **13** with Ra-Ni[23] (96%) provided the key interme-

Scheme III



[a] (a) (i) Phenylthioacetone, AcOH, ↑↓; (ii) Ra-Ni, EtOH, ↑↓; (b) method A: acid chloride, $AlCl_3$, CH_2Cl_2; (c) method B: 4-methoxybenzoyl acetone, AcOH, ↑↓; (d) method C: 4-methoxybenzoyl acetone, $C_6H_5CH_3$, H^+, ↑↓; then (e) AcOH, ↑↓.

Scheme IV



[a] (a) (i) $SOCl_2$; (ii) $HN(CH_3)OCH_3$; (b) MeMgBr; (c) NaH, EtOAc, *p*-dioxane; (d) (*R*)-**17**, method C.

(13) (a) Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and Characterization of a Cannabinoid Receptor in Rat Brain. *Mol. Pharmacol.* **1988**, *34*, 605–613. (b) Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; DeCosta, B. R.; Rice, K. C. Cannabinoid Receptor Localization in Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1932–1936. (c) Howlett, A. C.; Bidaut-Russell, M.; Devane, W. A.; Melvin, L. S.; Johnson, M. R.; Herkenhan, M. The Cannabinoid Receptor: Biochemical, Anatomical and Behavioral Characterization. *Trends Neurosci.* **1990**, *13*, 420–423. (d) Howlett, A. C. Reverse Pharmacology Applied to the Cannabinoid Receptor. *Trends Pharmacol. Sci.* **1990**, *11*, 395–397. (e) Makriyannis, A.; Rapaka, R. S. The Molecular Basis of Cannabinoid Activity. *Life Sci.* **1990**, *47*, 2173–2184.

(14) Stanton, J. L.; Ackerman, M. H. Synthesis and Anticonvulsant Activity of Some Tetracyclic Indole Derivatives. *J. Med. Chem.* **1983**, *26*, 986–989.

(15) A somewhat related hydrogenation procedure, utilizing Pd catalyst to targets more closely resembling **5**, has been reported: Vecchietti, V.; Mussini, E.; Ferrari, G. N_1-Acyl-3-amino-1,2,3,4-tetrahydroquinoline compounds. Eur. Pat. Appl. Ep 72070 A2, 16 Feb 1983, 29 pp; *Chem. Abstr.* **1983**, *99*, 139794z.

(16) Bouveault, L.; Blanc, G. Preparation of Primary Alcohols from the Corresponding Acids. *C. R. Hebd. Seances. Acad. Sci.* **1903**, *136*, 1676–1678.

(17) Use of $NaBH_3CN$ in AcOH (ref 18) also worked well in converting **4** to **5** on a small scale. On a larger scale, however, the *N*-ethyl derivative of **5** predominated.

(18) (a) Gribble, G. W.; Heald, P. W. Reactions of Sodium Borohydride in Acidic Media; III. Reduction and Alkylation of Quinoline and Isoquinoline with Carboxylic Acids. *Synthesis* **1975**, 650–652. (b) Glennon, R. A.; Jacyno, J. M.; Salley, J. J., Jr. 2,3-Dihydro and Carbocyclic Analogues of Tryptamines: Interaction with Serotonin Receptors. *J. Med. Chem.* **1982**, *25*, 68–70. (c) Gribble, G. W.; Nutaitis, C. F. Sodium Borohydride in Carboxylic Acid Media. A Review of the Synthetic Utility of Acyloxyborohydrides. *Org. Prep. Proced. Int.* **1985**, *17*, 317–384.

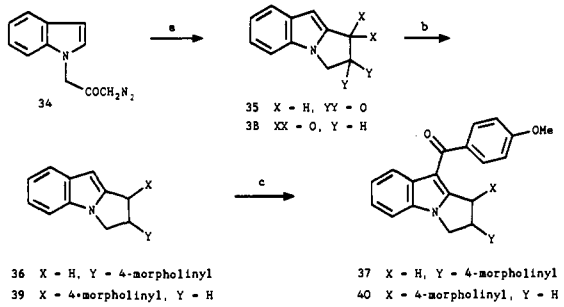
(19) Kurihara, T.; Takeda, H.; Ito, H.; Sagawa, K. Phenyl glycidyl ether derivatives. II. Synthesis of 3-substituted 3,4-dihydro-2H-1,4-benzoxazine and 3-substituted 3,4-dihydro-2H-1,4-benzothiazine. *Yakugaku Zasshi* **1968**, *88*, 1118–1122; *Chem. Abstr.* **1969**, *70*, 37748w.

(20) Omura, K.; Sharma, A. K.; Swern, D. Dimethyl Sulfoxide–Trifluoroacetic Anhydride: a New Reagent for Oxidation of Alcohols to Carbonyls. *J. Org. Chem.* **1976**, *41*, 957–962.

(21) (a) Other attempts at this oxidation ((1) CrO_3, H_2SO_4; (2) (i) HCl, (ii) CrO_3, H_2SO_4 with Na citrate workup (ref 22); or (3) PDC, CH_2Cl_2) were unsuccessful. (b) The ketone **13** has been observed to decompose at temperatures above 40 °C or even upon standing in the presence of basic impurities. Purified **13** is stable for long periods in the cold under a nitrogen atmosphere. Herrmann, J. L., Department of Chemical Development, Sterling Research Group, Rensselaer, NY, private communication.

(22) Mueller, R. H.; DiPardo, R. M. Chromium(VI) Oxidations of Secondary Alcohols in the Presence of Amino Groups, or How to Solubilize Chromium(III) in Base. *J. Org. Chem.* **1977**, *42*, 3210–3212.

Scheme V



[a] (a) $[Rh(OAc)_2]_2$, C_6H_6; (b) morpholine, $NaBH_3CN$, H^+; (c) *p*-anisoyl chloride, $AlCl_3$, CH_2Cl_2.

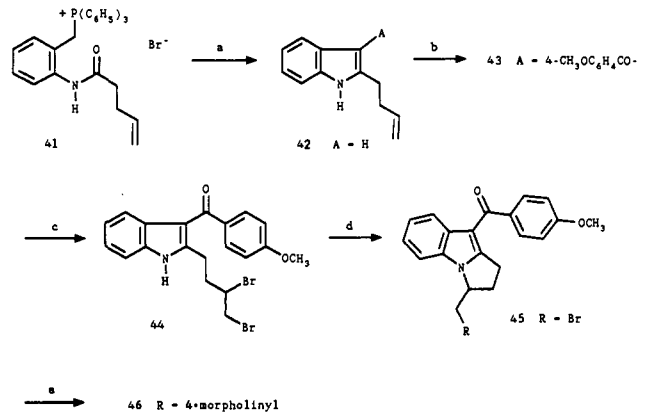
diate 14.[24] Conversion of 14 to the target 20 followed the indolization sequence and subsequent transformations analogous to those used for the synthesis of 10 in Scheme I.

For the synthesis of analogues of 20 by replacing the 4-methoxyphenyl ring with other aromatic systems (Table II), the precursor 19 presented a common intermediate, one step removed from the final targets. The first analogue prepared was 21, which was obtained by Friedel-Crafts acylation (hereinafter referred to as method A) of 19 with 1-naphthoyl chloride in 51% yield. Attempts to optimize this process and prepare larger quantities of 21 using excess $AlCl_3$ were unsuccessful.[25] These results, when coupled with our findings that the synthesis of the enantiomers of 20 and 21 would likely require the resolution of the intermediate 14, encouraged us to explore an alternative, shorter process from 14 to both 20 and 21.

Analogous to the reaction of the hydrazine 17 with phenylthioacetone in refluxing AcOH to afford directly the indole 18 (Schemes II and III), treatment of 17 with (4-methoxybenzoyl)acetone[26] in refluxing AcOH (hereinafter referred to as method B) afforded 20 directly in 27% yield from 17 (Scheme III). Even unoptimized, this three-step sequence from 14 to 20 represents an improvement over the original sequence of five steps in 16% overall yield. For the synthesis of the enantiomers of 20 from optically pure 14, even better yields were obtained when 17 was converted to the enamino ketone 22 in a separate step, then followed by refluxing in AcOH (hereinafter referred to as method C). Thus, in this manner a 53% yield of (*S*)-(−)-20 was obtained from (*S*)-(−)-14 and a 44% yield of (*R*)-(+)-20 from (*R*)-(+)-14.

Similar iterations afforded the remaining analogues in the benzoxazine subseries (Table II). Optically pure targets were obtained from enantiomerically pure 14, which was resolved by crystallizations with (+)- and (−)-dibenzoyltartaric acid (DBT). Absolute configuration was established by X-ray crystallographic analysis of the salt of (*R*)-(+)-14 with (−)-DBT. Analytical HPLC experiments were used to verify that the unlikely racemization of the enantiomers of 20 and 21 had not occurred in their preparation from optically pure 14.

Scheme VI



[a] (a) NaOtBu, $C_6H_5CH_3$, ↑↓; (b) (i) CH_3MgBr, Et_2O; (ii) *p*-anisoyl chloride; (c) Br_2, CH_2Cl_2; (d) NaH, DMF; (e) morpholine, 80 °C.

The synthesis of the radioligand precursor (*R*)-(+)-29 (Scheme IV) involved treatment of the novel dibromonaphthyl diketone 33 with (*R*)-17. The synthesis of 33 utilized the naphthoic acid 30.[27] The precursor (*R*)-(+)-29 was doubly tritiated to provide [^{3}H]-(*R*)-(+)-21.

The key intermediate in the synthesis of the target 37 was 35 (Scheme V), which resulted from an intramolecular carbenoid cycloaddition.[28] The α-diazo ketone 34 was synthesized from indole-1-acetic acid[29] using conventional procedures. When treated with a catalytic quantity of rhodium(II) acetate dimer in benzene at 25 °C, 34 cyclized cleanly to pyrroloindole 35. Borch amination of 35 with morpholine and sodium cyanoborohydride[30] gave rise to 36 (51% from 34), which was acylated with *p*-anisoyl chloride to afford the target 37. In similar fashion the analogue 40 was prepared from the ketone 38.[31]

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(25) Use of excess $AlCl_3$ gave a mixture of 3- and 4-monoacyl- and 3,4-diacylindole derivatives in addition to unreacted starting material (19).

(26) (a) Sardella, D. J.; Heinert, D. H.; Shapiro, B. L. Nuclear Magnetic Resonance Studies of Enol–Enol and Keto–Enol Equilibria in Substituted Benzoylacetones. *J. Org. Chem.* **1969**, *34*, 2817–2821. (b) Sprague, J. M.; Beckham, L. J.; Adkins, H. Preparation of 1,3-Diketones by the Claisen Reaction. *J. Am. Chem. Soc.* **1934**, *56*, 2665–2668.

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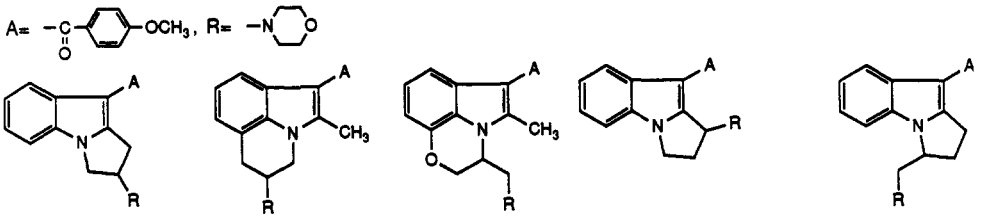
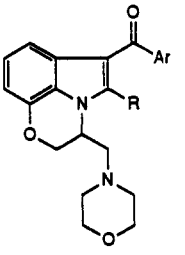
Table I. Side Chain Conformationally Restrained Analogues of Pravadoline

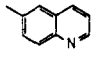
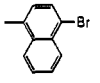


compd	MVD activity:[a] IC_{50} ± SE, μM (n)	PG synthesis inhibition:[b] IC_{50}, μM	ACh writhing:[c] ED_{50}, mg/kg po	[^{3}H]-AAI binding:[d] IC_{50}, nM (n)
1	0.453 ± 0.019 (4)	5 (1.7–13.3)	26 (19–35) (iv) 4 (2–7)	3155 ± 54 (3)
10	>10 (2)	12% @ 30 μM	30% @ 100 mg/kg	1.4% @ 1 μM
20	0.123 ± 0.013 (4)	72 (32–166)	30% @ 100 mg/kg (iv) 3.4 (0.3–37)	152 ± 17 (3)
37	>10 (2)	18% @ 30 μM		−13% @ 1 μM
40	>10 (2)	49% @ 30 μM	91 (54–143)	−16% @ 1 μM
46	0.32 (2)			3053 (1)
(*R*)-(+)-**20**	0.044 ± 0.013 (6)	50 (15–162)		106 ± 11 (3)
(*S*)-(−)-**20**	>10 (4)	30 (6–138)		17500 ± 6.19 (3)
indomethacin	>10	0.5 (0.4–0.7)		
desacetyllevonantrodol	0.0023 ± 0.00077			0.46 ± 0.019 (3)

[a] Mouse vas deferens inhibitory activity. Effects of (aminoalkyl)indole analogues in isolated tissue preparations in vitro. Values are the mean ± SE of *n* determinations. [b] Inhibition of PG synthesis in mouse brain microsomes, as described in ref 5. Values are the IC_{50} (with 95% confidence limits) or % inhibition @ 30 μM (*n* = 1). [c] ACh writhing assay in mice, as described in ref 5. Values are the ED_{50} with confidence limits in parentheses or the % inhibition @ 100 mg/kg. [d] (Aminoalkyl)indole (AAI) binding. Concentration of compound required to displace 50% of 0.5 nM [^{3}H]-(*R*)-(+)-**21** binding in rat cerebellum membranes as described in ref 11. Values are IC_{50} ± SE or % inhibition of binding @ 1 μM (*n* = 1).

Table II. Structure–Activity Relationships among Benzoxazine Analogues



compd	Ar	R	MVD activity:[a] IC_{50} ± SE, μM (n)	PG synthesis inhibition:[b] % at 30 μM	ACh writhing:[c] ED_{50}, mg/kg, iv	[^{3}H]-AAI binding:[d] IC_{50}, nM (n)
23	4-CH_3O-C_6H_4	H	0.0445 ± 0.0098 (2)	−9	2 (0.8–5)	249 ± 17 (3)
20	4-CH_3O-C_6H_4	CH_3	0.123 ± 0.013 (4)	12	3.4 (0.3–37)	152 ± 17 (3)
24	4-CH_3O-C_6H_4	C_2H_5	28% @ 10 μm (3)			27% @ 1 μM
25	2-F-C_6H_4	CH_3	0.076 ± 0.004 (2)	42		1426 ± 54 (3)
26	1-naphthyl	H	0.002 ± 0.00003 (2)			7.37 ± 0.92 (3)
21	1-naphthyl	CH_3	0.006 ± 0.0006 (4)	−35	0.25 (0.13–0.48) (po) 33 (12–86)	5.56 ± 0.41 (3)
27		CH_3	0.021 ± 0.00065 (4)	26	(po) 27 (16–45)	132.5 ± 14.53 (3)
28		CH_3	0.0022 ± 0.0002 (4)			2.32 ± 1.42 (3)
(*R*)-(+)-**21**			0.00043 ± 0.0001 (6)	9	(po) 33 (20–56)	2.77 ± 0.22 (3)
(*S*)-(−)-**21**			>10 (7)	10	(po) 10% @ 100	8002 ± 260 (3)
(*R*)-(+)-**29**						48.3 ± 3.18 (3)

For description of footnotes, refer to Table I.

The preparation of **46** is derived from the butenylindole **42**, which was obtained through an intramolecular Wittig-type reaction of the phosphonium amide **41** (Scheme VI).[32,33] Subsequent bromination of **43** afforded **44** (58%), treatment of which with NaH in DMF afforded a mixture of three products in which the pyrrolo[1,2-*a*]indole **45** was isolated in 11% yield. The desired **45** was converted to the target **46** using standard conditions.

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Biological Evaluation

The compounds were tested and compared to pravadoline (Tables I and II) for inhibition of PG synthesis in mouse brain microsomes[5] and tested in isolated mouse vas deferens preparations for inhibition of electrically stimulated contractions.[6] They were also tested in the mouse acetylcholine writhing assay for in vivo antinociceptive effect. Data are included for the reference compounds indomethacin (an NSAID) and desacetyl levonantrodol (a cannabinoid).

The radioligand [^{3}H]-(*R*)-(+)-**21** was prepared as part of these studies, and binding was characterized in rat brain membranes. This is described elsewhere.[11,34] An AAI binding assay in rat cerebellum membranes has been developed.[11] The compounds in Tables I and II were also tested in retrospective fashion for inhibition of radioligand binding in this assay.

Structure–Activity Relationships

The racemic derivatives **10**, **20**, **37**, and **40** (Table I) are much weaker than pravadoline (**1**) as inhibitors of PG synthesis. Restraining the amine side chain in these analogues has thus seriously weakened the NSAID component of pravadoline's profile. These results are not useful in identifying whether or not the morpholine in pravadoline can be suitably oriented for optimal PG synthesis inhibition, since the compounds in this study are poor substrates for this activity.

In contrast to the results for PG synthesis, the benzoxazine **20** inhibited electrically stimulated contractions in mouse vas deferens preparations (MVD activity), the racemate being 3-fold more potent (IC_{50} = 0.123 μM) than pravadoline (IC_{50} = 0.453 μM). It was subsequently determined that the activity of **20** is specific to the *R* enantiomer, with an eudismic ratio[10] >227. These preliminary results suggest that the optimum location of the morpholine for the MVD component of pravadoline's profile is in the lower right quadrant, beneath the plane defined by the indole ring (see Figure 1).

It was noted that the three MVD inactive analogues **10**, **37**, and **40** have the morpholine directly attached to the heterocyclic nucleus. The morpholine in these compounds is thus likely restricted to lie in or near the plane of the indole. In **20** the morpholine is positioned one carbon removed from the heterocyclic nucleus. To better characterize MVD activity with respect to the three-dimensional relationship of the morpholine side chain with the heterocyclic nucleus, we subsequently prepared the pyrroloindole **46**. In this compound, like **20**, the morpholine is separated from the heterocyclic nucleus by a methylene group. The compound was active in the MVD assay, although 2–3-fold less potent than **20**. This result supports a premise that the morpholine nitrogen is required to be held out of the plane of the heterocyclic nucleus. Furthermore, it is likely that the morpholine ring is better positioned for interaction with the biological target in **20** than in **46**. Preliminary modeling studies, excluding energetics, indicate that the conformations of **20** and **46** may share similar vectorial relationships in a defined three-point pharmacophore search.[35b] A more precise statement regarding the optimum location of the morpholine in pravadoline analogues for MVD activity, however, may be better supported by the synthesis and evaluation of further targets.

An extensive structure–activity study of simpler pravadoline analogues has been conducted.[3,9] In general we have found that at C-2 of the indole nucleus a hydrogen substituent is optimum for MVD activity, with H > CH_3 ≫ C_2H_5. For inhibition of PG synthesis, however, the methyl or ethyl substituent is best, with both more active than H. More importantly, it has also been demonstrated that a bicyclic aroyl substituent, such as naphthoyl, in place of the anisoyl group of pravadoline, confers significantly enhanced potency in the MVD assay, to the detriment of effects on PG synthesis.[3,9] In terms of side-chain length, MVD activity is optimal in an aminoethyl side chain; activity is usually lost or dramatically decreased in higher homologues.[9] These studies show that simple variations in the (aminoalkyl)indole structure can be used to synthesize either MVD-selective or NSAID-selective derivatives in this series.

Structure–activity relationships of a limited number of aroyl variations as well as substitutions at C-2 of the indole nucleus on the racemic benzoxazine **20** are presented in Table II. As inhibitors of PG synthesis, most of the compounds are inactive or at best weakly active (e.g., **27**). A significant range in potency was obtained, however, in the MVD assay. The three compounds, **20**, **23**, and **24** yielded results consistent with simpler analogues regarding C-2 indole substitution: H > CH_3 ≫ C_2H_5. The 2-ethylindole derivative **24** is essentially inactive. Similarly for the naphthoyl derivatives **21** and **26**, the 2-H analogue appears to be the more potent. Earlier studies analyzed the effects of C-2 indole substitution on the conformation of the aroyl group in simpler pravadoline derivatives.[3] Spectroscopic evidence, most notably NMR chemical shifts and NOE effects, support a major conformational difference of the aroyl group between the 2-H/2-CH_3 indole pair.[3] Similar NMR chemical shift differences are observed in comparison of the spectra of **20** and **23**. Since the morpholine side chain is similarly restrained in each, the observed change in MVD activity in changing indole substitution from 2-CH_3 to 2-H is likely the result of the aroyl conformational changes.[3,9]

The bicyclic aroyl derivatives **21** and **26–28** were more potent (2–21 nM) than the monocyclic 4-methoxybenzoyl of **20** (123 nM), which was expected upon the basis of earlier studies.[3,9] Like **20** (Table I), the activity of **21** was found to be specific to the *R* enantiomer. This eutomer is one of the most MVD-potent of the (aminoalkyl)indoles (AAIs) to date, with an IC_{50} of 0.43 nM and a eudismic ratio[10] greater than 23 000.

Several of these compounds have demonstrated in vivo antinociceptive activity in laboratory models. In the ACh-induced writhing assay in mice, **20** and **23** had antinociceptive ED_{50}'s in the range of 2–4 mg/kg upon intravenous administration. The most potent compound tested was **21**, which was at least 1 order of magnitude more potent than this. Though limited, these results are consistent with the more extensively studied correlation

(34) In these studies (ref 11) it was determined that the ligand bound to a single class of sites (K_d = 2 nM; B_{max} = 1.2 pmol/mg of protein) and was saturable, heat-labile, reversible, and stereospecific. Analogues of pravadoline completely inhibited radioligand binding with potencies extending over a 1000-fold range. IC_{50} values in this binding assay were correlated with MVD activity. Only cannabinoids completely inhibited binding in a competitive manner.

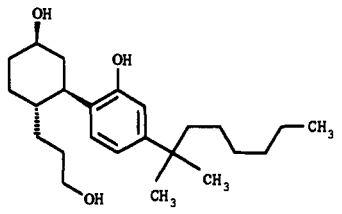
(35) (a) Bell, M. R.; D'Ambra, T. E.; Daum, S. J.; Eissenstat, M. A.; Estep, K. G.; Olefirowicz, E. M.; Ward, S. J. A Potential New Mechanism for Antinociception: Preliminary Structure–Activity Relationships at a Putative Aminoalkylindole Receptor. *Dialog in Science*, New Strategies in Pain Control, July 24, 1989, Princeton, NJ. (b) Olefirowicz, E. M.; Estep, K. G., Department of Medicinal Chemistry, Sterling Research Group, Rensselaer, NY, unpublished results.

of MVD activity with in vivo antinociceptive potency.[7]

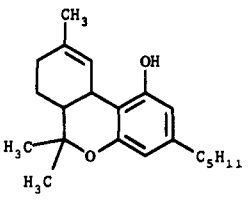
A potential correlation of MVD activity with observations of behavioral depression in mice has also been reported for simpler analogues of pravadoline at doses at and above the ED_{90} (iv and po) in the ACh writhing assay.[7,9,12] Behavioral depression in the ACh writhing assay for the analogues in Table II was noted in investigator observations of apparent sedation and/or ataxia at doses above ED_{50} values. Consistently, whereas the *R* enantiomer of **21** was antinociceptive in the ACh writhing assay and exhibited signs of behavioral depression, the *S* enantiomer was inert.

For further study into the nature of this MVD activity, we prepared a tritiated derivative of one of the more potent compounds to use as the ligand for a binding assay and for potential autoradiographic studies. A number of MVD-active (aminoalkyl)indoles have been profiled through Novascreen,[36a] initially to gain further insights into the MVD mechanism and later as a means of testing the specificity of some of the more potent MVD agonists. The choice of tritiating (*R*)-(+)-**21** was supported by its specificity; it did not appreciably displace radioligands in a large number of binding assays.[36] The dibromo radioligand precursor (*R*)-(+)-**29** was therefore synthesized and subsequently converted to the bis-tritiated analogue [^{3}H]-(*R*)-(+)-**21**.[37] The development and validation of the binding assay in rat brain homogenates utilizing [^{3}H]-(*R*)-(+)-**21**,[11] has demonstrated a close similarity of the AAI binding site with the synthetic cannabinoid (CP-55,940) binding site described by Howlett and colleagues.[11,13]

The analogues listed in Tables I and II have been evaluated in the AAI binding assay. The correlation between AAI binding potency and activity in the MVD assay has previously been demonstrated among a number of structural variations of pravadoline,[9,11] and the data for the analogues in Table II is generally consistent. Worth highlighting are critical correlations: (i) stereoselectivity, the *R* enantiomer is the eutomer (**20** and **21**), and (ii) compounds which are inactive in the MVD functional assay are also inactive in the binding assay (**10**, **24**, **37**, and **40**).



CP - 55,940



Δ^9 - THC

Discussion

It is not apparent from a quick inspection of structures that these (aminoalkyl)indoles would interact with the cannabinoid receptor at a site close to or equivalent to where the classical cannabinoids bind. The AAIs have significantly different structural features when compared to the tricyclic benzopyran skeleton of the cannabinoids. Most obvious is the presence of an amine which exhibits a stereochemical specificity for activity. This is in contrast to the phenolic, lipophilic cannabinoids. Cannabinoids have, however, been shown to display stereoselective properties.[38]

Cannabinoids and AAIs do share many biological properties. For example, cannabinoids are antinociceptive[39] and representative analogues possess analgesic potency approaching that of morphine in hot plate and tail-flick models of pain.[40] Both cannabinoids and AAIs inhibit adenylate cyclase in certain cell types,[8,41] and do so in a G-protein-dependent manner.[8b,41b] Animal behavioral effects of cannabinoids include ataxia in dogs, immobility and a biphasic change in spontaneous locomotor activity in rodents,[42] as well as behavioral depression, hypothermia, and catalepsy in mice.[38] It is clear from these reports and the data for the AAIs reported herein and elsewhere[7,8,11] that the similarities reported in the binding assays for the AAIs[11,12] and the classical cannabinoids[13] are mirrored in a wide range of in vitro and in vivo effects.

Pharmacophore development and modeling studies of the cannabinoid nucleus have identified proposed binding requirements and common features of nonclassical cannabinoids[41a] and cannabimimetic benzofurans.[43] The discovery of cannabinoid-mimetic AAIs presents an opportunity to further refine these models. An AAI pharmacophore hypothesis has been developed, and preliminary results have been reported.[35] The proposed pharmacophore model and its implications to previous models will be further described in due course.

Summary

We have prepared a number of side chain conformationally restrained analogues of pravadoline (**1**). Doing so resulted in a significant decrease in the ability of these analogues to inhibit PG synthesis relative to the activity of pravadoline. In contrast, MVD activity is sensitive to the location of the amine in three-dimensional space and was significantly enhanced in the conformationally restrained analogue **20**. This apparent optimum orientation of the morpholine, within the limits of the compounds

(36) (a) Novascreen, Nova Pharmaceutical Corporation, Baltimore, MD. (b) The effect of (*R*)-(+)-**21** was evaluated through Novascreen and elsewhere on the displacement of radioligands for the following binding sites: α_1-adrenergic, α_2-adrenergic, β-adrenergic, dopaminergic, histamine, muscarinic, nicotinic, adenosine, $GABA_B$, opioid, 5HT, substance P, excitatory amino acid, Arg vasopressin, VIP, PDE, nitrendipine, Ang II, bombesin, PDGF, EGF, LTB_4, and PCP. A listing for the methodology of each assay as well as the specific radioligand evaluated is described in ref 6.

(37) This conversion was performed under contract with E. I. du Pont de Nemours and Co. Medical Products Division, Boston, MA.

(38) Little, P. J.; Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacology and Stereoselectivity of Structurally Novel Cannabinoids in Mice. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1046–1051.

(39) Melvin, L. S.; Johnson, M. R.; Harbert, C. A.; Milne, G. M.; Weissman, A. A Cannabinoid Derived Prototypical Analgesic. *J. Med. Chem.* **1984**, *27*, 67–71.

(40) Wilson, R. S.; May, E. L.; Martin, B. R.; Dewey, W. L. 9-Nor-9-hydroxyhexahydrocannabinols. Synthesis, Some Behavioral and Analgesic Properties, and Comparison with the Tetrahydrocannabinols. *J. Med. Chem.* **1976**, *19*, 1165–1167.

(41) (a) Howlett, A. C.; Johnson, M. R.; Melvin, L. S.; Milne, G. M. Nonclassical Cannabinoid Analgetics Inhibit Adenylate Cyclase: Development of a Cannabinoid Receptor Model. *Mol. Pharmacol.* **1988**, *33*, 297–302. (b) Howlett, A. C.; Qualy, J. M.; Khachatrian, L. L. Involvement of G_i in the Inhibition of Adenylate Cyclase by Cannabimimetic Drugs. *Mol. Pharmacol.* **1986**, *29*, 307–313. (b) Rowley, J. T.; Rowley, P. T. Tetrahydrocannabinol Inhibits Adenyl Cyclase in Human Leukemia Cells. *Life Sci.* **1990**, *46*, 217–222.

(42) (a) Razdan, R. K. Structure–Activity Relationships in Cannabinoids. *Pharmacol. Rev.* **1986**, *38*, 75–149. (b) Compton, D. R.; Martin, B. R. Pharmacological Evaluation of Water Soluble Cannabinoids and Related Analogs. *Life Sci.* **1990**, *46*, 1575–1585.

(43) Mechoulam, R.; Breuer, A.; Jarbe, T. U. C.; Hiltunen, A. J.; Glaser, R. Cannabimimetic Activity of Novel Enantiomeric, Benzofuran Cannabinoids. *J. Med. Chem.* **1990**, *33*, 1037–1043.

Table III. Compounds Synthesized by Procedures Illustrated by Representative Examples

compd	method of synthesis[a]	% yield	formula	recrystn solvent	mp, °C	analysis
20	A	55	$C_{24}H_{26}N_2O_4$	EtOAc/hexane	182–189	C,H,N
(*R*)-(+)-**20**[b]	C	44	$C_{24}H_{26}N_2O_4$	Et_2O	151–153	C,H,N
21	A	51	$C_{27}H_{26}N_2O_3 \cdot CH_3SO_3H$	CH_3OH/Et_2O	257–260	C,H,N,S[c]
(*S*)-(−)-**21**[d]	C	44	$C_{27}H_{26}N_2O_3 \cdot CH_3SO_3H$	CH_3OH/Et_2O	256–260	C,H,N
(*R*)-(+)-**21**[e]	C	51	$C_{27}H_{26}N_2O_3 \cdot CH_3SO_3H$	CH_3OH/Et_2O	256–259	C,H,N
24[f]	B	12	$C_{25}H_{28}N_2O_4$	EtOAc/hexane	185.5–187.0	C,H,N
25	C	39	$C_{23}H_{23}FN_2O_3 \cdot CH_3SO_3H$	CH_3OH/Et_2O	241–245	C,H,N,F,S
26	C	15	$C_{26}H_{24}N_2O_3$	EtOAc	190–193	C,H,N
27	A	59	$C_{26}H_{25}N_3O_3$	EtOAc/hexane	192–198	C,H,N
28	C	36	$C_{27}H_{25}BrN_2O_3 \cdot CH_3SO_3H$	CH_3OH/Et_2O	281–286	C,H,N,Br,S
29[g]	C	23	$C_{27}H_{24}Br_2N_2O_3$	EtOAc/hexane	120	C,H,N[h]
37	A	50	$C_{23}H_{24}N_2O_3 \cdot CH_3SO_3H$	2-PrOH	138–142	C,H,N
40	A	55	$C_{23}H_{24}N_2O_3$	CH_3CN	168–171	C,H,N

[a] Refers to representative procedure described in the Experimental Section. [b] $[\alpha]^{25}_D$ = +55.1° (*c* = 1, $CHCl_3$). [c] Anal. Calcd for $C_{23}H_{23}FN_2O_3 \cdot CH_3SO_3H$: C, 64.35; H, 5.79; N, 5.36; S, 6.13. Found: C, 63.90; H, 5.91; N, 5.26; S, 6.47. [d] $[\alpha]^{25}_D$ = −39.4° (*c* = 1, DMF). [e] $[\alpha]^{25}_D$ = +40.2° (*c* = 1, DMF). A sample of the methanesulfonate salt was converted to the free base, $[\alpha]^{25}_D$ = +47.4° (*c* = 1, $CHCl_3$). Enantiomeric purity was found to be >99% by HPLC analysis. [f] The sodium salt of 1-(4-methoxyphenyl)-1,3-pentanedione (ref 26b) was refluxed with **17** for 4 h in AcOH. [g] $[\alpha]^{25}_D$ = +44.4° (*c* = 1, $CHCl_3$). [h] Anal. Calcd for $C_{27}H_{24}Br_2N_2O_3$: C, 55.50; H, 4.14; N, 4.79. Found: C, 55.95; H, 4.49; N, 4.56.

studied, was determined to be the lower right quadrant of the molecule, beneath the plane defined by the indole ring. A number of nanomolar potent analogues in the benzoxazine subseries have been prepared. Through the development of a binding assay using tritiated (*R*)-(+)-**21**, it has been shown that the AAI binding site is functionally equivalent with the CP-55940 cannabinoid binding site. A number of parallels between the in vitro and in vivo effects of both the (aminoalkyl)indoles and the cannabinoids exist. These novel indole structures represent a new class of compounds which possess cannabinoid agonist activity.

Experimental Section

Proton (^1H) NMR spectra were measured at 270 MHz on a JEOL FX instrument, at 300 MHz on a General Electric QE-300 instrument, or at 60 MHz on a Varian T60 instrument using $CDCl_3$ or DMSO-d_6 as solvent. Carbon (^{13}C) NMR spectra were measured at 67.8 MHz using the JEOL FX instrument or at 75.5 MHz on the General Electric instrument. Infrared spectra were measured on a Nicolet 20 SX FT IR or on a Perkin-Elmer Model 467 instrument. Ultraviolet spectra were measured on a Gilford Response UV-vis spectrophotometer. Mass spectra were measured on a JEOL JMS-01SC. Optical rotations were obtained on an Autopol III polarimeter (Rudolph Research). Elemental analyses were performed by Galbraith Laboratories of Knoxville, TN. Melting points are uncorrected. All structures were consistent with NMR, IR, MS, UV, and TLC. Unless specifically designated, all structures represent racemic mixtures.

Reactions requiring anhydrous or oxygen-free conditions were conducted in oven-dried (120 °C) glassware under an atmosphere of dry N_2. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl under a nitrogen (N_2) atmosphere.

Analytical thin-layer chromatography (TLC) was performed on E. Merck 5 × 20 cm Kieselgel 60 F-254 plates. Preparative high-pressure liquid chromatography (HPLC) was performed on a Waters Prep 500 instrument using two SiO_2 cartridges. Flash chromatography was performed using 230–400 mesh Kieselgel 60 (E. Merck).

The enantiomeric purity of **14** was determined on the in situ generated trifluoroacetanilide derivative using a Chirasil-Val III column (25 m × 0.25 mm, i.d., FSOT) on a Varian 3700 gas chromatograph instrument with a flame ionization detector. The oven temperature was 185 °C, the injection port temperature was 260 °C, and the detector temperature was 280 °C. The nitrogen flow rates were as follows: carrier flow of 1 mL/min; splitter flow of 50 mL/min; makeup flow of 25 mL/min. This procedure provided base line separation of the enantiomers with accuracy to within ±0.1%. Additional details on this derivatization follow the description of the preparation of **14**.

An HPLC method was also employed for enantiomeric excess (ee) determinations of **14**. The chromatographic column was an alpha-1 acid glycoprotein immobilized on 5-μm silica gel (Enantiopac, Pharmacia LKB Biotechnology), 10 cm in length, 4.0 mm i.d. The chromatographic system consisted of a Beckman 110A solvent-delivery pump, a Kratos 757 ultraviolet/visible variable-wavelength detector monitored at 240 nm and equipped with a flow cell of 8.0-mm path length, and a Micromeritics 728 Autosampler equipped with an electrically actuated Valco six-port injection valve fitted with a 10-μL loop. The mobile phase consisted of 3% 2-PrOH in 20 mM sodium phosphate buffer, pH 7.0, prepared by dilution of a 0.4 M aqueous stock solution. The solvent was delivered at a flow rate of 0.3 mL/min.

For ee determinations of **21**, the Beckman HPLC system was used with the following variations: the detector was set to 228 nm, the column was a Chiralcel OD (tris 2,5-dimethylphenyl carbamate cellulose) 5-μm particles, 25-cm length (Daicel). The mobile phase was CH_3OH/2-PrOH/CH_3CN (90:10:1) at 1.0 mL/min. Representative retention times were 9.1 min for the *S*-(−)-isomer and 11.7 min for the *R*-(+)-isomer.

The radioligand [^3H]-(*R*)-(+)-**21** was prepared at Du Pont/NEN by the catalytic (10% Pd/C) tritiation of the precursor (*R*)-(+)-**29** in EtOH overnight. After labile tritium removal, the compound was purified on TLC. In our laboratories, a control experiment using H_2 gas and Pd/C was performed, and the resulting (*R*)-(+)-**21** was determined by HPLC to be enantiomerically pure.

3-(4-Morpholinyl)quinoline (4). To a suspension of 30 g (0.75 mol) of 60% NaH in 500 mL of DMF was added dropwise a solution of 40 g (0.28 mol) of 3-aminoquinoline in 100 mL of DMF. After this addition was complete, 46 mL (0.39 mol) of 2-chloroethyl ether was added at once. The mixture was stirred at ambient temperature. As TLC analysis (1:1 hexane/EtOAc) indicated the reaction to be incomplete, additional NaH and alkylating agent were added. The mixture was then allowed to stir at ambient temperature for 3 days. Excess hydride was then carefully quenched by the dropwise addition of H_2O. The reaction mixture was partitioned between EtOAc and H_2O. The aqueous layer was extracted further with another portion of EtOAc. The combined organic fractions were washed with saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated to about 200 mL. The resulting suspension was diluted with hexane, and the solids were collected and washed with more hexane to afford 38.4 g (64%) of **4**. This material was routinely used directly in the next step. An elemental analysis was performed on the hydrochloride salt, recrystallized from 2-PrOH: mp 113–115 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.89 (d, *J* = 2.1 Hz, 1 H), 8.56 (m, 1 H), 7.94 (d, *J* = 1.2 Hz, 1 H), 7.87 (m, 1 H), 7.72 (m, 2 H), 3.93 (m, 4 H), 3.40 (m, 4 H). Anal. Calcd for $C_{13}H_{14}N_2O \cdot 1.33HCl \cdot 2.25H_2O$: C, 51.46; H, 6.59; N, 9.23; Cl, 15.58. Found: C, 51.38; H, 6.73; N, 9.31; Cl, 15.70.

1,2,3,4-Tetrahydro-3-(4-morpholinyl)quinoline (5). A mechanically stirred solution of 76.4 g (0.36 mol) of **4** in 1300 mL of *n*-BuOH in a 3-L, three-neck flask under N_2 was brought to reflux. To this solution was added over 1 h at a rate to maintain a reflux 60 g (2.6 mol) of sodium pellets. Additional sodium was added until all of **4** had been consumed, as measured by TLC

analysis (1:1 hexane/EtOAc). The mixture was then cooled to room temperature, 1 L of H_2O was added, and the mixture was stirred until all solids had dissolved. The phases were separated and the *n*-BuOH phase was washed with 1 L of saturated aqueous NaCl. The combined aqueous fractions were extracted with EtOAc. The combined organics were concentrated under reduced pressure. The concentrate was diluted with EtOAc, filtered, and then concentrated again. The residue was dissolved in EtOAc, flashed through a plug of SiO_2, and concentrated to a solid. The solid was combined with that from a previous experiment. An amount of 3.5 g (16.4 mmol) of **4** was purified by preparative HPLC, eluting with 1:1 hexane/EtOAc. So obtained was 40.9 g (50%) of **5**. An analytical sample was prepared by recrystallization from EtOAc: mp 99.5–102.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (m, 2 H), 6.63 (dd, J = 7.6 and 7.1 Hz, 1 H), 6.48 (d, J = 8.4 Hz, 1 H), 3.74 (m, 4 H), 3.48 (m, 1 H), 3.10 (m, 1 H), 2.92 (m, 1 H), 2.77 (m, 2 H), 2.65 (m, 4 H). Anal. ($C_{13}H_{18}N_2O$) C, H, N.

5,6-Dihydro-2-methyl-5-(4-morpholinyl)-1-(phenylthio)-4*H*-pyrrolo[3,2,1-*ij*]quinoline (8). To a mechanically stirred solution of 28.7 g (0.13 mol) of aniline **5** in 600 mL of 2 N HCl at 0 °C was added over 10 min a solution of 10.3 g (0.15 mol) of $NaNO_2$ in 100 mL of H_2O. The mixture was stirred for 1 h at 0 °C, then diluted with 700 mL of H_2O and 700 mL of EtOAc, and was made neutral by the cautious addition of solid $NaHCO_3$. The organic phae was washed with 1 L of saturated aqueous NaCl, dried over $MgSO_4$, and filtered. Concentration under reduced pressure afforded **6** as a solid. This solid was reduced directly as follows.

To 1 L of THF under N_2 at 0 °C in a 3-L, three-neck flask fitted with a mechanical stirrer was cautiously added 8 g (0.21 mol) of powdered $LiAlH_4$. The nitrosamine **6** was added as a solution in 100 mL of THF. After the addition was complete, the mixture was refluxed for 1 h. **Caution**: *it should be noted that in the $LiAlH_4$ reduction of this and related nitrosamines described herein that there is/can be an induction period. The out-of-control refluxing and frothing that have resulted can be controlled by using oversized glassware and cautiously warming the mixture to reflux.* The reaction mixture was then cooled in an ice bath, and 30 mL of saturated aqueous Na_2SO_4 was cautiously added dropwise. The resulting thick suspension was stirred for a few minutes and then filtered through a plug of Celite, and the filter cake was washed thoroughly with EtOAc. The organics were concentrated under reduced pressure to afford the hydrazine **7**.

The hydrazine **7** and 23.7 g (0.14 mol) of phenylthioacetone were dissolved in 500 mL of glacial AcOH and refluxed for 1 h. The reaction mixture was allowed to cool and then diluted with 2 L of H_2O and 1500 mL of EtOAc. The resulting mixture was made neutral by the careful, slow addition of solid $NaHCO_3$. The organics were dried over $MgSO_4$, filtered, and concentrated. This material was purified by preparative HPLC, eluting with 2:1 hexane/EtOAc. The product-containing fractions afforded 20 g (42% yield for three steps) of (phenylthio)indole **8** as a solid. A 3-g sample was recrystallized from EtOAc/hexane to afford 2.3 g of analytically pure **8**: mp 147.5–149.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 7.6 Hz, 1 H), 7.16–6.98 (m, 6 H), 6.93 (d, J = 7.1 Hz, 1 H), 4.32 (m, 1 H), 3.92 (dd, J = 12.4 and 10.0 Hz, 1 H), 3.77 (m, 4 H), 3.27 (m, 2 H), 3.06 (dd, J = 15.1 and 10.8 Hz, 1 H), 2.76 (m, 4 H), 2.47 (s, 3 H). Anal. ($C_{22}H_{24}N_2OS$) C, H, N, S.

5,6-Dihydro-2-methyl-5-(4-morpholinyl)-4*H*-pyrrolo[3,2,1-*ij*]quinoline (9). To a mechanically stirred solution of 17.7 g (48.6 mmol) of (phenylthio)indole **8** in 1 L of absolute EtOH was added 8 spoonfuls of Ra-Ni, and the mixture was refluxed for 1 h. At this time, TLC analysis (3:3:94 2-$PrNH_2$/CH_3OH/$CHCl_3$) indicated that the conversion was approximately two-thirds complete. An additional four teaspoons of Ra-Ni was added, and the mixture was refluxed for 70 min more. The mixture was cooled, and the Ra-Ni was allowed to settle. While still warm, the majority of the solution was decanted and filtered through Celite (the Ra-Ni was kept "wet" under some solvent due to its potentially flammable nature). In similar fashion, the Ra-Ni was washed three times with EtOAc, by allowing the catalyst to settle and decanting off the solvent through Celite. The organics were concentrated under reduced pressure. The residue was dissolved in EtOAc, filtered through a plug of SiO_2, and concentrated to a solid. This solid was dissolved in CH_2Cl_2 and purified by preparative HPLC, eluting with 2:1 hexane/EtOAc to afford 9.8 g (79%) of **9**. An analytical sample was prepared by recrystallization of 2.2 g from EtOAc/hexane (1:2) to afford 1.4 g of **9**: mp 112–114 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, J = 7.8 Hz, 1 H), 6.96 (dd, J = 7.8 and 7.1 Hz, 1 H), 6.85 (d, J = 7.1 Hz, 1 H), 6.18 (s, 1 H), 4.25 (m, 1 H), 3.83 (dd, J = 12.4 and 10.0 Hz, 1 H), 3.77 (m, 4 H), 3.20 (m, 2 H), 3.00 (dd, J = 15.2 and 10.9 Hz, 1 H), 2.75 (m, 4 H), 2.40 (s, 3 H). Anal. ($C_{16}H_{20}N_2O$) C, H, N.

[5,6-Dihydro-2-methyl-5-(4-morpholinyl)-4*H*-pyrrolo-[3,2,1-*ij*]quinolin-1-yl](4-methoxyphenyl)methanone (10). A representative procedure for method A follows: To a suspension of 7.1 g (53.3 mmol) of $AlCl_3$ in 100 mL of CH_2Cl_2 was added rapidly 5.9 mL (35.7 mmol) of *p*-anisoyl chloride. The resulting mixture was stirred for 1 h. This solution was then added dropwise over 10 min to a solution of 7.6 g (29.7 mmol) of indole **9** in 100 mL of CH_2Cl_2. The red mixture was then gently refluxed for 20 min. The mixture was cooled and poured into 300 mL of ice/water, and the CH_2Cl_2 was evaporated under reduced pressure. The remaining mixture was diluted with EtOAc and neutralized by the cautious addition of solid $NaHCO_3$. The mixture was filtered through Celite, and the organics were washed with saturated aqueous NaCl, dried over $MgSO_4$, and filtered. Concentration under reduced pressure afforded a white solid. The solid, which TLC analysis (1:1 hexane/EtOAc) showed to contain unreacted indole **9**, was dissolved in EtOAc, treated with charcoal, and filtered through SiO_2. Partial concentration under reduced pressure afforded 2.0 g of pure **10** and 4.0 g of a mixture of **9** and **10** in the mother liquors. The solid obtained from the mother liquors was recrystallized from EtOAc, affording an additional 1.0 g of **10**, for a combined yield of 3.0 g (26%). An analytical sample was prepared by recrystallization from EtOAc, mp 200.5–202.0 °C. Anal. ($C_{24}H_{26}N_2O_3$) C, H, N.

3-(4-Morpholinyl)-1-(2-nitrophenoxy)-2-propanone (13). To a −78 °C solution of 12 mL (0.169 mol) of DMSO in 90 mL of CH_2Cl_2 was added slowly 21 mL (0.148 mol) of trifluoroacetic anhydride. The mixture was stirred for 15 min after which a solution of 30 g (0.106 mol) of the alcohol **12**[44] in 40 mL of CH_2Cl_2 was added dropwise over 15 min. The resulting mixture was stirred at −78 °C for 30 min, and then 60 mL (0.434 mol) of triethylamine was added rapidly. The cold bath was removed, and the mixture was gradually warmed to ambient temperature. After stirring for 30 min, the reaction mixture was diluted with 250 mL of saturated aqueous $NaHCO_3$ and the phases were mixed. The organics were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a solid. The solid was slurried with Et_2O, filtered, and washed further with Et_2O to afford 24 g (81%) of **13** as a white solid. This was used directly in the subsequent reduction step, as it was observed in previous experiments that the material could readily decompose: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.93 (dd, J = 8 and 2 Hz, 1 H), 7.57 (m, 1 H), 7.12 (dd, J = 8 and 7.5 Hz, 1 H), 6.98 (d, J = 7.5 Hz, 1 H), 4.78 (s, 2 H), 3.74 (m, 4 H), 3.56 (s, 2 H), 2.58 (m, 4 H); IR (KBr) 2930, 2825, 1728, 1605, 1518, 1349 cm^{-1}.

3,4-Dihydro-3-[(4-morpholinyl)methyl]-2*H*-1,4-benzoxazine (14). To a solution of 22 g (78.6 mmol) of **13** in 1250 mL of EtOAc (reductions of **13** were limited by its poor solubility) was added 4 spoonfuls of Ra-Ni, and the suspension was hydrogenated on a Parr shaker at 45 psi of H_2 pressure for 6 h. The solution was decanted from the Ni and filtered through Celite. The filtrate was concentrated under reduced pressure to afford 17.7 g (96% yield) of **14** as a light brown solid. An analytical sample was prepared by treatment of an EtOAc solution of **14** with charcoal followed by recrystallization to afford a white powder: mp 117–121 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (m, 2 H), 6.63 (m, 2 H), 4.34 (br s, 1 H), 4.21 (dd, J = 10.4 and 2.7 Hz, 1 H), 3.85 (dd, J = 10.4 and 7.4 Hz, 1 H), 3.72 (m, 4 H), 3.56

(44) (a) Nordmann, J.; Faure, R.; Loiseau, G.; Mattioda, G. 1-Amino-3-(aryloxy)-2-propyl Nicotinate Pharmaceuticals. *Fr. Demande* 2,223,006, 25 Oct 1974, 9 pp: *Chem. Abstr.* **1975**, *82*, 156088p. (b) Nordmann, J.; Faure, R.; Mattioda, G.; Loiseau, G.; Millischer, R. Esters of p-Chlorophenoxyisobutyric Acid Useful as Drugs. *Fr. Demande* 2,228,480, 06 Dec 1974, 11 pp: *Chem. Abstr.* **1975**, *83*, 27940g.

(m, 1 H), 2.58 (m, 2 H), 2.42 (m, 2 H), 2.37 (d, J = 6.6 Hz, 2 H). Anal. ($C_{13}H_{18}N_2O_2$) C, H, N.

Derivatization Procedure and Gas Chromatographic Analysis of 14 for Enantiomeric Purity. Approximately 5 mg of 14 or the dibenzoyltartrate salt of 14, as subsequently described, was dissolved in 2 mL of EtOAc in a 1-dram vial. To this solution was added 0.1 mL of Et_3N and 0.1 mL of trifluoroacetic anhydride, and the mixture was refluxed for 15 min. To the cooled solution was added, just prior to chromatography, 2 mL of 5% aqueous NH_4OH with adequate mixing. A 1-mL aliquot of the EtOAc phase was then transferred to another vial containing 100 mg of $MgSO_4$. A 1-μL aliquot of this EtOAc solution was then injected onto a Chirasil-Val III column for separation of enantiomers. Representative retention times were 15.9 min for the S isomer and 16.2 min for the R isomer, with base-line separation.

(*R*)-(+)-3,4-Dihydro-3-[(4-morpholinyl)methyl]-2*H*-1,4-benzoxazine (14). Many initial attempts to resolve either enantiomer of 14 as the dibenzoyltartaric acid salts crystallizing from acetone/hexane mixtures only resulted in modest enantiomeric enhancements in ratios of approximately 45:55. Resolution was ultimately achieved on these mixtures by crystallization from CH_3OH. Thus 23.5 g of the (-)-dibenzoyltartrate salt of 14 was recrystallized twice from CH_3OH to afford an off-white solid containing enantiomerically pure (*R*)-(+)-14 by GC analysis. Characterization revealed this salt to be a 2:1 complex with 14/dibenzoyltartaric acid, respectively: mp 177.5–179.5 °C; $[\alpha]_D$ = -39.8° (c = 1 in DMF). A sample of these crystals was submitted for X-ray structural analysis. Anal. ($C_{13}H_{18}N_2O_2 \cdot 0.5C_{18}H_{14}O_8$) C, H, N.

The free base of (*R*)-(+)-14 was made by partitioning the salt between EtOAc and saturated aqueous Na_2CO_3. An analytical sample was prepared by recrystallization from EtOAc/hexane: mp 94–96 °C; $[\alpha]_D$ = +28.1° (c = 1 in $CHCl_3$). Anal. ($C_{13}H_{18}N_2O_2$) C, H, N.

(*S*)-(-)-3,4-Dihydro-3-[(4-morpholinyl)methyl]-2*H*-1,4-benzoxazine (14). In a similar manner, the levorotatory enantiomer of 14 was prepared by recrystallizations of the (+)-dibenzoyl-D-tartaric acid salt from CH_3OH, enantiomerically pure to GC analysis. The free base was prepared as above to provide (*S*)-(-)-14: mp 93–95 °C; $[\alpha]_D$ = -28.0° (c = 1 in $CHCl_3$). Anal. ($C_{13}H_{18}N_2O_2$) C, H, N.

1-Amino-3,4-dihydro-3-[(4-morpholinyl)methyl]-2*H*-1,4-benzoxazine (17). In a procedure analogous to the nitrosation of 5, 55.6 g (0.24 mol) of 14 in 1 L of 2 N aqueous HCl at 0 °C was reacted with 18 g (0.26 mol) of an aqueous solution of $NaNO_2$. The resulting nitrosamine was reduced with $LiAlH_4$ in THF, similar to the procedure described for the reduction of 6 to afford 58.4 g of the hydrazine 17. *Caution is advised, refer to the procedure for the reduction of 6 to 7.* 17: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.08 (m, 1 H), 6.90 (m, 1 H), 6.73 (m, 2 H), 4.23 (m, 2 H), 3.95 (br s, 2 H), 3.70 (m, 4 H), 3.52 (m, 1 H), 2.77 (dd, J = 13 and 5.5 Hz, 1 H), 2.60–2.40 (m, 5 H).

2,3-Dihydro-5-methyl-3-[(4-morpholinyl)methyl]-6-(phenylthio)pyrrolo[1,2,3-*de*]-1,4-benzoxazine (18). A solution of 57.9 g of the hydrazine 17 obtained in the previous experiment and 40 g (0.24 mol) of phenylthioacetone in 500 mL of AcOH was refluxed for 1 h. The reaction was worked up as in the synthesis of 8. The product residue was dissolved in EtOAc and treated with charcoal. The solution was filtered through SiO_2 and concentrated to about 500 mL, whereupon solids crystallized out, yielding 25 g after collecting and rinsing with Et_2O. The remaining material was purified by preparative HPLC eluting with 2:1 hexane/EtOAc to afford an additional 4.7 g (combined yield, 29.7 g (33%)) of 18 and 25.5 g (45%) of recovered 14. An analytical sample of 18 was prepared by two recrystallization from EtOAc/hexane (1:3) to afford an off-white solid: mp 161–164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 (m, 3 H), 7.02 (m, 4 H), 6.66 (d, J = 7.3 Hz, 1 H), 4.80 (d, J = 11.4 Hz, 1 H), 4.40 (m, 1 H), 4.27 (dd, J = 11.4 and 1.2 Hz, 1 H), 3.69 (m, 4 H), 2.73 (dd, J = 13.2 and 8.8 Hz, 1 H), 2.51 (s, 3 H), 2.50 (m, 5 H). Anal. ($C_{22}H_{24}N_2O_2S$) C, H, N, S.

2,3-Dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazine (19). In a procedure analogous to the desulfurization of 8, 29.7 g (78 mmol) of 18 was reacted with Ra-Ni in refluxing EtOH to afford 18.5 g (87%) of 19 after recrystallization from 800 mL of EtOH. An analytical sample was prepared by recrystallization from EtOAc/hexane: mp 178–180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, J = 7.7 Hz, 1 H), 6.92 (dd, J = 7.7 and 7.7 Hz, 1 H), 6.59 (d, J = 7.7 Hz, 1 H), 6.18 (s, 1 H), 4.79 (d, J = 11.0 Hz, 1 H), 4.32 (m, 1 H), 4.19 (dd, J = 11.0 and 1.2 Hz, 1 H), 3.71 (m, 4 H), 2.67 (dd, J = 13.0 and 9.4 Hz, 1 H), 2.58–2.38 (m, 5 H), 2.45 (s, 3 H). Anal. ($C_{16}H_{20}N_2O_2$) C, H, N.

(*S*)-(-)-20. A representative procedure for method C follows: A solution of 5.8 g of (*S*)-17, 5 g (0.026 mol) of 1-(4-methoxyphenyl)-1,3-butanedione[26] and 0.5 g of pyridinium 3-nitrobenzenesulfonic acid in 300 mL of H_2O was refluxed for 3 h using a Dean–Stark water trap connected to a reflux condenser. The cooled solution was filtered and concentrated under reduced pressure.

The concentrate was dissolved in 250 mL of AcOH and refluxed for 1 h. The cooled solution was then concentrated under reduced pressure, and the residue was partitioned between H_2O and EtOAc. Concentrated aqueous NH_4OH was added until the aqueous phase was alkaline. The organics were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Chromatography on 200 g of SiO_2, eluting with 1:1 hexane/EtOAc, and crystallization from Et_2O afforded 5.1 g of (*S*)-(-)-20. An additional 0.5 g was obtained by further chromatography of mixed fractions, for a combined yield 5.6 g (53%) from (*S*)-(-)-14. An analytical sample was prepared by recrystallization from Et_2O: mp 149–150 °C; $[\alpha]_D$ = -53.8° (c = 1 in $CHCl_3$). Anal. ($C_{24}H_{26}N_2O_4$) C, H, N.

[2,3-Dihydro-3-[(4-morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](4-methoxyphenyl)methanone (23). A representative procedure for method B follows: A solution of 13 g (52.2 mmol) of hydrazine 17 and 10.2 g (57.3 mmol) of 1-(4-methoxyphenyl)-1,3-propanedione[26b] in 300 mL of AcOH was refluxed for 1 h. The product was purified by preparative HPLC, eluting with 1:2 hexane/EtOAc to afford 1.8 g (9%) of 23 after recrystallization from EtOAc: mp 209–214 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J_{AB} = 8.6 Hz, 2 H), 7.84 (s, 1 H), 7.78 (d, J = 8.0 Hz, 1 H), 7.18 (dd, J = 8.0 and 7.8 Hz, 1 H), 7.01 (d, J_{AB} = 8.6 Hz, 2 H), 6.79 (d, J = 7.8 Hz, 1 H), 4.46 (m, 3 H), 3.91 (s, 3 H), 3.73 (br t, J = 4.6 Hz, 4 H), 2.80–2.60 (m, 4 H), 2.52–2.43 (m, 2 H). Anal. ($C_{23}H_{24}N_2O_4$) C, H, N.

1-(4-Bromo-1-naphthalenyl)-1,3-butanedione. To a 1-L, three-neck oven-dried flash charged with 5.3 g (0.13 mol) of 60% NaH, 300 mL of anhydrous Et_2O, and 1 mL of EtOH under a N_2 atmosphere was added at ambient temperature over 5 min a solution of 30 g (0.12 mol) of (4-bromo-1-naphthalenyl)methyl ketone[45] in 50 mL of anhydrous Et_2O followed by the rapid addition of 47 mL (0.48 mol) of EtOAc. The mixture was refluxed for 3 h. The solution was diluted with 500 mL of H_2O and stirred, and the organic phase was extracted further with 300 mL of H_2O. The combined aqueous fractions were added to 500 mL of EtOAc and acidified with stirring by the addition of concentrated HCl. The organic phase was dried over $MgSO_4$, filtered, and concentrated to afford 24.2 g (70%) of a tan solid.

1-(5,7-Dibromo-1-naphthalenyl)ethanone (32). The acid chloride made from two batches (11.5 g (35 mmol) and 8.2 g (25 mmol)) of 30,[27] and 6.0 g (61.5 mmol) of N,O-dimethylhydroxylamine hydrochloride were dissolved in 750 mL of CH_2Cl_2 and cooled to 0 °C. A solution of 60 mL (431 mmol) of Et_3N in 250 mL of CH_2Cl_2 was added dropwise, and the mixture was stirred overnight at ambient temperature. The mixture was then filtered and concentrated under reduced pressure. The resulting solids were slurried in CH_2Cl_2 and filtered, and the filtrate was chromatographed, eluting with 9:1 hexane/EtOAc, to afford 12 g (74%) of 31 as a yellow solid: ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.35–7.75 (m, 3 H), 7.65–7.40 (m, 2 H), 3.43 (s, 3 H), 3.37 (s, 3 H).

To this solid (44.3 mmol) in 400 mL of freshly distilled THF under N_2 at 0 °C was added 29.5 mL (88.6 mmol) of 3 M CH_3MgBr solution in Et_2O. After 10 min, an additional 7.5 mL (22.5 mmol) of 3 M CH_3MgBr was added as TLC analysis (4:1 hexane/EtOAc) indicated that the reaction was not complete. The resulting mixture was stirred for an additional 10 min, and then quenched

(45) Jacobs, T. L.; Winstein, S.; Ralls, J. W.; Robson, J. H. Substituted α-Dialkylaminoalkyl-1-naphthalenemethanols. II. 1-Halo-naphthalenes in the Friedel and Crafts Reaction. *J. Org. Chem.* **1946**, *11*, 27–33.

with H_2O and concentrated under reduced pressure. The concentrate was partitioned between EtOAc and H_2O, and both phases were filtered through Celite. The organics were dried over $MgSO_4$, filtered, and concentrated again to afford 9.9 g (69%) of **32** as a yellow solid: ^1H NMR (60 MHz, $CDCl_3$) δ 8.85 (br s, 1 H), 8.26 (d, J = 8.2 Hz, 1 H), 7.91 (d, J = 8.2 Hz, 1 H), 7.80 (br s, 1 H), 7.45 (dd, J = 8.2 and 8.2 Hz, 1 H), 2.69 (s, 3 H).

1-(5,7-Dibromo-1-naphthalenyl)-1,3-butanedione (33). To a warm solution of 8.5 g (26 mmol) of **32** and 3 mL (31.2 mmol) of EtOAc in 200 mL of freshly distilled *p*-dioxane under N_2 was added in portions 1.05 g (26 mmol) of 60% NaH. The resulting mixture was stirred overnight at ambient temperature. The mixture was then concentrated under reduced pressure, and the concentrate was partitioned between H_2O and EtOAc. Upon acidification and further workup of the aqueous phase, there was obtained only 300 mg of orange material. Therefore, the organics were concentrated, and the concentrate was dissolved in CH_2Cl_2 and treated with 5 N NaOH. The resulting "oily" solids were filtered, partitioned between H_2O and EtOAc, and acidified with concentrated HCl. The organics were dried, filtered, and concentrated to afford 1.9 g (20%) of **33**, which was used directly: ^1H NMR (60 MHz, $CDCl_3$) δ 8.55 (br s, 1 H), 8.25 (br d, J = 8.5 Hz, 1 H), 7.95–7.10 (m, 3 H), 5.90 (br s, 1 H), 2.20 (br s, 3 H).

Hydrogenation of (*R*)-(+)-29 to (*R*)-(+)-21. A solution of 80 mg (0.14 mmol) of (*R*)-(+)-**29** in EtOAc in a Parr bottle was treated with 20 mg of 10% Pd/C and hydrogenated at 50 psi H_2 pressure. After 1 h, 0.2 mL (1.4 mmol) of Et_3N was added, and hydrogenation at 50 psi was continued for an additional 2 h, at which time TLC analysis (1:1 hexane/EtOAc) revealed that all of (*R*)-(+)-**29** had reacted. The solution was then filtered through a pad of Celite, and the filtrate was washed with 10 mL of 2 N aqueous HCl. The organics were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford 15 mg (26%) of (*R*)-(+)-**21**, which was determined to be of >99% ee by HPLC analysis: $[\alpha]_D$ = +49.6° (c = 1 in $CHCl_3$).

1-Diazo-3-indol-1-yl-2-propanone (34). To a solution of 2.63 g (15.0 mmol) of indole-1-acetic acid[29] and 2.09 mL (15.0 mmol) of triethylamine in 60 mL of THF at 0 °C was added dropwise over 5 min 2.95 mL (15.0 mmol) of isobutyl chloroformate. After the resulting mixture was stirred for 30 min at 0 °C, an ethereal solution of diazomethane was added in 1-mL portions with continued ice bath cooling. The mixture was allowed to warm slowly to ambient temperature with stirring over 3.5 h. The mixture was then filtered through Celite, and the filtrate was concentrated under reduced pressure. Chromatography on silica gel, eluting with 4:1 hexane/EtOAc, afforded 1.61 g (54%) of **34** as a yellow oil: ^1H NMR (200 MHz, $CDCl_3$) δ 7.66 (m, 1 H), 7.17 (m, 3 H), 7.08 (d, J = 3 Hz, 1 H), 6.61 (d, J = 3 Hz, 1 H), 4.81 (s, 2 H), 4.60 (s, 1 H).

2,3-Dihydro-1*H*-pyrrolo[1,2-*a*]indol-2-one (35). A solution of 1.61 g (8.09 mmol) of **34** in 400 mL of benzene containing 13 mg (0.36 molar equiv) rhodium(II) acetate dimer was stirred at ambient temperature for 15 h. Removal of the solvent under reduced pressure afforded **35** as a brown solid: ^1H NMR (200 MHz, $CDCl_3$) δ 7.60 (m, 1 H), 7.17 (m, 3 H), 6.35 (s, 1 H), 4.40 (s, 2 H), 3.67 (s, 2 H).

2,3-Dihydro-2-(4-morpholinyl)-1*H*-pyrrolo[1,2-*a*]indole (36). The ketone **35** was treated sequentially with 4.22 mL (48.4 mmol) of morpholine, 4.6 mL of 5 N HCl/CH_3OH and 507 mg (8.07 mmol) of $NaBH_3CN$. Chromatography on SiO_2, eluting with 1:1 hexane/EtOAc, afforded 990 mg (51%) of **36** as a dark brown oil which solidified on standing: ^1H NMR (60 MHz, $CDCl_3$) δ 7.42 (m, 1 H), 7.22–6.86 (m, 3 H), 6.04 (s, 1 H), 4.24–3.30 (m, 8 H), 2.95 (m, 1 H), 2.41 (m, 4 H).

2,3-Dihydro-1-(4-morpholinyl)-1*H*-pyrrolo[1,2-*a*]indole (39). To a solution of 8.83 g (101 mmol) of morpholine and 6.8 mL of 5 N HCl/CH_3OH in 42 mL of CH_3OH was added sequentially 2.89 g (16.9 mmol) of **38**[31] and 743 mg (11.8 mmol) of $NaBH_3CN$, and the resulting mixture was stirred for 20 h at ambient temperature. After workup, chromatography on silica gel and elution with 1:1 EtOAc/hexane afforded 3.70 g (91%) of **39** as a dark orange gum: ^1H NMR (200 MHz, $CDCl_3$) δ 7.56 (m, 1 H), 7.21–7.00 (m, 3 H), 6.28 (s, 1 H), 4.22 (m, 1 H), 4.10–3.82 (m, 2 H), 3.68 (m, 4 H), 2.75–2.37 (m, 6 H).

2-(3-Butenyl)-1*H*-indole (42). To a suspension of 197 g (440 mmol) of (2-aminobenzyl)triphenylphosphonium bromide[32] in 2 L of CH_2Cl_2 at ambient temperature was added 57.4 g (440 mmol) of 4-pentenoyl chloride[46] over 25 min. The resulting homogeneous solution was stirred further for 6 h. Concentration of the mixture afforded **41** as a white solid. A portion of this material (82.3 g, 155 mmol) was suspended in 675 mL of toluene in a three-neck flask equipped with a mechanical stirrer and reflux condenser. A solution of 19.4 g (202 mmol) of NaOtBu in 100 mL of THF was added over 15 min. After refluxing for an additional 45 min, the partially cooled red solution was filtered through a pad of Celite. The mixture was partially concentrated and the solids were removed by filtration through a plug of SiO_2. Subsequent chromatography on SiO_2 eluting with hexane, followed by 9:1 hexane/EtOAc afforded 4.51 g (17%) of **42** as a yellow oil: ^1H NMR (300 MHz, $CDCl_3$) δ 7.97 (br s, 1 H), 7.50 (m, 1 H), 7.31 (d, J = 8.0 Hz, 1 H), 7.10 (m, 2 H), 6.28 (s, 1 H), 6.01–5.88 (m, 1 H), 5.14 (dd, J = 18.9 and 1.0 Hz, 1 H), 5.07 (dd, J = 10.3 and 1.0 Hz, 1 H), 2.89 (t, J = 7.5 Hz, 2 H), 2.52 (m, 2 H).

[2-(3-Butenyl)-1*H*-indol-3-yl](4-methoxyphenyl)methanone (43). This material was prepared from 8.31 g (48.6 mmol) of **42**, 21 mL (63.2 mmol) of a 3 M solution of CH_3MgBr, and 8.55 g (48.6 mmol) of *p*-anisoyl chloride in Et_2O.[3] Chromatography on SiO_2, eluting with 4:1 hexane/EtOAc and then with 7:3 hexane/EtOAc, afforded 12.2 g (83%) of **43** as an orange oil which solidified on standing: ^1H NMR (300 MHz, $CDCl_3$) δ 8.85 (br s, 1 H), 7.80 (m, 2 H), 7.31 (d, J = 8.4 Hz, 2 H), 7.14 (dd, J = 8.0 and 7.5 Hz, 1 H), 7.04 (dd, J = 8.0 and 7.5 Hz, 1 H), 6.93 (m, 2 H), 5.82 (m, 1 H), 5.04 (dd, J = 15.8 and 1.3 Hz, 1 H), 5.00 (d, J = 11.2 Hz, 1 H), 3.88 (s, 3 H), 3.09 (t, J = 7.4 Hz, 2 H), 2.47 (m, 2 H).

[2-(3,4-Dibromobutyl)-1*H*-indol-3-yl](4-methoxyphenyl)methanone (44). To a solution of 1.77 g (5.80 mmol) of **43** in 50 mL of CH_2Cl_2 at 0 °C was added over 20 min a solution of 300 μL (5.80 mmol) of Br_2 in 8 mL of CH_2Cl_2. After further stirring at 0 °C for 30 min, the mixture was poured into saturated aqueous $NaHCO_3$. The aqueous layer was washed with CH_2Cl_2, and the combined organics were dried over $MgSO_4$, filtered, and concentrated. Chromatography on SiO_2, eluting with 4:1 hexane/EtOAc, afforded 1.57 g (58%) of **44** as a pale yellow foam: ^1H NMR (300 MHz, $CDCl_3$) δ 9.11 (br s, 1 H), 7.80 (m, 2 H), 7.34 (d, J = 8.0 Hz, 1 H), 7.29 (d, J = 8.0 Hz, 1 H), 7.17 (dd, J = 8.0 and 8.0 Hz, 1 H), 7.06 (dd, J = 8.0 and 8.0 Hz, 1 H), 6.93 (m, 2 H), 4.03 (m, 1 H), 3.88 (s, 3 H), 3.77 (dd, J = 10.5 and 4.5 Hz, 1 H), 3.56 (dd, J = 10.3 and 9.0 Hz, 1 H), 3.31 (m, 1 H), 3.08 (m, 1 H), 2.63 (m, 1 H), 2.12 (m, 1 H).

[2,3-Dihydro-3-(bromomethyl)-1*H*-pyrrolo[1,2-*a*]indol-9-yl](4-methoxyphenyl)methanone (45). To a solution of 1.95 g (4.19 mmol) of **44** in 15 mL of DMF was added in portions 200 mg (5.03 mmol) of 60% NaH. The resulting mixture was stirred for 90 min. To the mixture was then added 10 mL of H_2O followed by 20 mL of CH_2Cl_2. The organics were washed with H_2O, dried over $MgSO_4$, filtered, and concentrated. Analysis of the crude reaction mixture (300 MHz, ^1H NMR) indicated an approximate 1:6:1 ratio of three products. Chromatography on SiO_2 eluting with 4:1 hexane/EtOAc afforded 175 mg (11%) of **45**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.96 (m, 1 H), 7.73 (m, 2 H), 7.32 (m, 1 H), 7.21 (m, 2 H), 6.95 (m, 2 H), 4.85 (m, 1 H), 3.88 (s, 3 H), 3.82 (dd, J = 11.0 and 3.2 Hz, 1 H), 3.69 (dd, J = 11.0 and 7.0 Hz, 1 H), 3.13 (m, 1 H), 2.90 (m, 1 H), 2.77 (m, 1 H), 2.56 (m, 1 H).

[2,3-Dihydro-3-[(4-morpholinyl)methyl]-1*H*-pyrrolo[1,2-*a*]indol-4-yl](4-methoxyphenyl)methanone (46). A solution of 115 mg (0.30 mmol) of **45** in 2.5 mL of morpholine was heated in an oil bath at 80 °C for 2 h. The reaction mixture was concentrated under reduced pressure, and the product was chromatographed on SiO_2, eluting with 1:1 hexane/EtOAc, to afford 62 mg (53%) of **46** as a pale yellow solid. A sample was recrystallized from EtOAc: mp 149–150 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.93 (m, 1 H), 7.71 (d, J = 8.6 Hz, 2 H), 7.48 (m, 1 H), 7.19 (m, 2 H), 6.94 (d, J = 8.6 Hz, 2 H), 4.63 (m, 1 H), 3.88 (s, 3 H), 3.70 (m, 4 H), 3.09 (m, 1 H), 2.92–2.78 (m, 2 H), 2.72–2.58 (m, 2 H), 2.54–2.42 (m, 4 H). Anal. Calcd for $C_{24}H_{26}N_2O_3 \cdot {}^1/_4H_2O$: C, 72.98; H, 6.76; N, 7.09. Found: C, 73.17; H, 6.63; N, 7.14.

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Pharmacology Procedures. Procedures for the biological testing reported herein have been published.[5,6,11]

(Aminoalkyl)indole Binding Assay. Radioligand binding studies were performed as described[11] using male Sprague-Dawley rat cerebellar membranes from a 48000*g* pellet which was washed twice by suspension in 20 mM HEPES buffer, pH 7. The pellet was suspended (1:120 w/v) in buffer, kept on ice, and used within 1 h.

The assay was started with the addition of homogenate containing 100-120 μg of cerebellar membrane protein. The 1-mL final assay volume contained: 20 mM HEPES pH 7; 0.5 nM [^{3}H]-(*R*)-(+)-**21** (59-60 Ci/mmol, 99% purity, Du Pont/NEN); 1 mg/mL BSA (Sigma A-7030); 100-120 μg of cerebellar membrane protein; and varying concentrations of competing compounds. Nonspecific binding was determined in the presence of 1 μM unlabeled (*R*)-(+)-**21**.

Compounds were solubilized in (i) a mixture of methanesulfonic acid/ethanol, (ii) ethanol, or (iii) DMSO. The experiments were controlled for vehicle effects. Further dilutions of compounds or radioligand were in buffer containing 5 mg/mL BSA to prevent absorption to glass.

The incubation was carried out at 30 °C for 90 min and stopped by rapid filtration and rinsing with 20 mL of 20 mM HEPES, pH 7.0, containing 0.5 mg/mL BSA over Whatman GF/B filters (presoaked in 5 mg/mL BSA-Buffer) on a 48-channel cell harvester. Radioactivity on the filters was measured by liquid scintillation spectrometry. Specific binding was defined as the difference in binding in the presence and absence of 1 μM (*R*)-(+)-**21**. Assays were performed in triplicate, and each experiment was repeated at least three times.

Binding data was analyzed using the radioligand binding analysis program EBDA[47] and LIGAND.[48] Protein was determined by the method of Lowry et al.[49]

Acknowledgment. We are grateful to John Herrmann, Garry Pilling, and Dick Philion for the preparation of bulk intermediates, to Steve Clemans and his staff for spectroscopic measurements, and to Sol Daum, Lynn McNaughton, and Jeff Abt for technical assistance.

Supplementary Material Available: (1) NMR chemical shift data for **20**, **21**, **24**, **29**, and **40** and (2) tables listing atomic coordinates, bond distances and angles, and thermal parameters for the (-)-dibenzoyl-L-tartaric acid salt complex of (*R*)-(+)-**14** (1:2) (10 pages). Ordering information is given on any current masthead page.

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2β-Substituted Analogues of Cocaine. Synthesis and Inhibition of Binding to the Cocaine Receptor

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The potencies of a series of 2β-substituted cocaine analogues to displace [^{3}H]-3β-(*p*-fluorophenyl)tropane-2β-carboxylic acid methyl ester binding in rat striatal membranes demonstrate the requirement for a 2β-substituent with two hydrogen-bond acceptors. The insensitivity of the ester moiety to steric and electronic factors suggests its modification to provide site-specific irreversible ligands.

The natural component of coca leaves (*Erythroxylum coca*), known as (-)-cocaine, is a psychostimulant and a powerful reinforcer[1,2] known to bind to specific sites in mammalian brain.[3-7] A correlation of the potencies of cocaine and cocaine analogues in drug self-administration with their potencies to inhibit dopamine uptake and with their binding affinities has supported the existence of a cocaine receptor at the dopamine transporter.[8] To elucidate the nature of this putative pharmacophore, we initiated a program to systematically examine the effects of structure variation on binding affinity. Recently, we reported on the stereoselectivity of the cocaine binding site,[9] the effect of substitution at C-3,[10-12] and the effects of the location of the nitrogen atom and of its substitution pattern.[13] As part of this continuing investigation, we now report on the effect of substitution at the 2-position.

Results

Synthesis. The cocaine analogues, **2-20**, which were synthesized and studied are listed in Table I. Scheme I summarizes the procedures utilized to prepare **2-15**. Hydrolysis of (-)-cocaine (**1**) gave benzoylecgonine (**10**);[14] reduction of **10** with diborane afforded the alcohol **12** which, when treated with acetic anhydride, gave **13**. Treatment of **10** with *N,N*-formyldiimidazole or thionyl chloride gave the imidazolide **22a** or acid chloride **22b**,

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