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C-ATTACHED AMINOALKYLINDOLES: POTENT CANNABINOID MIMETICS

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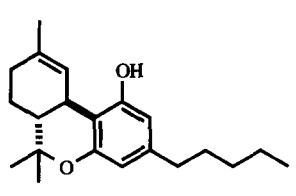
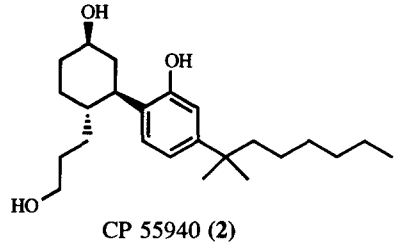
Abstract. Aminoalkylindoles (AAIs) with potent cannabinoid agonist activity have been synthesized where the aminoalkyl chain is attached to the indole ring via a carbon atom of the cyclic amine.

Introduction. Constituents of marijuana such as Δ^1 THC (**1**) have long been of interest because of their central nervous system (CNS) activity. Among the most interesting properties of cannabinoids are their psychotropic,[1] analgesic,[2] antiemetic,[3] and ocular pressure lowering properties.[4] These properties of cannabinoids have been extensively explored in the search for therapeutic utilities, but clinical utility has been limited.[5]

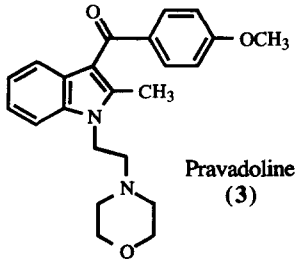
Figure 1



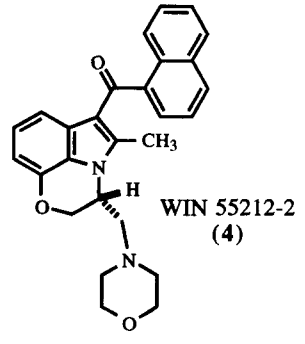
Δ^1-THC (**1**)



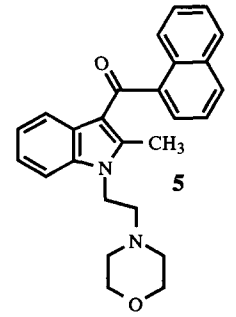
CP 55940 (**2**)



Pravadoline (**3**)



WIN 55212-2 (**4**)



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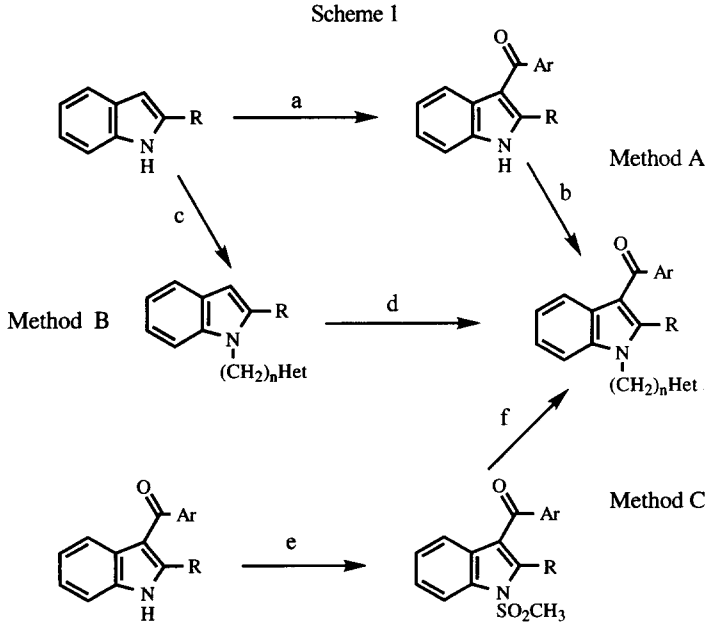
The work of several groups on the synthesis of cannabinoid mimetics has provided compounds which are useful tools for better understanding cannabinoid pharmacology. We have demonstrated that a series of aminoalkylindole (AAI) antinociceptive agents, originally designed as non-ulcerogenic non-steroidal antiinflammatory drugs (NSAIDS), e.g. pravadoline (**3**), is also associated with a second mechanism of action, manifested by potent activity at inhibiting electrically-induced contractions of mouse vas defera (MVD).[6] CP 55940 (**2**) and Win 55212-2 (**4**), a conformationally restricted AAI, were used in the development of radioligand binding assays and in the localization of cannabinoid binding sites in brain.[7] These efforts pointed to the presence of a specific cannabinoid receptor. The cloning and expression of such a receptor has since been reported.[8]

We have recently described the cannabinoid binding SAR for (N-attached) AAIs in which the heterocyclic amine nitrogen is attached to the indole ring nitrogen via a carbon chain.[9] Compound **5** is the most potent compound described in that work. This report describes a subseries of AAIs where the heterocyclic amine is attached to the indole ring via a carbon atom of the heterocycle (C-attached) rather than via the nitrogen atom. This variation provided potent, stereoselective cannabinoid agonists as reflected by activity in the [^{3}H]-Win-55212-2 binding assay and inhibition of electrically induced contractions of MVD.

Chemistry. Many of the new compounds described herein were synthesized in a similar manner to our earlier work.[6b,9] In Method A an appropriately functionalized indole is aroylated at the 3-position followed by N-alkylation (Scheme 1). Alternatively N-alkylation could be accomplished first, followed by acylation of the indole

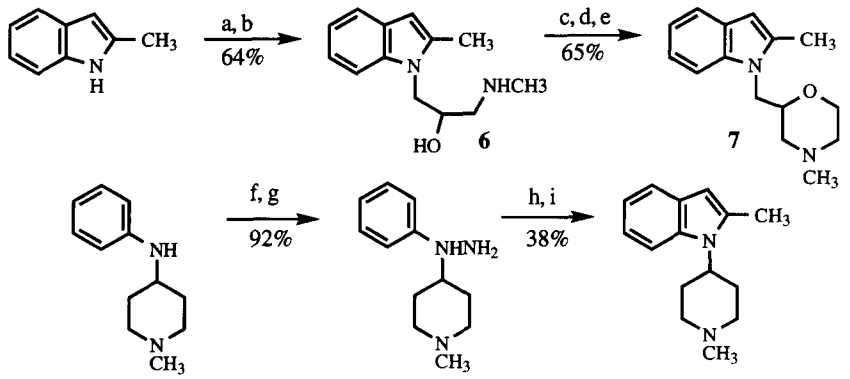
Scheme 1



Method A: a. MeMgX, Et_2O, ArCOCl; b. NaH, DMF, Het$(CH_2)_nX$. Method B: c. KOH, Het$(CH_2)_nX$, DMSO; d. ArCOCl, $AlCl_3$ or $EtAlCl_2$, CH_2Cl_2. Method C: e. 50% NaOH, $MeSO_2Cl$, $(Bu)_4NHSO_4$, CH_2Cl_2; f. NaH, Het$(CH_2)_nOH$, K_2CO_3, Toluene, Δ. (Het = heterocyclic amine, X = halo)

at C_3 (Scheme 1, Method B). For acid sensitive analogs $EtAlCl_2$ was used in place of $AlCl_3$ in the Friedel-Crafts acylation. A new method was developed for the synthesis of certain analogs in which the alkylation proceeded poorly, in part because the aminoalkylhalide was not readily available in a pure state. In this activation-transfer method an N-sulfonylated indole is treated with an alkoxide to generate an indole anion and the sulfonate of the alcohol *in situ*. These subsequently react to provide the AAI (Scheme 1, Method C).[10]

Scheme 2



a. KOH, epibromohydrin, DMSO; b. 40% $MeNH_2$; c. $ClCH_2COCl$, Et_3N; d. KOtBu, THF; e. BH_3, THF; f. $NaNO_2$, HCl; g. LAH, THF; h. phenylthioacetone, HOAc, reflux; i. Ra-Ni, EtOH, reflux.

Certain examples required building either the amine or the indole rings. The morpholine ring in compound **7**, the precursor to analog **21**, was synthesized as shown in scheme 2. The piperidinoindole precursor **10** for compound **17** was synthesized from hydrazine **9** using a Fischer indole protocol (Scheme 2). The other methods of synthesizing specific analogs are summarized in the footnotes for table 1.

Results and Discussion. The activities of C-attached analogs at inhibiting ^{3}[H]-Win 55212-2 binding and electrically induced MVD contractions are described in table 1. Activity in the binding assay has been shown to be reflective of cannabinoid activity,[7e] and the MVD was used as a functional assay reflective of agonist effects.[6] Several compounds were inactive in the binding assay, yet were active in the MVD. Such compounds might be interacting with other receptors known to have MVD activity (e.g. opiate or α_2). Alternatively such compounds might be acting at a different cannabinoid subreceptor or might show activity if a full dose-response curve were run in the binding assay. Compounds which are active in the binding assay but inactive as agonists in the MVD would be possible cannabinoid antagonists. No such compounds were observed in this subseries. With the few exceptions noted above, the activity in the binding assay paralleled the activity in the MVD.

In our initial work on C-attached analogs the *p*-anisoyl 3-substituent found in pravadoline (**3**) was left constant and the indole 1-substituent varied. As in the N-attached series of pravadoline analogs,[9] compounds where the amine nitrogen was separated from the indole nitrogen by two carbons as in (**11**) were the most potent.

Direct attachment of the heterocycle to the indole was disfavored even when two carbons separated the amine nitrogen and the indole (**12**), presumably because the reduced flexibility of such compounds does not allow the amine to adopt an optimum binding conformation.[9] In the N-attached series the nature of the amine affects activity, with morpholine and thiomorpholine generally being most potent. In contrast, for the C-attached series with the 3-anisoyl substituent, the N-methylpiperidine (**11**), N-methylpyrrolidine (**16**), N-methyl(thiomorpholine) (**22**), N-methylmorpholine (**20**), and 1,4-dimethylpiperazine (**19**) bound with roughly descending potency. As is seen in the N-attached series, replacing the anisoyl by a 1-naphthoyl (**28-33**) markedly increased potency, presumably in part because of an enhanced lipophilic interaction.

As had been noted in our earlier work, removing the 2-methyl group gave increased potency. A clear example of this effect was the 2-H analog **26** which was 70x more potent than the 2-methyl analog **11**. This potency increase may be related to the ability of the 1-substituent to adopt a more bioactive conformation near the 2-position.[7d,9] Removing the N-methyl substituent (**24**) from the heterocyclic amine decreased activity as did adding a carbon to give an N-ethyl analog (**25**).

Combining the optimal 3-(1-naphthoyl), 2-H, and 1-piperidinyl substituents provided 3-(1-naphthoyl)-2-H-1-(N-methylpiperidinyl-2-methyl) indole analog **30**, which was extremely potent both in the binding assay (IC_{50} = 1.2 nM) and in the MVD (IC_{50} = 0.47 nM). This compound was the most potent compound uncovered in our work, and is comparable in potency to the most potent cannabinoids of more traditional structure.

Most of the compounds described herein have an optical center, and data is only reported for the racemate. For compound **16**, the two enantiomers **[17 (R), 18 (S)]** were easily accessible because of the availability of the enantiomers of N-methyl-prolinol. High stereoselectivity was seen in the binding assay. Both enantiomers were potent in the MVD, but only compound **17** showed a full dose-response. The activity in the MVD for compound **18** was likely due, at least in part, to binding at α_2 receptors (data not shown).[11] Because of the high potency of analog **30**, efforts were initiated to determine the activity of the enantiomers. Racemic **30** was resolved by HPLC using a semi-preparative CHIRALCEL® OD column (10 x 250 mm) using 20% ethanol-hexane. Biological testing of these compounds in a [^{3}H]CP-55940 cannabinoid binding assay[12] demonstrated high enantioselectivity, with the more active enantiomer (K_i = 0.27 nM) approximately 3 orders of magnitude more potent than the less active enantiomer (K_i = 217 nM).[13]

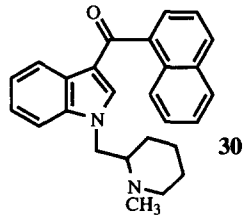


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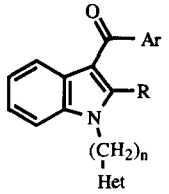
Table I. [^{3}H]-Win 55212-2 binding and MVD activity of AAIs



Cmpd	Ar	R	n	Het[a]	Methd[b] (% Yld)	mp °C	Salt	IC_{50} (nM) [^{3}H]-Win-55212-2 Binding[c]	IC_{50} (nM) MVD[d]
3	p-OMePh	Me	2	4-morph				3155 ± 54	319 ± 63
11	p-OMePh	Me	1	2-(1-Me-pip)	A (55)	122-123		699 ± 63	134 ± 42
12	p-OMePh	Me	0	3-(1-Me-pip)	D (65)	190-193		-34% at 1000	partial[e]
13	p-OMePh	Me	2	2-(1-Me-pip)	A (52)	120-121	HCl·1/2 H_2O	-25% at 1000	>10,000
14	p-OMePh	Me	1	3-(1-Me-pip)	A (70)	91.5-93.5		-23% at 1000	>10,000
15	p-OMePh	Me	0	4-(1-Me-pip)	A (58)	147-149		-25% at 1000	>10,000
16	p-OMePh	Me	1	2-(1-Me-pyr)	A (66)	235.5-237.5	HCl	53% at 1000	63 ± 6
17 (R)	p-OMePh	Me	1	2-(1-Me-pyr)	A (35)	235-238	HCl	497 ± 52	37 ± 5
18 (S)	p-OMePh	Me	1	2-(1-Me-pyr)	A (30)	235-238	HCl	7% at 1000	partial[e]
19	p-OMePh	Me	1	2-(1,4-di-Me-piperaz)	A (57)	128-131		-8% at 1000	1428 ± 528
20	p-OMePh	Me	1	3-(4-Me-morph)	E (70)	125-126		43% at 3000	210 ± 170
21	p-OMePh	Me	1	2-(4-Me-morph)	B (74)	169-170		-23% at 1000	>10,000
22	p-OMePh	Me	1	3-(4-Me-thiomorph)	C (64)	127.5-129		40% at 1000	1148 ± 728
23	p-OMePh	Me	1	3-(4-Me-thio-morph), S-oxide	F (67)	172-174		-10% at 1000	>10,000
24	p-OMePh	Me	1	2-pyr	G (65)	170-172	HCl	-24% at 1000	220 ± 43
25	p-OMePh	Me	1	2-(1-Et-pyr)	A (73)	115-116	HCl·1/4 H_2O	-29% at 1000	>10,000
26	p-OMePh	H	1	2-(1-Me-pip)	A (76)	159-160		9.7 ± 1.7	2.6 ± 0.6
27	p-OMePh	H	1	2-(1-Me-pyr)	A (68)	106-107	HCl·1/4 H_2O		8.0 ± 4.0
5	1-naphthyl	H	2	4-morph				7.8 ± 0.3	6.3 ± 1.2
28	1-naphthyl	Me	1	2-(1-Me-pyr)	A (85)	110.5-112.5		6.5 ± 0.6	2.4 ± 0.4
29	1-naphthyl	Me	1	2-(1-Me-pip)	A (68)	140-141		5.4 ± 0.9	1.2 ± 0.7
30	1-naphthyl	H	1	2-(1-Me-pip)	A (71)	134.5-136.5		1.22 ± 0.02	0.47 ± 0.12
31	1-naphthyl	H	1	3-(4-Me-morph)	E (78)	165-167		3.0 ± 0.16	0.40 ± 0.20
32	1-naphthyl	H	1	2-(1,4-di-Me-piperaz)	C (40)	68-75		51	15 ± 0
33	1-naphthyl	H	1	2-pip	D (56)	282-284	HCl	6.6 ± 0.2	3.0 ± 0.8
1								5.8 ± 0.7	4.0 ± 0.5

[a]Abbreviations: morph = morpholinyl, pip = piperidinyl, pyr = pyrrolidinyl, piperaz = piperazinyl, thiomorph = thiomorpholinyl. [b]Methods A, B, and C: see text. Method D: Catalytic hydrogenation of the pyridinium salt over PtO_2. Method E: Reductive methylation of the NH analog using formaldehyde/formic acid. Method F: Oxidation of compound **22** using 30% H_2O_2 in hexafluoroacetone. Method G: Hydrogenolysis of the N-benzyl analog with ammonium formate and 10% Pd/C in methanol. [c]Concentration of compound required to inhibit 50% of 0.5 nM [^{3}H]-Win 55212-2 binding in rat cerebellum membranes as described in reference 7e. Values are the IC_{50} or % inhibition at the highest tested dose (nM). Negative values connote stimulation rather than inhibition. [d]Concentration of compound required to inhibit electrically induced contractions in isolated mouse vas defera (MVD) preparations in vitro as described in reference 6a. Values are the IC_{50} or the highest tested dose (nM). [e]Partial agonists showed a maximal inhibition of 60 – 80%.

Conclusions. C-attached AAIs where the heterocyclic amine was attached from the alpha-carbon of the heterocycle via a methylene to the indole nitrogen were generally more potent than N-attached analogs where the attachment is from the nitrogen of the heterocycle. Potency was optimum for 3-(1-naphthoyl)-2-H-1-(N-methylpiperidinyl-2-methyl) indole (**30**), and activity resided predominantly in a single enantiomer. The C-attached subseries of AAIs, with novel structures and favorable physical properties (increased solubility), thus provides new tools for studying structure and function of cannabinoid receptors.

References and Notes

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⊥ All research by these authors prior to manuscript submission was carried out at the Sterling Winthrop Pharmaceuticals Research Division.

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11. However, most of the compounds in this series which have been tested at α_2 or other receptors showed little or no binding.
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13. The enantioselectivity could be even greater, since contamination of the less active enantiomer by the more active has not been ruled out. Further studies on these enantiomers including determination of their absolute configuration are in progress.

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