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MORPHOLINOALKYLINDENES AS ANTINOCICEPTIVE AGENTS: NOVEL CANNABINOID RECEPTOR AGONISTS

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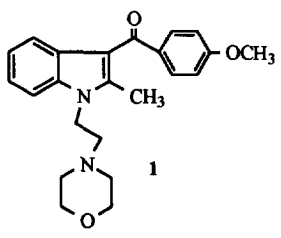
Abstract: Indene analogs of pravadoline exhibited antinociceptive activity in several animal models. The inhibition of prostaglandin (PG) synthesis in mouse brain microsomes was diminished in these pravadoline analogs, but they were potent in inhibiting electrically stimulated contractions in the mouse vas deferens (MVD) preparations. Binding studies with ligand WIN 55212-2 have aided to demonstrate that the morpholinoalkyl-indene binding site is functionally equivalent with cannabinoid binding site. The antinociceptive activity of the indene derivatives appears to be mediated by increased affinity for the cannabinoid receptor.

Nonsteroidal antiinflammatory drugs (NSAIDS) are widely used drugs for the management of pain and inflammation. Pravadoline (1) and related (aminoalkyl)indoles (AAIs) are a new type of antinociceptive agents in which the classical acidic function of the NSAIDS is replaced by an amine (morpholine). These AAIs are close relatives of the analgesic clometacin (2) which in turn is a positional isomer of indomethacin (3).[1,2] It was established in preclinical studies that pravadoline does not produce gastrointestinal irritation (GI) following either acute or chronic administration.[3] In several animal models it has been shown that the antinociceptive effects of



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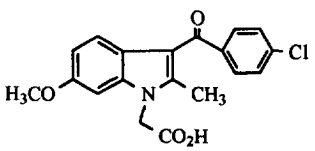

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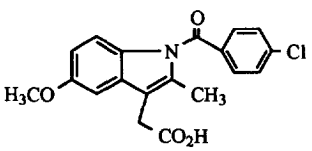

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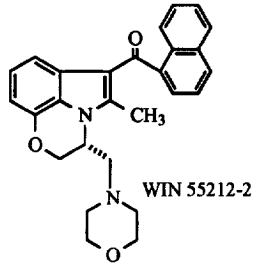
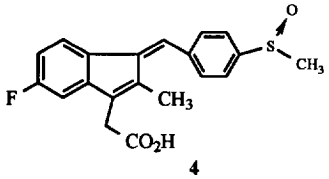
pravadoline are mediated by two mechanisms. Similar to NSAIDS, pravadoline inhibits prostaglandin (PG) synthesis *in vitro* and *ex vivo* in mouse brain preparations.[3] The other antinociceptive mechanism is related to the inhibition of the neuronally stimulated contractions of guinea pig ileum and mouse vas deferens (MVD) preparations. These latter activities are not associated with NSAIDs.[4] Using radiolabelled WIN 55212-2, a binding assay was developed which helped to establish the second mechanism of antinociception as cannabinoid.[2,5]



WIN 55212-2

In early clinical trials of pravadoline in humans, there were indications of central nervous system (CNS) side effects and we undertook an approach, exemplified by sulindac (**4**), to prepare indene analogs (**5**). Sulindac (**4**), an indene isoster of indomethacin (**3**)[6] was discovered in an effort to circumvent the CNS side effects of indomethacin. In sulindac, the trigonal center of N-acyl indole of indomethacin (**3**) was replaced with a benzylidene indene. The result was antiinflammatory activity of sulindac (**4**) comparable to indomethacin coupled with an improved over all profile. The indene derivatives of pravadoline were then evaluated for PG synthesis inhibition activity, acetylcholine (ACh) writhing, and mouse vas deferns (MVD) activities.



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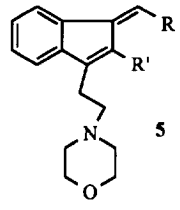

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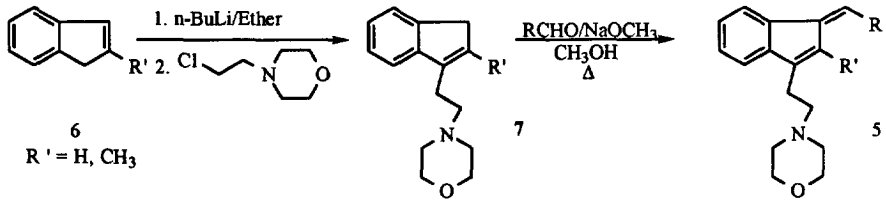
R = aryls
R' = H, CH_3

The benzylidene indene derivatives were prepared as depicted in Scheme I. The anion generated at room temperature either from indene (**6**, R' = H)[7] or 2-methylindene (**6**, R' = CH_3)[8] was reacted with the 2-chloroethylmorpholine to give a mixture of olefinic isomers [**6**, R' = H; ^{1}H NMR, δ 6.40 (d, J = 3.5 Hz, 1H), 6.65 (d, J = 3.2 Hz, 1H) and 3.25 (br s, 2H), 6.10 (br s, 1H)]. The mixture was then isomerized by warming with

acetic acid to the tri- and tetra-substituted olefins 7 in 80-84% overall yield. Condensation of 7 with various aromatic aldehydes in presence of sodium methoxide/methanol[6] gave the desired compounds (**5**) as a mixture of geometrical isomers. In compound **5**, the cis isomer (> 95%) predominated when R' = H and it was a mixture of cis and trans (4:1) when R' = CH_3.

Scheme I



These indene analogs, **5a-k**, were compared with pravadoline (Table 1 and 2) for inhibition of PG synthetase in mouse brain microsomes and inhibition of contractions of mouse vas deferens (MVD) preparations.[3,4] The compounds were also evaluated for *in vivo* antinociceptive activity in animal models. The oral acetylcholine (ACh) writhing test served as the initial assay for the evaluation of the antinociceptive activity *in vivo*. Displacement of the [^{3}H]-(R)-(+)-WIN 55212-2 binding in rat cerebellar membranes, as described earlier,[2] assessed the cannabinoid agonist activity.[9]

In contrast to pravadoline, the indene analogs (**5a-f**) were much weaker inhibitors of PG synthesis indicating that the oxygen atom of the aroyl group of pravadoline may play a significant role in this activity. The naphthyl analogs (**5g-h**) were inactive in this assay (Table 1). This result was not surprising since it has been shown that the naphthoyl analog of pravadoline also lacked the PG synthetase activity.[1,2] Thus, the replacement of the indole ring with indene and/or the lack of an oxygen atom at the proper region of the molecule has resulted in reducing the NSAID component in these molecules.

On the other hand, the oral ACh writhing activity of the indene compounds were comparable to pravadoline and naphthylidene analogs (**5g**, **5i**, and **5k**) were among the most potent compounds of this series. In general, the 2-methyl indene compounds (**5c**, **5h**, and **5j**) were less potent in the ACh writhing assay than the indene analogs. These results argue against the observed *in vivo* activity of indenes being a consequence of PG synthetase inhibitory activity.

Table 1: The physical properties, inhibition of PG synthetase and ACh writhing activity of indene analogs (5).

Compound	R	R'	mp (°C)	Yield (%)	PG Synthetase IC_{50} (μm)[a]	Ach ED_{50} mg/kg(po)[b]
	Pravadoline				3.5	41.0
5a	Phenyl	H	197-199	54	20.0	65.0
5b	(4-CH_3O)-phenyl	H	175-177	76	58% @ 30	46.0
5c	(4-CH_3O)-phenyl	CH_3/E/Z(4:1)	230-232	28	22% @ 30	30% @ 100
5d	(3,4-di-CH_3O)-phenyl	H	199-201	37	11% @ 30	19.0
5e	(4-CH_3S)-phenyl	H	233-235	78	NT[c]	50.0
5f	(4-CH_3SO)-phenyl	H	122-124	58	55% @ 30	25.0
5g	1-naphthyl	H	206-208	61	0% @ 30	83% @ 10
5h	1-naphthyl	CH_3/E/Z(4:1)	282-284	47	2% @ 30	34
5i	4-CH_3O-1-naphthyl	H	249-251	52	NT	3.2
5j	4-CH_3O-1-naphthyl	CH_3/E/Z(4:1)	265-267	32	NT	39% @ 100
5k	4-HO-1-naphthyl	H	219-221	53	NT	2.7

[a]Inhibition of PG synthesis in mouse brain microsomes, as described in ref 3. Values are the IC_{50} (with 95% confidence limits) or % inhibition @ 30 μM ($n = 1$).

[b]ACh writhing assay in mice, as described in ref 3. Values are the ED_{50} or the % inhibition @ 100 mg/kg.

[c]NT = not tested

Indene analogs inhibited the electrically stimulated contraction of MVD and were found to be more potent than pravadoline (Table II). A correlation between MVD activity, *in vivo* antinociceptive potency, and interaction with cannabinoid receptor has been demonstrated for amino(alkyl)indoles.[2,10] The indene analogs were also active in the displacement of the ligand [^{3}H]-(R)-(+)-WIN 55212-2 (Table 2), especially the naphthyl analogs, **5g** and **5i**, are very potent in this assay, suggesting the cannabinoid mechanism for the observed antinociception. The testing in isolated tissue preparations and the radioligand binding assays has ruled out the interaction of pravadoline[2] and these indene compounds with opiate and related receptors.

Table 2 : MVD activity and cannabinoid receptor binding affinity of indene derivatives (5).

Compound	R	R'	MVD IC_{50} (μM)[a]	Cannabinoid Receptor Binding[b] IC_{50}(nM)	% @1μM
	Pravadoline		0.53	3155.0	
5a	Phenyl	H	0.079		33.0
5b	(4-CH_3O)-phenyl	H	0.08	58.0	
5c	(4-CH_3O)-phenyl	CH_3	NT[c]		45.0
5d	(3,4-di-CH_3O)-phenyl	H	0.019	28.0	
5e	(4-CH_3S)-phenyl	H	NT		96.0
5f	(4-CH_3SO)-phenyl	H	0.19		NT
5g	1-naphthyl	H	0.007	1.0	
5h	1-naphthyl	CH_3	0.045	10.0	
5i	4-CH_3O-1-naphthyl	H	0.008	0.9	
5j	4-CH_3O-1-naphthyl	CH_3	0.35	NT	
5k	4-HO-1-naphthyl	H	NT		97.0

[a]Mouse vas deferens inhibitory activity, ref 3.

[b]Concentration of compound required to displace 50% 0f 0.5 nM [^{3}H]-(R)-(+)-WIN 55212-2 binding in rat cerebellar membranes as described in ref 5 and 11.

[c]NT = not tested

In summary , we have prepared indene analogs of pravadoline to probe for antinociceptive activity. Most of these compounds inhibited electrically stimulated contractions in the MVD preparation and the evidence suggests that potent cannabinoid activity *in vitro* parallels the antinociceptive activity *in vivo*. The naphthylidene analogs, **5g** (IC_{50} = 1.0 nM) and **5i** (IC_{50} = 0.9 nM) are potent cannabinoid receptor agonists.

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