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C-3 Amido-Indole Cannabinoid Receptor Modulators

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Abstract—C-3 Amido-indoles were found to selectively bind to the CB2 receptor. SAR studies led to optimized compounds with excellent in vivo potency against LPS induced TNF-α release in murine models of cytokine production.

The discovery of the human peripheral cannabinoid (CB2) receptor,[1] has focused a significant amount of research effort on the development of CB2 selective ligands.[2] The CB2 receptor, a member of the GPCR superfamily, is expressed in cells of the immune system,[3] whereas the CB1 receptor is predominantly in the central nervous system.[4] While the endogenous role of the CB2 receptor has yet to be identified, CB2 selective agonists are considered to be useful for the treatment of inflammatory disorders such as rheumatoid arthritis (RA), asthma, and chronic obstructive pulmonary disease (COPD) while being devoid of psychotropic effects associated with CB1 agonism.[5] The objective of our study was to prepare an orally active CB2 selective agonist.

Initiation of our efforts to find CB2 selective agonists started with a survey of known CB2 ligands.[6] The structurally dissimilar WIN-55212–2 (**1**)[6b] and the Japan Tobacco cinnamides[7] (**2** and **3**) served as reference compounds in our efforts (Fig. 1, Table 1). From our initial studies we discovered that indoles such as **4**, having novel C-3 amide substitution, are moderately potent CB2 ligands (**4**, CB2 K_i 250 nM).[8] Additionally, incorporation of a C-7 methoxy[9] gave **5a**, which had a CB2 K_i of 8 nM and was 500-fold selective for the CB2 receptor. It had been previously reported that CB2 agonists inhibit activation of nuclear factor-kappa B (NFκB) induced by lipopolysaccharide (LPS).[10] Since NFκB is required for TNF production,[11] we chose to utilize the LPS activation of human PBMC's as our secondary assay.[12] Compound **5a** was shown to inhibit LPS stimulated TNF-α production with an IC_{50} of 12 μM.[13]

Chiral discrimination was observed with these systems since the enantiomer of **5a** had minimal CB2 affinity.

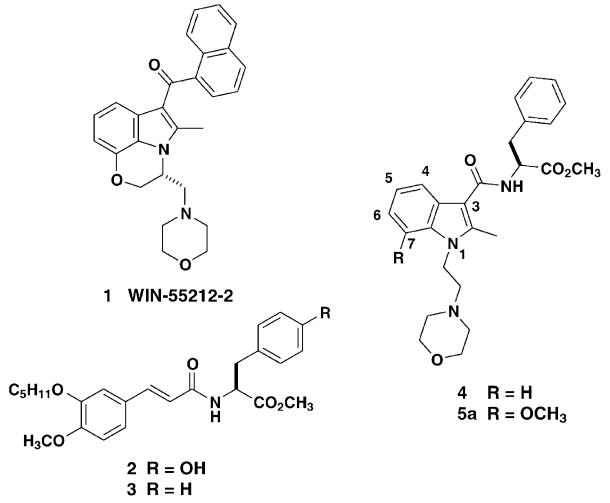

Figure 1. Development of the C-3 amido-indole CB2 ligands.

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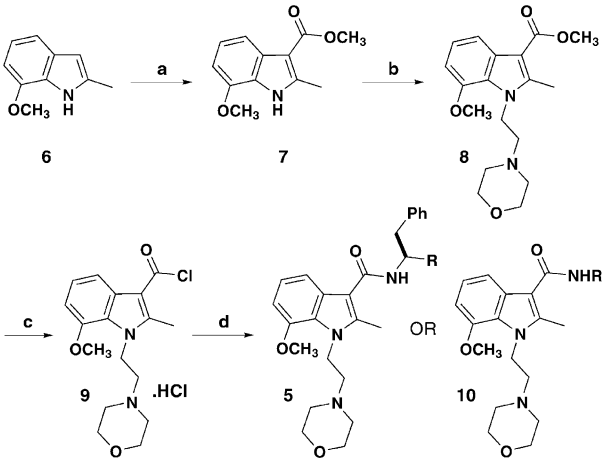
Table 1. CB2 and CB1 binding constants for compounds **1**–**5**

Compd	K_i (nM)		CB1/CB2	LPS-TNF
	CB2	CB1		IC_{50} (μM)
1	4	40	10	8
2	120	5700	48	—
3	86	51%[a]	—	—
4	250	—[b]	—	—
5a	8	4000	500	12

[a]% Inhibition at 1 μM.
[b]Not tested.

Preliminary metabolic stability studies with **5a** demonstrated that the ester moiety was susceptible to microsomal hydrolysis and therefore, our initial focus centered on finding a suitable replacement via precedented ester isosteres and 5-membered heterocycles. Additionally, high throughput synthesis was used to explore alternative C-3 amide substituents.

Starting with 2-methyl-7-methoxy indole **6** (Scheme 1),[14] conversion to **7** was carried out using a mild, 2 step acylation protocol developed in our laboratories.[15] Alkylation at N-1 with chloroethylmorpholine hydrochloride gave **8**, which was saponified and converted to the acid chloride to give **9** as a stable salt. Exposure of **9** to a variety of amines[16] generated the C-3 amide analogues **5** or **10**.



Scheme 1. (a) $ClCOCCl_3$, collidine, MeCN; KOH, MeOH; (b) NaH, 2-chloroethyl morpholine.HCl, DMF; (c) 3 N aq NaOH, MeOH then $SOCl_2$, DCE; (d) RNH_2, TEA, DCE.

The results of these efforts, outlined in Table 2, demonstrate that there is limited substitution tolerability at C-3 apart from ester substituents (**10b**–**d**). Ketones **5h** and **5i**, oxime **5j**, and isoxazole **5m** were the few non-esters that achieved binding affinities under 200 nM. Fenchyl amide **10a** and the enantiomer (**ent-10a**) were the first non-ester replacements at C-3 with appreciable affinity to CB2.[17] The (*S*)-fenchyl analogue **10a** was selective (CB1 K_i 10 μM) and showed moderate functional cell activity (LPS-TNF IC_{50} 33 μM). The tetrasubstituted cyclohexylamine **10e** had comparable affinity to **10a**. In addition, approximately 25 anilines of varied substitution patterns were coupled to **9**. Compounds of this

Table 2. SAR of C-3 amide substitution

Compd	R	CB2 K_i (nM)
5a	CO_2CH_3	8
5b	CH_3	263
5c	CH_2CH_3	18%[a]
5d	CN	18%
5e	CH_2OCH_3	6%
5f	$CONHCH_3$	294
5g	$CON(CH_3)_2$	29%
5h	$COCH_3$	8
5i	$COCH_2CH_3$	24
5j	$C(NOCH_3)CH_3$	184
5k	3-Pyrazole	7%
5l	3-Isoxazole	235
5m	5-Isoxazole[b]	169
10a	(*S*)-fenchyl	30
Ent-10a	(*R*)-fenchyl	41
10b	L-3-Chlorophenylalanine-OMe	31
10c	3-(4-Thiazolyl)-L-alanine-OMe	20
10d	3-(2-Thienyl)-L-alanine-OMe	12
10e	2,2,5,5-Tetramethylcyclohexyl	38
10f	2,2-Dimethylcyclopentyl	205

[a]Percent values indicate % inhibition at 100 nM ligand concentration.
[b]Racemic.

class failed to demonstrate appreciable CB2 affinity or functional activity. A comparison of the dose related inhibition of LPS stimulated TNF-α production for **5a**, **10a**, and WIN-55212 (**1**) is shown in Figure 2.

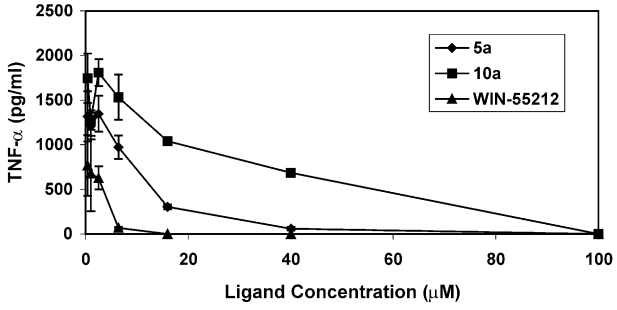
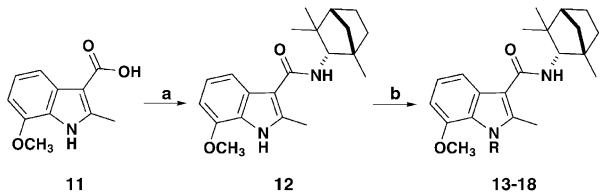


Figure 2. In vitro inhibition of LPS induced TNF-α with **5a** and **10a**.

Having identified a non-ester lead (i.e., **10a**), the SAR at N-1 was investigated. In an effort to maximize synthetic throughput, we undertook an approach that would allow for incorporation of the SAR variant in the last synthetic step. To this end, 2-methyl-7-methoxy indole-3-carboxylic acid **11**[18] was coupled with (*S*)-fenchyl amine followed by chemoselective alkylation at N-1 with an alkyl halide and NaH (Scheme 2).

Representative compounds are listed in Table 3 with their CB2 K_i. Improvement in CB2 binding affinity relative to **10a** was limited to **13**, however, this com-



Scheme 2. (a) *S*-fenchylamine, EDC/HOBt/DCM; (b) RBr or RCl, NaH/DMF, 0–80 °C.

Table 3. SAR at N-1 of *S*-fenchyl amide indoles

Compd	R	CB2 K_i (nM)
13	*n*-Pentyl	5
14	*N*,*N*-dimethylamino ethyl	505
15	Methoxy ethyl	60
16	*N*-morpholino propyl	25
17	*N*-piperidino ethyl	89
18	*N*-pyrrolidino ethyl	25%[a]

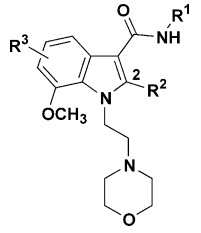
[a]Percent values indicate % inhibition at 100 nM ligand concentration.

pound failed to demonstrate significant functional activity (ca. 20% inhibition at 20 μM). Analogues **15** and **16** showed relatively good CB2 affinity but with no obvious advantage over **10a**.

In light of the limited improvements obtained from the N-1 substitution studies, focus shifted to C-2 and the phenyl ring of the indole core (Table 4). Replacement of the C-2 methyl with hydrogen generated **19a** with comparable CB2 binding affinity to **10a** and a 3-fold increase in functional cell potency (LPS-TNF IC_{50} 13 μM). This represented the first non-ester compound with comparable functional activity to the original lead **5a**. Compounds **19b–d** also maintained good CB2 affinity with this modification. Increasing the size of the C-2 substituent (**20a** and **b**) had a deleterious effect on binding and they were not pursued further.

Aryl ring substitution was next investigated. C-4 (**21a–c**) and C-6 (**21g–h**) and substitutions generally were not tolerated. C-5 modifications were limited to fluoro (**21f**) while maintaining the CB2 affinity observed with **19a**.

Table 4. SAR at C-2 and phenyl ring (C-4,5,6)



Compd	R^1	R^2	R^3	CB2 K_i (nM)
19a	(*S*)-fenchyl	H	H	12
19b	L-Amphetamine	H	H	71
19c	2,2,5,5-Tetramethyl cyclohexyl	H	H	22
19d	(*R*)-fenchyl	H	H	13
20a	(*S*)-fenchyl	ethyl	H	103
20b	(*S*)-fenchyl	*n*-pr	H	6%[a]
21a	(*S*)-fenchyl	CH_3	4-Cl	11%
21b	(*S*)-fenchyl	CH_3	4-Br	42%
21c	(*S*)-fenchyl	CH_3	4-CN	33%
21d	(*S*)-fenchyl	CH_3	5-Cl	251
21e	(*S*)-fenchyl	CH_3	5-OCH_3	0%
21f	(*S*)-fenchyl	CH_3	5-F	18
21g	(*S*)-fenchyl	CH_3	6-Cl	15%
21h	(*S*)-fenchyl	CH_3	6-OCH_3	0%

[a]Percent values indicate % inhibition at 100 nM ligand concentration.

Having a potent non-ester CB2 ligand (i.e., **19a**) in hand, we chose the murine model of LPS induced TNF-α production[19] to evaluate the in vivo efficacy of this compound. Amide **19a** displayed a dose dependant inhibition of TNF-α production with an ED_{50} of 5 mg/kg when administered intraveneously (Fig. 3).[20] When administered orally, however, TNF-α inhibition was not observed up to a dose of 50 mg/kg. Preliminary metabolism studies indicated that de-alkylation at N-1 contributed to the poor oral efficacy. Additionally, diminished receptor subtype selectivity was observed (CB1 K_i 380 nM).

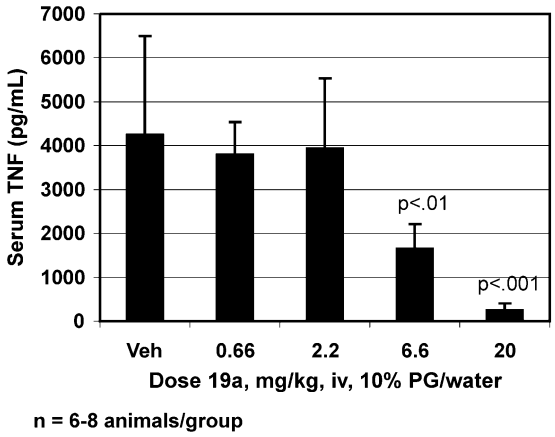


Figure 3. In vivo inhibition of LPS induced TNF-α with **19a**.

The results described herein represent our initial efforts toward the development of novel C-3 amido-indole derived CB2 receptor modulators. We have demonstrated that these compounds bind to the CB2 receptor and inhibit pro-inflammatory responses in a murine model of acute inflammation. Further studies addressing efforts to increase oral bioavailability will be reported in the near future.

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8. *CB2 and CB1 assay protocol*: The following assay has been carried out using the human cannabinoid receptor expressed in CHO cells. Radioactive tracer label (WIN 55,212–2 Mesylate [5,7–3H] for CB2, CP55,940 for CB1) and test compound are incubated together in a 96-well tissue culture plate. All reagents are dissolved or resuspended in binding buffer (10 mM HEPES, pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, 0.3% BSA). The reaction is initiated by the addition of membranes (50 μg) from CHO-K1 cells expressing either CB1 or CB2. The plates are incubated 2 h with shaking at room temperature and the reaction is harvested on a Wallac Filtermat B with 7 wash cycles using wash buffer (10 mM HEPES, pH 7.4, 0.1% BSA). The filter is counted in a Betaplate scintillation counter to ascertain the cannabinoid inhibitory activity of the test compound (activity inversely proportional to the amount of labeled WIN55,212-2 incorporated). Routinely the radiolabel was used at a concentration of 10 nM but the exact concentration of reagents and the amount of label can be varied as needed.

9. The incorporation of a C-7 methoxy has been described in 3-aroylindole CB ligands (ref 6c). A 2-fold increase in IC_{50} was observed.

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13. In vitro *functional cell assay protocol*: Freshly isolated human monocytes, or the human monocytic cell line THP-1, are incubated at 1×10^6 cells/mL in RPMI 1640 media containing 10%FBS with the test compound for 30 min and then stimulated by the addition of either lipopolysaccharide (LPS) or immune complexes (IC). Cells are incubated for 6 h at 37 °C at which time the cell supernatants are removed and assayed for cytokines (TNF, IL-1β, IL-6, and IL-8) using commercially available ELISA kits.

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16. Amines were purchased in the highest enantiopurity available or prepared using literature methods.

17. Fenchyl amine substitution has been used in the development of CB2 selective antagonists, ref 6g.

18. 2-Methyl-7-methoxyindole-3-carboxylic acid was prepared via the saponification of **7**.

19. In vivo inhibition of LPS stimulated TNF-α with Win-55212–2 has been demonstrated in a mouse model of pulmonary inflammation: Berdyshev, E.; Boichot, E.; Corbel, M.; Germain, N.; Lagente, V. *Life Sci.* **1998**, *63*, 125.

20. *TNF-α Production by LPS-Stimulated Mice*: Mice (Balb/c female, 6–8 weeks of age, Harlan Labs; $n=8$/treatment group) were injected intraperitoneally with 50 ug/kg lipopolysaccharide (LPS; *E. coli* strain 0111:B4, Sigma) suspended in sterile saline. 90 min later, mice were sedated by CO_2:O_2 inhalation and a blood sample was obtained. Serum was separated and analyzed for TNF-α concentrations by commercial ELISA assay per the manufacturer's instructions (R&D Systems, Minneapolis, MN). Test compounds were administered orally at various times before or at the time of LPS injection. The compounds were dosed either intraveneously or as solutions in various vehicles or solubilizing agents.