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Triaryl bis-sulfones as a new class of cannabinoid CB2 receptor inhibitors: identification of a lead and initial SAR studies

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Abstract—A novel class of cannabinoid CB2 receptor ligands is described. These triaryl bis-sulfones are nanomolar inhibitors of the CB2 receptor and show high selectivity over the cannabinoid CB1 receptor. One example of this new class decreases ligand-induced GTPγS binding to recombinant CB2 cell membranes, identifying the compound as a CB2-selective inverse agonist.

Cannabinoids produce a complex array of biological effects.[1] These effects are mediated primarily through one of two *G*-protein coupled receptors—CB1 and CB2. The cannabinoid CB1 receptor is expressed primarily in the CNS.[2] This receptor is believed to be the mediator of the psychological effects of Δ9-tetrahydrocannabinol (THC). There is also evidence that the CB1 receptor is involved in cytokine production and inflammation in several standard mouse inflammation models.[3] A second receptor, the cannabinoid CB2 receptor, exists primarily in immune related tissues and cells,[4] offering the possibility of modulating the immune system without causing the CNS effects associated with compounds that bind to the CB1 receptor. To date, reported functions of CB2 have included modulation of B-cell differentiation,[5] altered migration,[6] altered antigen processing[7] in macrophages, and altered cannabinoid-mediated anti-tumor activity.[8] Of the immunoregulatory activities ascribed to cannabinoid compounds, some appear to be centrally mediated through the CB1 receptor.[3] Some activities are receptor independent.[9] However, a number are pertussis-toxin sensitive and thus clearly receptor mediated. We embarked on a program to identify a novel class of highly selective cannabinoid CB2 compounds. Our goal was to find potent CB2 selective ligands to explore their immunological effects.

An interest in harnessing the therapeutic potential of the cannabinioid system has lead to the development of several unique classes of cannabinoid ligands.[10] A set of dihydrobenzopyran-type structures, including HU210[11] and the CB2-selective JWH-051[12] and L-759633[13] were derived from the structure of Δ9-tetrahydrocannabinol. Removing the dihydropyran ring of Δ9-tetrahydrocannabinol lead to CP55940.[14] Aminoalkylindoles were developed by Sterling Winthrop leading to development of Win 55,212-2, AM630 and related compounds.[15] Derivatives of the endocannabinoid anandamide[16] lead to a set of eicosanoid-like derivatives.[17] Finally, researchers from Sanofi have developed a set of biarylpyrazoles, including the CB1-selective SR141716A[18] and the CB2-selective SR 144528.[19] SR 141716A (called Rimonabant) is being evaluated by Sanofi for the control of obesity.[20]

Compound **1** was identified as a lead compound for the program with a CB2K_i = 31 nM. However, when a series of analogs of **1** was prepared, all of them had K_i values greater than 1 μM—including the acetamide and the methanesulfonamide. A new highly purified sample of **1** also had a CB2K_i greater than 1 μM. LCMS indicated the presence of two impurities present in the original

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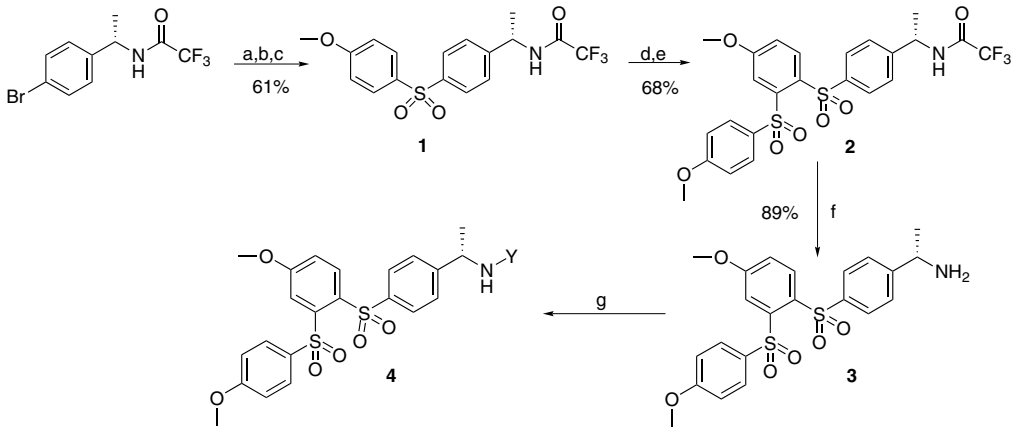

Scheme 1. Reagents and conditions: (a) 1.02 equiv CH_3Li, THF–hexanes, −78 °C; (b) 1.02 equiv *n*-BuLi; (c) 4-methoxyphenylsulfonyl fluoride; (d) 2.1 equiv *n*-BuLi, THF–hexanes, −78 °C, then 4-methoxyphenyl disulfide, −78 °C to rt overnight; (e) *m*-CPBA, CH_2Cl_2; (f) 1.0 M aq LiOH, dioxane; (g) electrophile, CH_2Cl_2, diisopropylethyl amine, rt overnight, then Tris (2-aminoethyl)amine-polystyrene.

sample of **1**. These impurities became targets for further investigation.

Compound **1** was resynthesized on large scale and all the reaction products were tested in the binding assay. The active impurity present in the screening sample of **1** was found to be compound **2**. Compound **2** is formed in 3% yield under the conditions used to prepare **1**, presumably by *ortho*-lithiation of **1** by excess butyl lithium in the reaction and subsequent trapping of the resulting dianion by the sulfonyl fluoride. Compound **2** is highly active in the CB2 inhibition assay showing sub-nanomolar potency with fair selectivity for CB2 in preference to CB1—(See Table 1).[21]

In order to study **2** and its analogs, we developed a synthesis of **2** based on the *ortho*-lithiation dianion chemistry. Compound **1** was treated with 2.1 equiv of *n*-butyl lithium, trapped with bis-(4-methoxyphenyl) disulfide, and oxidized to the corresponding sulfone. The *ortho*-lithiation was completely selective for the nonbenzamide ring, possibly because of electrostatic repulsion by the trifluoroacetamide anion that is initially formed. Compound **2** was then deprotected giving compound **3**, which had relatively weak activity (5 μM) at CB2, and treated with a series of acid halides, sulfonyl chlorides, and isocyanates to give amides, sulfonamides, and ureas as products (Scheme 1).[22]

Further development of this class of compounds is illustrated in Tables 1–3. Several SAR trends can be seen. The first is that for all amide and sulfonamide substituents, small hydrophobic alkyl groups are preferred for the CB2 receptor. Aromatic groups decrease activity at CB2, but can improve it at CB1 (**4i**, **4p**). Sulfonamides are more active at CB2 than the corresponding amides, while ureas with the same R group are relatively inactive. The compound with the best combination of CB2 potency and selectivity for CB2 over CB1 was the methanesulfonamide **4j**.

As an initial characterization of the pharmacology of this new class of cannabinoid compounds, **4j** was tested for the ability to modulate the binding of [^{35}S]GTPγS to the cannabinoid CB2 receptor.[23] Figure 1 shows that, like the Sanofi inverse agonist SR 144528, increasing concentrations of **4j** decreases [^{35}S]GTPγS binding to the CB2 membrane preparation. By comparison, the cannabinoid agonists WIN 55,212-2 and HU210 increase [^{35}S]GTPγS binding to the membrane

Table 1. Amide analogs of **4**: Y = (C=O)–R

Entry	R	K_iCB2 (nM)	K_iCB1 (nM)	Selectivity (CB1K_i/CB2K_i)
2	Trifluoromethyl	0.3	235	783
4a	Methyl	7.7	1779	231
4b	Ethyl	29	3,559	122
4c	Propyl	6.2	1173	189
4d	Cyclopropyl	15.7	10,625	676
4e	*t*-Butyl	38.6	2309	60
4f	Benzyl	412	478	1.2
4g	*p*-Toluyl	289	884	3
4h	4-Methoxyphenyl	1258	1172	0.9
4i	3,4-Dichlorophenyl	167	84	0.5

Table 2. Sulfonamide analogs of **4**: Y = (SO_2)–R

Entry	R	K_iCB2 (nM)	K_iCB1 (nM)	Selectivity (CB1K_i/CB2K_i)
4j	Methyl	0.4	905	2263
4k	Ethyl	1	1968	1513
4l	Butyl	41	1129	27
4m	Phenyl	239	855	3.6
4n	*p*-Toluyl	495	1923	3.9
4o	3,5-Dichlorophenyl	2476	117	0.05
4p	Benzyl	160	79	0.5

Table 3. Urea analogs of **4**: Y = (CONH)–R

Entry	R	K_iCB2 (nM)	K_iCB1 (nM)	Selectivity (CB1K_i/CB2K_i)
4q	Propyl	495	72,089	145
4r	*p*-Toluyl	802	100,000	125
4s	4-F-Phenyl	1136	17,588	15

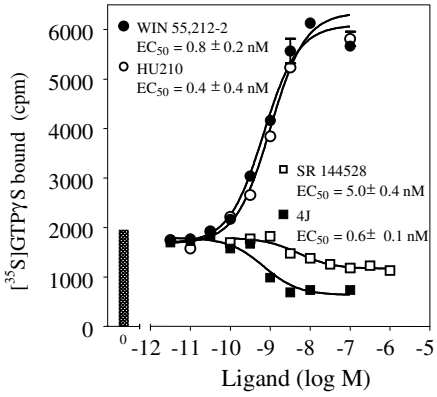

Figure 1. Effect of cannabinoids on [^{35}S]GTPγS binding in Sf9-hCB2 membranes.

preparations with increasing concentrations. These results are consistent with **4j** being an inverse agonist.

Several classes of CB2-selective inverse agonist compounds have been described. These include SR 144528[19] and JTE-907[24] which is highly potent and selective for rodent CB2 (rat K_i = 0.38 nM, 2760 selectivity; mouse K_i = 1.55 nM; 684 selectivity) but its potency for human CB2 is less impressive (K_i = 35.9 nM, 66 selectivity). Both compounds show significant dose-dependent anti-inflammatory activity, reducing carrageenan-induced paw edema in mice.[24] AM630[13] also exhibits a K_i for the human CB2 receptor (using [^{3}H] CP55,940 as radioligand) of 31.2 nM, with selectivity over human CB1 of 165.

The biologic roles of CB2-selective inverse agonists have yet to be fully understood. Most reported work with these agents demonstrates an ability to function as antagonists to added cannabinoid ligands. For example, in studies investigating the role of the CB2 receptor in pain, SR144528 has no significant effect on carrageenan-evoked thermal and mechanical hyperalgesia at concentrations shown to block the activity of CB2-selective agonists.[25] Studies are on-going to establish an in vivo biology for this class of compounds.

In summary, we have identified a novel class of cannabinoid CB2 specific ligands with high affinity for the human receptor. Initial pharmacologic characterization of **4j**, the most potent and selective compound of this series, suggests that the class are inverse agonists for the CB2 receptor and can have excellent selectivity relative to the CB1 receptor. It is hoped that with the discovery of increasingly potent and specific compounds the therapeutic potential of the cannabinoid CB2 receptor can be realized.

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22. All products gave satisfactory analytical data as indicated by ^{1}H NMR and LCMS. Physical data for **4j** is as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, 8.7 Hz, 1H), 7.95–7.90 (m, 6H), 7.46 (d, 9 Hz, 2H), 7.21–7.18 (m, 1H), 6.97 (d, 9 Hz, 2H), 4.74–4.60 (m, 1H), 4.60 (d, 6.3 Hz, 1H, (NH)), 3.95 (s, 3H), 3.85 (s, 3H), 2.65 (s, 3H), 1.53 (d, 6.9 Hz, 3H); LCMS EI exact mass calculated for $C_{23}H_{25}NO_8S_3$ (parent) 539.07 *m/z* found ($M+H^+$) 540.1. For experimental details of the synthesis and the determination of K_i values, see: Kozlowski, J. A.; Shih, N. -Y.;

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23. Triplicate serial dilutions of test compound, or cannabinoid agonists WIN55,212-2, HU210, and inverse agonist SR144528 were incubated with 2 μg Sf9 cell membranes expressing $G\alpha_{i3}$, $\beta_{1\gamma2}$, and hCB2 (7–14 pmol/mg) and 0.3 nM [^{35}S]GTPγS in 20 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, and 0.2% (w/v) BSA (Factor V, lipid free), pH 7.4 supplemented with 2.5 μM GDP. Following incubation for 30 min at 30 °C the reaction was terminated by rapid filtration through the microfiltration plates. The filters were washed 10 times at room temperature with 20 mM HEPES and 10 mM sodium pyrophosphate, and membrane-bound radioactivity was measured by liquid scintillation. Nonlinear regression analysis of the data was performed with Prism 2.0b (GraphPad, San Diego, CA). Additional information may be found in: Gonsiorek, W.; Lunn, C.; Fan, X.; Narula, S.; Lundell, D.; Hipkin, R. W. *Mol. Pharmacol.* **2000**, *57*, 1045.
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