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Conformationally constrained analogs of BAY 59–3074 as novel cannabinoid receptor ligands

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ABSTRACT

To obtain information on the pharmacophoric requirements of the CB1/CB2 partial agonist BAY 59–3074 we have synthesized a series of new conformationally constrained dibenzofuran (**4a–d**) and dibenzopyran analogs (**5**). All constrained analogs exhibited reduced binding affinity at both cannabinoid receptor subtypes, suggesting that planar conformations of these ligands are less favored by both receptors. We also found that **4c**, **4d**, and **5** exhibited 3- to 12-fold selectivity for hCB2 over rCB1 receptors and may serve as new chemotypes for the development of CB2-selective cannabinergics.

The endocannabinoid system plays a vital role in many pathophysiological processes and includes two cannabinoid receptors (CB),[1–6] CB1 and CB2, their endogenous ligands, arachidonoylethanolamine (AEA, anandamide)[7] and 2-arachidonoylglycerol (2-AG)[8,9] as well as several enzymes involved in their biosynthesis and bioinactivation. CB1 and CB2 belong to class-A (rhodopsin-like) of the superfamily of G-protein coupled receptors (GPCRs). While CB1 is the most abundant GPCR in the brain and also present in several peripheral tissues[3,10–12] CB2 is mainly found in the periphery[13,14] and to a small extent in brain.[15] However, its expression is upregulated during early stages of inflammation.[14] (−)Δ^9-Tetrahydrocannabinol (Δ^9-THC; **1**, Fig. 1), the main psychoactive constituent of marijuana[16] (*Cannabis sativa* L.), produces its physiological effects through interaction with both CB1 and CB2, while its psychoactive effects are attributable to interactions with CB1 receptors in the CNS.[17] Existing data suggest the potential usefulness of CB agonists for treating pain, gastrointestinal (GI) disorders, glaucoma, nausea and vomiting induced by chemotherapeutic agents, atherosclerosis, addiction, MS and tumorigenesis.[18,19]

Using high throughput screening (HTS), Bayer Pharmaceuticals identified a structurally novel chemotype that exhibits agonism at both CB receptors. BAY 38–7271 (**2**; Fig. 1; (−)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate), a structurally novel cannabinergic ligand, is a high affinity (K_i = 0.46–1.85 nM), full agonist at both CB receptors with pronounced neuroprotective properties.[20–23] Structural modification of the cyclopentyl ring fused to the aromatic ring led to another structurally novel, orally active, CB1/CB2 agonist, BAY 59–3074 (**3**; Fig. 1) that exhibits moderate affinity (K_i = 55.4, 48.3, and 45.5 nM for rat CB1, human CB1 and human CB2 receptors, respectively) and partial agonist properties at these receptors in [^{35}S]GTPγS binding assays.[24] BAY 59–3074 was shown to be selective for CB receptors with no significant interactions with other targets in a 214-target receptor, and enzyme activity screen. In rat models of chronic neuropathic and inflammatory pain, BAY 59–3074 exhibits pronounced antihyperalgesic and antiallodynic properties.[24,25]

There is limited SAR information on the two BAY templates in the literature. As both structures share common pharmacophoric features including an aryloxyphenyl template and the *meta* sulfonyl alkyl side chain, we hypothesized that both molecules may interact with the CB1 receptor in a similar fashion. With the objective of improving the affinity and potency of BAY 59–3074 and increasing our understanding of the conformational requirements for CB1 receptor binding and activation, we synthesized a novel series of analogs using the strategy of conformational restriction as a tool for molecular modification and design.

We report here, the design and synthesis of a series of such analogs in which rotation around the C1–O bond is restricted and orientation of the side chain is varied (Fig. 2). This was achieved by connecting the two rings of BAY 59–3074 through a single C2–C6′ (**4a**) or C4–C6′ (**4c**) bonds to form a dibenzofuran ring, or alternatively through a C2–C6′ methylene bridge and moving the side chain to the 4-position (**5**) (Fig. 2). To obtain information on the preferred relative orientation of the side chain with regard to the tricyclic template, we also synthesized their respective regioisomers **4b** and **4d**.

In all new compounds, all of the rings in the tricyclic heterocycle are coplanar or nearly coplanar.

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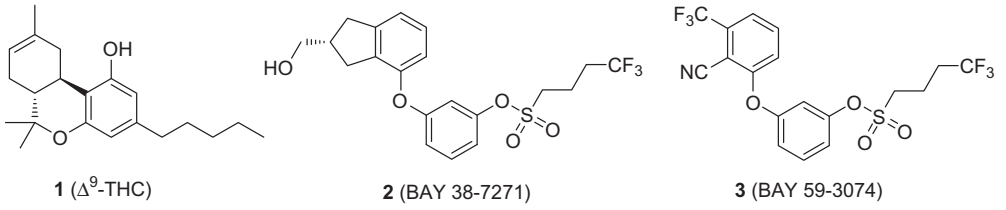


Figure 1. Structures of Δ^9-THC and BAY compounds.

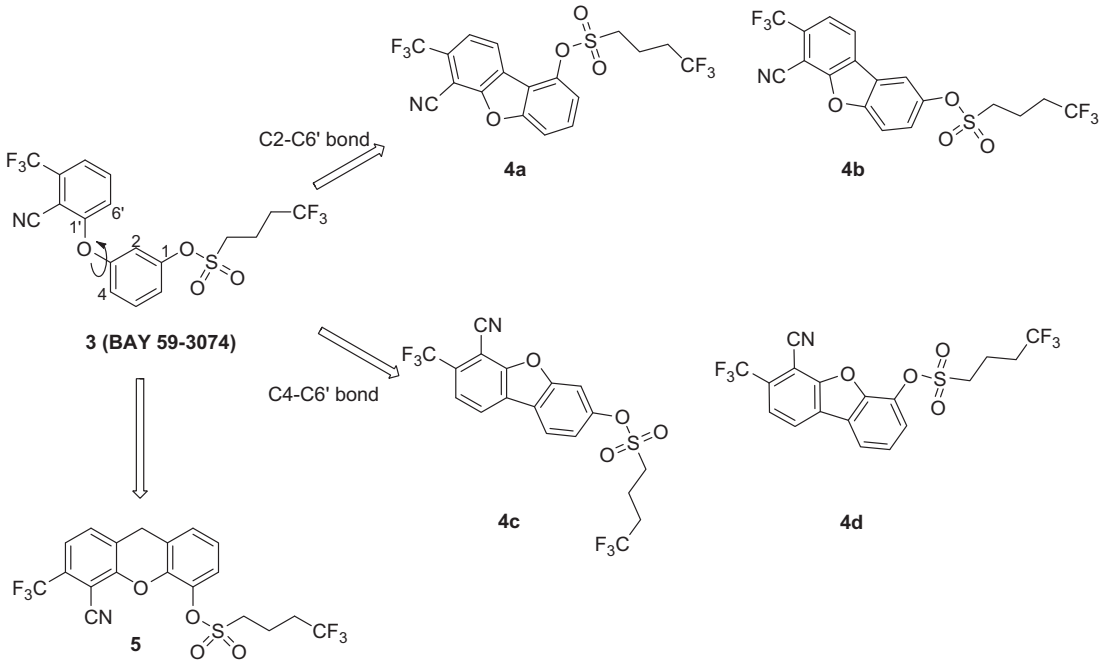
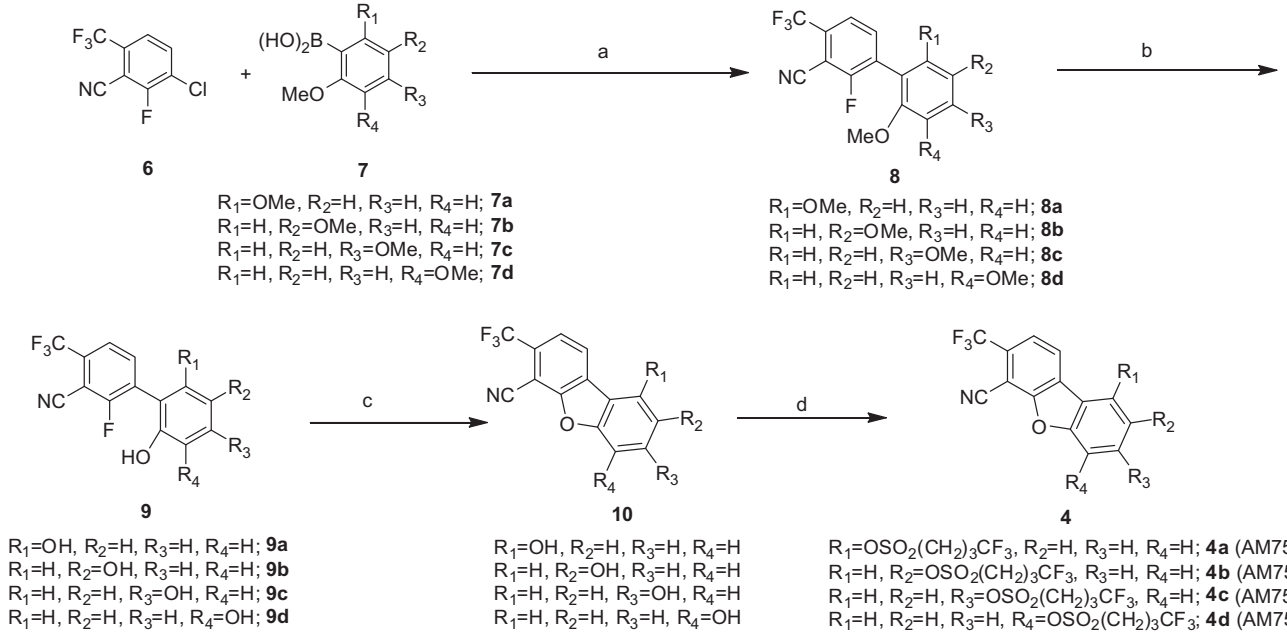
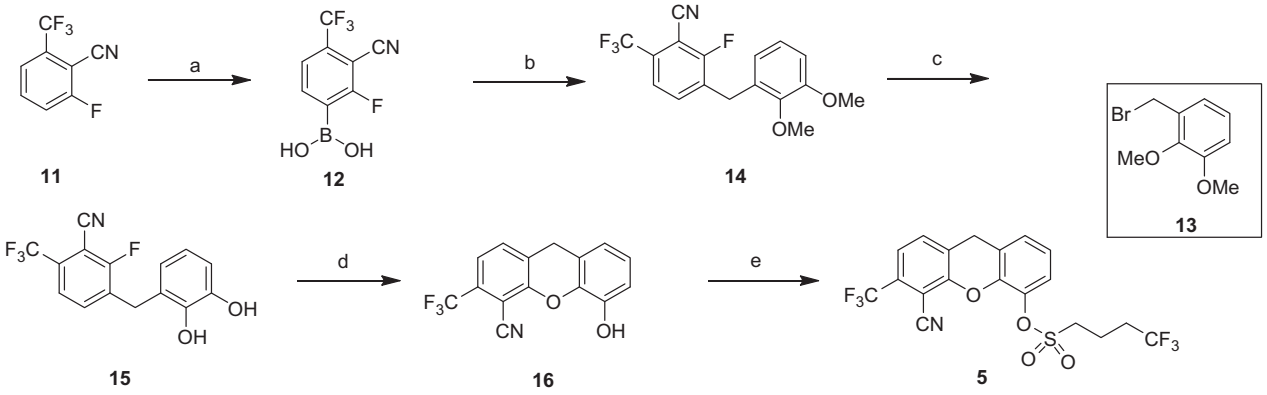


Figure 2. Structural development of tricyclic CB receptor ligands.



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_2$, $Ba(OH)_2{\cdot}8H_2O$, DME/H_2O, microwave, 120 °C, 15 min 42–64%; (b) BCl_3, TBAI, DCM, −78 °C to rt, overnight, 65–87%; (c) NaH, DMSO, rt, 1 h, 65–81%; (d) $ClOSO_2(CH_2)_3CF_3$, 45% NaOH, TBABr, DCM, 74–90%.



Scheme 2. Reagents and conditions: (a) LDA, THF, −78 °C, 2 h, trimethyl borate, −78 °C to rt, 16 h, 32%; (b) 1-(bromomethyl)-2,3-dimethoxybenzene, $Pd(PPh_3)_4$, $Ba(OH)_2{\cdot}8H_2O$, DME: H_2O, microwave, 34%; (c) BBr_3, CH_2Cl_2 −78 °C to rt, 65%; (d) NaOH, DMSO, rt, 54%; (e) 4,4,4-trifluorobutane-1-sulfonyl chloride, TBABr, 45% NaOH, CH_2Cl_2 0 °C to rt, 2 h, 75%.

Synthesis of conformationally constrained BAY 59–3074 analogs is outlined in Scheme 1. The common starting material 3-chloro-2-fluoro-6-(trifluoromethyl)benzonitrile (**6**) was prepared by dehydration of the corresponding benzamide using phosphorous oxychloride,[26] while the biaryl intermediates **8a**–**d**, were obtained by microwave accelerated Suzuki coupling of commercially available methoxyphenyl boronic acids **7** with **6** in 42–64% yields.[27] Deprotection of both methoxy groups in **8** by treatment with BCl_3 and TBAI in dichloromethane[28] at −78 °C led to biaryl phenols **9a**–**d** (obtained in 65–87% yield). These were subjected to intramolecular cyclization by treatment with sodium hydride in anhydrous DMSO to give dibenzofurans **10** in 65–81%. Treatment of **10** with commercially available 4,4,4-trifluorobutane-1-sulfonyl chloride in the presence of tetrabutylammonium bromide and 45% aq NaOH in dichloromethane gave the desired sulfonates **4a**–**d** in 74–90% yield.

Additionally, in order to explore a motif with limited conformational flexibility, we synthesized the xanthane analog **5** (Scheme 2), which connects the aryloxyphenyl template through a methylene bridge.

The synthesis of the dibenzopyran analog is summarized in Scheme 2. Metalation of commer cially available 2-fluoro-6-(trifluoromethyl) benzonitrile (**11**) with LDA followed by quenching with trimethyl borate gave boronic acid **12** in 32% yield. Suzuki-Miyaura coupling of **12** with 1-(bromomethyl)-2,3-dimethoxybenzene (**13**) under microwave accelerated conditions[27] gave **14** in 34% yield. This was demethylated using BCl_3/TBAI combination[28] to give **15** (65% yield) which upon treatment with sodium hydride in DMSO gave dibenzopyran intermediate **16** in 54% yield. The conformationally restricted analog **5** was obtained by treating **16** with 4,4,4-trifluorobutane-1-sulfonylchloride under phase-transfer conditions in 75% yield.

Table 1
Affinities for CB1 and CB2 receptors

Compound	rCB1 K_i (nM)	mCB2 K_i (nM)	hCB2 K_i (nM)	rCB1/hCB2
BAY 59–3074	55.4	—	45.5	1.21
AM-7526 (**4a**)	1900	676	1805	1.05
AM-7535 (**4b**)	2526	1709	3290	0.76
AM-7528 (**4c**)	1062	339	317	3.35
AM-7536 (**4d**)	2278	410	253	9.0
AM-7585 (**5**)	14,400	1130	1160	12.4

The affinities of all new compounds for both CB1 and CB2 cannabinoid receptors are listed in Table 1. rCB1, mCB2, and hCB2 binding affinities were determined by the radioligand competition

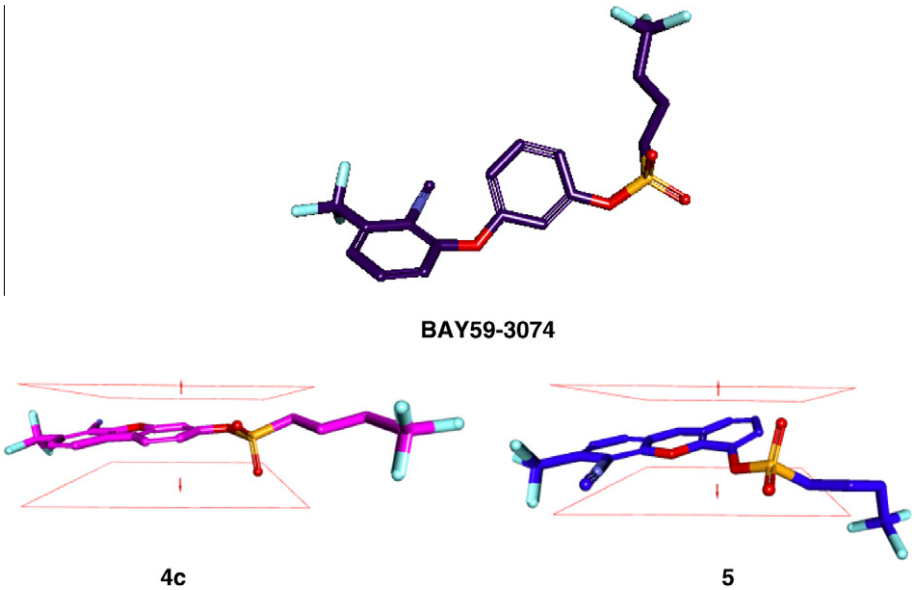


Figure 3. The preferred conformations of BAY 59–3074 (purple), **4c** (magenta) and **5** (blue) using Discovery Studio by Accelrys.

binding experiments using [^{3}H]CP55, 940, as the radioligand.[29,30] All conformationally constrained analogs had significantly reduced affinities for both receptors. Interestingly, two analogs AM-7528 (**4c**) and AM-7536 (**4d**) originating from direct C4–C6′ linkage exhibited selectivity for both mCB2 and hCB2 receptors compared to rCB1 receptors.

To explore the significant loss of affinity of the novel conformationally constrained analogs when compared to the parent ligand, BAY 59–3074, we examined their respective preferred conformations (Fig. 3) using Discovery Studio by Accelrys. It is clear that for analog **4c** the tricyclic ring system is planar. Expansion of the five membered furan ring of **4c** to a six membered ring in xanthane analog **5** avails some, albeit modest, conformational flexibility to the ring system. Here too, the overall tricyclic system is fixed into a quasi-planar conformation (2.16° from planar). Conversely, the parent compound assumes a conformation in which the planes of the two aryl rings are at 4.52° from planar and are capable of further accommodating additional thermodynamically allowable conformations capable of reacting favorably with the CB1 or CB2 targets. These results argue for a pharmacophoric conformation in which aryl rings A and B are not coplanar and may explain the loss of affinity for the novel conformationally constrained analogs described here.

In summary, to probe the bioactive conformation of BAY 59–3074 and to improve its binding profile at CB receptors, a series of conformationally constrained analogs were synthesized successfully. These constrained analogs represent a new CB chemotype of dibenzofuran class, with ligand **4d** exhibiting selectivity for both mouse and human CB2 receptors.

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