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Trisubstituted Sulfonamides: A New Chemotype for Development of Potent and Selective CB_2 Receptor Inverse Agonists

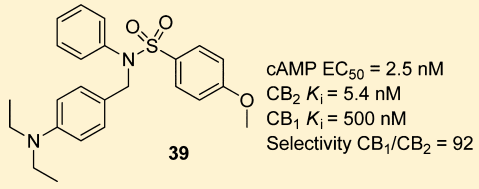
Qin Ouyang,[†,‡,@] Qin Tong,[†,‡] Rentian Feng,[†,‡] Kyaw-Zeyar Myint,[†,‡,∥] Peng Yang,[†,‡] and Xiang-Qun Xie*[,†,‡,∥,§,⊥]

[†]Department of Pharmaceutical Sciences and Computational Chemical Genomics Screening Center, School of Pharmacy, [‡]Drug Discovery Institute, [§]Pittsburgh Chemical Methods and Library Development Center, [∥]Department of Computational and Systems Biology, and [⊥]Department of Structural Biology, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

[@]College of Pharmacy, Third Military Medical University, Chongqing 400038, China

Supporting Information

ABSTRACT: An extensive exploration of the structure−activity relationship of a trisubstituted sulfonamide series led to the identification of **39**, which is a potent and selective CB_2 receptor inverse agonist [$K_i(CB_2)$ = 5.4 nM, and $K_i(CB_1)$ = 500 nM]. The functional properties measured by cAMP assays indicated that the selected compounds were CB_2 inverse agonists with high potency values (for **34**, EC_{50} = 8.2 nM, and for **39**, EC_{50} = 2.5 nM). Furthermore, an osteoclastogenesis bioassay indicated that trisubstituted sulfonamide compounds showed great inhibition of osteoclast formation.



KEYWORDS: *Cannabinoid receptors, inverse agonists, trisubstituted sulfonamides, osteoclast inhibitors*

Cannabinoid receptors 1 and 2 (CB_1 and CB_2, respectively) were identified in the early 1990s as members of the G protein-coupled receptor (GPCR) superfamily.[1,2] While CB_1 receptors are primarily found in the central nervous system (CNS), CB_2 receptors are predominantly located in tissues and cells of the immune system, such as tonsils, spleen, macrophages, and lymphocytes.[3] Also, there is some evidence of the presence of the CB_2 receptor in the CNS.[4,5]

Recently, numerous agonists and antagonists of cannabinoid receptors have been explored because of the important role of the endocannabinoid system in various diseases and disorders.[6] Among these, CB_1 receptor ligands have been developed for pain, appetite stimulation, nausea, neurodegeneration, hypermotility, and inflammation.[7] However, the CB_1 receptor ligands are known to cause side effects in the CNS such as cognitive dysfunction, motor incoordination, and sedation.[8] Because of differences in receptor distribution and signal transduction mechanisms, CB_2-selective ligands are considered as medications without CNS side effects,[9] and such ligands are being actively investigated for use in a multitude of diseases and pathological conditions,[10] such as atherosclerosis,[11] myocardial infarction,[12] stroke,[13] gastrointestinal inflammatory,[14] autoimmune,[15] and neurodegenerative[16] disorders, bone disorders,[17−19] and cancer.[20]

The development of CB_2 receptor-selective ligands has attracted significant attention because of the therapeutic potential of CB_2 receptor modulation.[21−23] The first CB_2 inverse agonist is SR144528, which is extensively used as the standard to measure the specificity of various cannabinoid inverse agonists for CB_2 in animal models.[24] Other notable examples of CB_2 receptor agonists and antagonists include AM630,[25] JTE-907,[26] Sch225336,[27] and JWH-133.[28] Recently, on the basis of the research in three-dimensional CB_2 receptor structure model[29,30] and pharmacophore database searches, our group also reported the discovery of novel bis-amide derivatives [**1** (Figure 1)] as CB_2 receptor inverse agonists and osteoclast inhibitors.[31] However, the optimization of bis-amide derivatives is limited by the synthesis method and symmetrical scaffold.

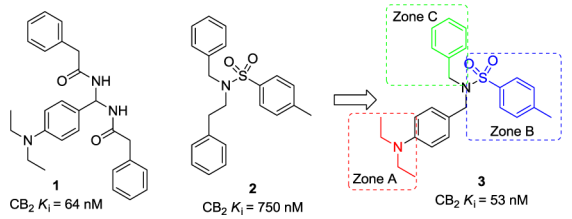


Figure 1. Structures of CB_2 receptor inverse agonists and new scaffold discovery.

On the basis of continuing virtual screening and QSAR results,[32] we designed and synthesized **2**, with a trisubstituted sulfonamide scaffold, as a novel chemotype with CB_2 binding activity [$K_i(CB_2)$ = 750 nM]. Compared with the structure of **1** and considering the QSAR results, we believed that a longer chain in zone A was important for the CB_2 inverse agonist (Figure 1). Compound **3** with a diethylamino group was synthesized and confirmed to have a better CB_2 binding affinity [$K_i(CB_2)$ = 53 nM] and a good CB_2 selectivity index [SI = 43,

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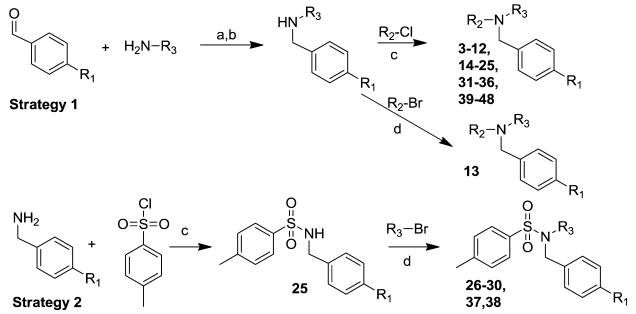
calculated as the $K_i(CB_1)/K_i(CB_2)$ ratio]. Given this promising result, **3** was chosen as a prototype for further structure−activity relationship (SAR) studies. Herein, we reported the design and synthesis of novel trisubstituted sulfonamide derivatives as CB_2 inverse agonists. Binding activities were investigated to define their SAR and ligand functionality. After we modified the groups at zones A−C, some derivatives, such as **34** [$K_i(CB_2)$ = 5.5 nM, and SI = 15], **39** [$K_i(CB_2)$ = 5.4 nM, and SI = 92], and **45** [$K_i(CB_2)$ = 4.0 nM, and SI = 120], were identified as CB_2-selective ligands with improved CB_2 binding affinity and high selectivity. These compounds were selected for the functional property investigation by a cAMP assay, which showed their high potency (for **34**, EC_{50} = 8.2 nM, and for **39**, EC_{50} = 2.5 nM) as CB_2 inverse agonists. Moreover, these compounds also showed great inhibition activity with osteoclast cells.

The trisubstituted sulfonamide derivatives were synthesized by two general strategies (Scheme 1). In the first strategy, the

Scheme 1. Synthesis of Trisubstituted Sulfonamides[a]



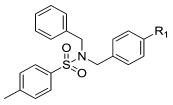
[a]Reagents and conditions: (a) CH_3OH, reflux; (b) CH_3OH, $NaBH_4$; (c) Et_3N, CH_2Cl_2; (d) K_2CO_3, CH_3COCH_3, reflux.

different imine intermediates, synthesized from reductive amination reactions of substituted aryl aldehydes and various amines, were reacted with different sulfonyl chlorides to prepare compounds **3**−**12**, **14**−**25**, **31**−**36**, and **39**−**48**, as well as with benzyl bromide to obtain **13**. Compounds **26**−**30**, **37**, and **38** were obtained by the reaction of intermediate **25** with various bromides. The structures of all the compounds were characterized by ^{1}H NMR and ESI-HRMS spectra; purity was confirmed by HPLC.

Biological data of compounds with different substituents of zone A are listed in Table 1. Some alkyl chains, whose substituents had been shown in our previous study to be favorable for CB_2 receptor affinity,[31] were introduced at position 4 of phenyl, such as the bialkyl chain (dimethylamino, isopropoxyl, and isopropyl), monoalkyl chain substituents (ethoxyl, propoxyl, and butyl), and cycloalkanes (1-piperidinyl). Among them, **3** (R_1 = diethylamino) and **6** (R_1 = 1-piperidinyl) showed higher affinities for the CB_2 receptor (53 and 44 nM, respectively) with a good CB_2 selective index (SI = 43 and 28, respectively). These results indicated that longer chains and double chains are necessary for improving binding affinity in this scaffold.

The modification of zone B was based on maintenance of the diethylamino group in zone A, as shown in Table 2. Compound **13**, in which the sulfo group was replaced with a CH_2 group, showed a slightly lower affinity. A similar result was found when a CH_2 group was added between the sulfo and the phenyl group (**14**). Many substituents were introduced at position 4 of

Table 1. CB_1 and CB_2 Receptor Affinities of Compounds 3−12 with a Modified Zone A



compd	R_1	K_i (nM) CB_2[a,b]	K_i (nM) CB_1[a,b]	SI[c]
3	-N(CH_2CH_3)$_2$	53 ± 6	2300 ± 200	43
4	-N(CH_3)$_2$	680 ± 10	NT	
5	-($CH_2CH_2Cl_2$)$_2$	166 ± 4	NT	
6	1-piperidinyl	44 ± 8	1300 ± 200	28
7	-OCH(CH_3)$_2$	174 ± 7	NT	
8	-OCH_2CH_3	230 ± 60	NT	
9	-$CH_2CH_2CH_3$	130 ± 20	NT	
10	-CH_2CH=CH_2	130 ± 40	NT	
11	-*n*-butyl	280 ± 50	NT	
12	-CH(CH_3)$_2$	230 ± 40	NT	
SR144528[d,e]		2.1 ± 0.4	NT	
SR141716[d,f]		NT	11 ± 1	

[a]Binding affinities of compounds for CB_1 and CB_2 receptors were evaluated using the [^{3}H]CP-55,940 radioligand competition binding assay. Data are means ± the standard error of the mean of at least three experiments performed in duplicate. [b]NT, not tested. [c]SI, selectivity index for CB_2, calculated as the $K_i(CB_1)/K_i(CB_2)$ ratio. [d]The binding affinities of reference compounds were evaluated in parallel with compounds **3**−**12** under the same conditions. [e]CB_2 reference compound. [f]CB_1 reference compound.

the sulfophenyl, such as Cl, F, H, acetylamide, isopropyl, methoxyl, isopropoxyl, and trifluoromethoxyl. Other than **20** (R_2 = 4-methoxyphenyl), which had a binding affinity for the CB_2 receptor (K_i = 46 nM) similar to that of **3**, **13**−**22** did not show obvious increased binding affinity. Large groups, like naphthyl and 1,1′-biphenyl, were also used to replace the 4-methylphenyl. This replacement resulted in a lower binding affinity. These results indicated that 4-methylphenyl and 4-methoxylphenyl are the best groups among our modifications of zone B. Moreover, the CB_2 selective indexes of **15**, **16**, **19**, and **20** were quite low, ranging from 6 to 15.

After investigation of the SAR of R_1 and R_2 in zones A and B, the binding affinities were not obviously improved. The following modification of group R_3 in zone C was based on maintenance of the R_1 and R_2 groups in **3** (Table 3). As expected, group R_3 is a key substituent for enhancing the CB_2 affinity. First, another CH_2 group was introduced at the N atom and the phenyl ring; compound **26** showed a lower CB_2 binding affinity (K_i = 120 nM). The same reduced trends were found when diethylamino and halogen (Cl and F) were added to the phenyl ring in zone C. The binding affinities for the CB_2 receptor of **27**−**30** were 540, 210, 390, and 120 nM, respectively. After substitution of the phenyl in zone C with cyclohexyl, a small increase in affinity was achieved [for **31**, $K_i(CB_2)$ = 34 nM], while replacement by five-membered heterocyclic moieties (in **32** and **33**) enhanced affinity dramatically, yielding a K_i values of 9.5 and 14 nM at CB_2 receptors and 570 and 610 nM at CB_1 receptors, respectively. It seemed that a smaller group in zone C is preferable for CB_2 binding activity, so some compounds with smaller group were synthesized. Compound **34** with the reduced CH_2 between the N atom in the core and phenyl group showed a higher CB_2 binding affinity (K_i = 5.5 nM, and SI = 15). However, replacement with other smaller groups, such as cyclohexyl (**35**),

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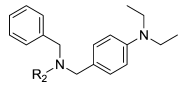
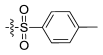
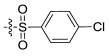
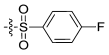
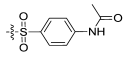
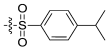
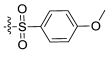
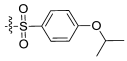
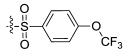
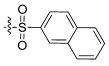
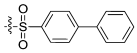
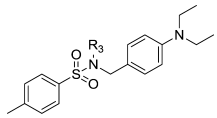
Table 2. CB_1 and CB_2 Receptor Affinities of Compounds 13−24 with a Modified Zone B

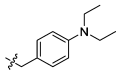


cmpd. no.	R_2	*Ki* (nM) CB_2[b]	*Ki* (nM) CB_1[a,c]	SI[d]
3		53 ± 6	2300 ± 200	43
13		150 ± 20	NT	
14		262 ± 5	NT	
15		73 ± 5	430 ± 90	6
16		102 ± 3	930 ± 90	9
17		170 ± 30	NT	
18		470 ± 60	NT	
19		71 ± 7	520 ± 50	7
20		46 ± 5	690 ± 90	15
21		94 ± 4	NT	
22		577 ± 7	NT	
23		190 ± 10	NT	
24		166 ± 7	NT	
SR144528[e,f]		2.1 ± 0.4	NT	
SR141716[e,g]		NT	11 ± 1	

[a−g]Same as the footnotes of Table 1.

Table 3. CB_1 and CB_2 Receptor Affinities of Compounds 25−38 with a Modified Zone C



Cmpd. no.	R_3	*Ki* (nM) CB_2[b]	*Ki* (nM) CB_1[a,c]	SI[d]
3		53 ± 6	2300 ± 200	43
25	H	3600 ± 500	NT	
26		120 ± 10	NT	
27		540 ± 60	NT	
28		210 ± 30	NT	
29		390 ± 20	NT	
30		120 ± 10	2250 ± 80	20
31		34 ± 2	560 ± 10	16
32		9.5 ± 0.9	570 ± 10	60
33		14 ± 2	610 ± 90	44
34		5.5 ± 0.7	81 ± 13	15
35		18 ± 4	210 ± 20	12
36		14 ± 2	340 ± 30	24
37		36 ± 3	500 ± 60	14
38		66 ± 10	1250 ± 60	19
SR144528[e,f]		2.1 ± 0.4	NT	
SR141716[e,g]		NT	11 ± 1	

[a−g]Same as the footnotes of Table 1.

5-methylthiazolyl (**36**), cyclopentyl (**37**), and allyl (**38**), significantly reduced the binding activity [$K_i(CB_2)$ = 18, 14, 36, and 66 nM, respectively]. Especially, **25**, without any substituted group except with an H atom at R_3, showed quite low CB_2 binding activity [$K_i(CB_2)$ = 3600 nM]. This result suggests that the space of the phenyl group is suitable for zone C and thus addition of a larger or smaller group results in lower CB_2 binding activity.

On the basis of the SAR research of zones A−C, we found that the introduction of some groups at R_1−R_3 could improve CB_2 binding activity. Further SRA research was based on the compound library of combinations of these groups: R_1 = diethylamino and 1-piperidinyl, R_3 = phenyl, cyclohexyl, furan-2-ylmethyl, and 5-methylthiazolyl, and R_4 = methoxyl, Cl, isopropyl, and methyl. The results are listed in Table 4 with the CB_2 binding affinity ranging from 4 to 310 nM and the selective index ranging from 9 to 120. Among them, two compounds were identified with better CB_2 binding activity and selectivity, **39** [$K_i(CB_2)$ = 5.4 nM, and SI = 92] and **45** [$K_i(CB_2)$ = 4.0 nM, and SI = 120].

Functional properties were investigated in cAMP assays by using cell-based LANCE cAMP assays as our published protocol[33] to measure the agonistic or antagonistic functional activities of the CB_2-selective compounds. Because CB_2 is a $G_{\alpha i}$-coupled receptor, an agonist inhibits the forskolin-induced cAMP production, resulting in an increase in the magnitude of the LANCE signal, while an antagonist or inverse agonist decreases the magnitude of the LANCE signal. In addition to **34**, **39**, **45**, and **47**, with respective modifications in zones A−C for good binding activity, were selected for cAMP assays. As shown in Figure 2, reduction of the magnitude of the LANCE signal occurred with increasing concentrations of **34**, **39**, **45**, **47**, and SR144528 with EC_{50} values of 8.2 ± 3.1, 2.5 ± 1.4, 73 ± 2, 49 ± 2, and 14 ± 3 nM, respectively. Such a contrary phenomenon was observed with agonists CP55,940 and HU308,[34] which showed cAMP production with EC_{50} values of 11 ± 2 and 85 ± 5 nM, respectively. On the basis of the LANCE signal change and the high EC_{50} value closely correlated with the high affinity value, it suggests that **34**, **39**, **45**, and **47** behaved as CB_2 receptor inverse agonists.

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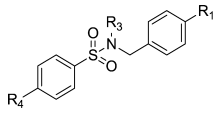
Table 4. CB_1 and CB_2 Receptor Affinities of Compounds 39−48



compd	R_1	R_3	R_4	K_i (nM) CB_2[a,b]	K_i (nM) CB_1[a,b]	SI[c]
39	-N(CH_2CH_3)$_2$	phenyl	-OCH_3	5.4 ± 0.5	500 ± 60	92
40	-N(CH_2CH_3)$_2$	phenyl	-$CH(CH_3)_2$	30 ± 4	2000 ± 200	66
41	-N(CH_2CH_3)$_2$	furan-2-ylmethyl	-OCH_3	20 ± 2	840 ± 80	42
42	-N(CH_2CH_3)$_2$	furan-2-ylmethyl	-$CH(CH_3)_2$	31 ± 4	1300 ± 100	42
43	-N(CH_2CH_3)$_2$	cyclohexyl	-OCH_3	75 ± 6	660 ± 60	9
44	-N(CH_2CH_3)$_2$	cyclohexyl	-$CH(CH_3)_2$	55 ± 2	1400 ± 100	25
45	-N(CH_2CH_3)$_2$	5-methylthiazolyl	-OCH_3	4.0 ± 0.6	600 ± 10	120
46	1-piperidinyl	phenyl	-CH_3	310 ± 20	NT	
47	1-piperidinyl	phenyl	-OCH_3	32 ± 7	590 ± 50	18
48	1-piperidinyl	phenyl	-Cl	140 ± 10	NT	
SR144528[d,e]				2.1 ± 0.4	NT	
SR141716[d,f]				NT	11 ± 1	

[a−f]Same as the footnotes of Table 1.

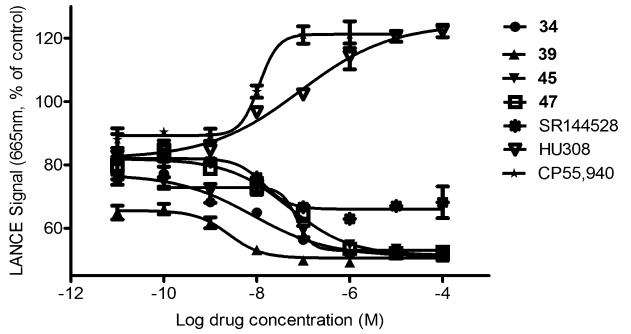


Figure 2. Comparisons of the LANCE signal of different CB_2 receptor ligands in stably transfected CHO cells expressing human CB_2 receptors in a concentration-dependent fashion. EC_{50} values of compounds **34**, **39**, **45**, **47**, and SR144528 are 8.2 ± 3.1, 2.5 ± 1.4, 73 ± 2, 49 ± 2, and 14 ± 3 nM, respectively. EC_{50} values for CP55,940 and HU308 are 11 ± 2 and 85 ± 5 nM, respectively. Data are means ± the standard error of the mean of one representative experiment of two or more performed in duplicate or triplicate.

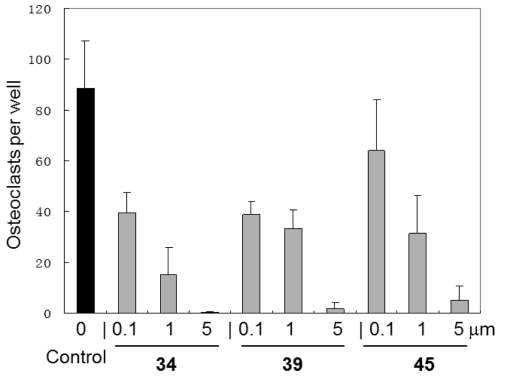


Figure 3. Inhibition of osteoclastogenesis by CB_2 ligands **34**, **39**, and **45**. All experiments were performed in triplicate. Results are means ± the standard deviation. Note that the control is vehicle control.

Modulating osteoclast function is a well-known activity of CB_2 receptor agonists[35] and inverse agonists.[19] Three compounds, **34**, **39**, and **45**, were selected on the basis of the results of binding affinity and selectivity, as well as their functionality as candidate inhibitors of osteoclast (OCL) formation. As shown in Figure 3, we tested the effects of these most promising CB_2 ligands on osteoclast (OCL) formation using mouse bone marrow mononuclear cells treated by the mouse receptor activator of NF-κB ligand (RANKL) plus macrophage-colony-stimulating factor (M-CSF) (see the Supporting Information). These three compounds exhibited strong inhibition of osteoclastogenesis. Among them, **34** showed the most favorable activity. At 0.1, 1, and 5 μM, it suppressed osteoclast formation by 55, 83, and 99.7%, respectively. To investigate their cell toxicity, **34** was tested in a cytotoxicity assay without showing any cytotoxic effects at a concentration of 5 μM. These results indicate that our compounds possess favorable therapeutic indexes and the inhibition of osteoclastogenesis is not a result of their cytotoxicity.

In summary, we have discovered the trisubstituted sulfonamide chemotype as a novel series possessing significant cannabinoid CB_2 receptors affinity. Some compounds with high binding affinities and selective indexes of CB_2 receptors were identified by optimization of zones A−C. The potencies of the novel compounds were measured in functional assays, with high potency values (represented by EC_{50}) that are closely correlated with the high affinities (expressed as K_i), revealing that the novel series behaves as CB_2 receptor inverse agonists. The promising inhibition activity to osteoclast cells of this novel series of compounds offers an attractive starting point for further optimization.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic experimental details, analytical data, and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: (412) 383-5276. Fax: (412) 383-7436. E-mail: xix15@pitt.edu.

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Notes

The authors declare no competing financial interest.

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