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Novel sulfamoyl benzamides as selective CB_2 agonists with improved in vitro metabolic stability

Ian Sellitto [a], Bertrand Le Bourdonnec [a,*], Karin Worm [a], Allan Goodman [a], Markku A. Savolainen [a], Guo-Hua Chu [a], Christopher W. Ajello [a], Christopher T. Saeui [a], Lara K. Leister [a], Joel A. Cassel [b], Robert N. DeHaven [b], Christopher J. LaBuda [b], Michael Koblish [b], Patrick J. Little [b], Bernice L. Brogdon [c], Steven A. Smith [c], Roland E. Dolle [a]

[a] Department of Chemistry, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, USA
[b] Department of Pharmacology, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, USA
[c] Department of DMPK, Adolor Corporation, 700 Pennsylvania Drive, Exton, PA 19341, USA

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ABSTRACT

A lead optimization campaign in our previously reported sulfamoyl benzamide class of CB_2 agonists was conducted to improve the in vitro metabolic stability profile in this series while retaining high potency and selectivity for the CB_2 receptor. From this study, compound **14**, *N*-(3,4-dimethyl-5-(morpholinosulfonyl)phenyl)-2,2-dimethylbutanamide, was identified as a potent and selective CB_2 agonist exhibiting moderate in vitro metabolic stability and oral bioavailability. Compound **14** demonstrated in vivo efficacy in a rat model of post-surgical pain.

The cannabinoid receptors CB_1 and CB_2 belong to the super family of G-protein coupled receptors. While CB_1 receptors are expressed in the central nervous system (CNS), CB_2 receptors are mainly localized in peripheral nerve terminals and in the tissues of the immune system.[1] These receptors are activated by cannabinoid ligands that are either produced endogenously (known as endocannabinoids) or administered exogenously in the form of natural or synthetic compounds. *Cannabis sativa* contains the active component Δ^9-tetrahydrocannabinol (THC, **1**) that displays a wide range of beneficial pharmacological effects including analgesia and anticonvulsive activity.[2–6] THC also elicits a number of undesirable CNS effects such as psychotomimetic effects and impaired memory function. These undesired effects are believed to be mediated via activation of the CB_1 receptors in the CNS.[7] Separating the therapeutic effects of cannabinoid agonists from their undesired effects could be accomplished by either preventing the ligands from crossing the blood-brain barrier[8] or by increasing the selectivity of the ligands for the CB_2 receptor.[9] Several CB_2 selective agonists have been previously reported.[10] GW405833 (**2**, Fig. 1) was shown to produce CB_2 mediated antihyperalgesic activity in numerous rodent models of pain.[11] AM1241 (**3**, Fig. 1) reversed carrageenan induced inflammatory thermal hyperalgesia in rats. This effect was blocked by pretreatment with a CB_2 selective antagonist but not by a CB_1-selective antagonist.[12,13] These results support the development of novel small molecule CB_2 agonists for the treatment of pain.[14] More recent research has led to the identification of structurally diverse classes of CB_2 agonists, including benzimidazoles (**4**),[15] oxadiazoles (**5**),[16] aryl sulfonamides (**6**),[17] and pyrimidines (**7**).[18]

We identified recently a novel series of phenylsulfonamides as selective CB_2 agonists.[19,20] Compound **8**,[20] the lead derivative in this series displayed good affinity at the CB_2 receptor (K_i = 23 nM) and 150-fold selectivity over the CB_1 receptor; however, this compound displayed poor metabolic stability in rat and human liver microsomes (Fig. 2). We report here the summary of the lead optimization campaign conducted to improve upon the metabolic stability of **8**, while retaining potent affinity and selectivity at the CB_2 receptor.

Compounds **9–21** were prepared according to Scheme 1. Chlorosulfonylation of commercially available substituted nitrobenzenes (**32a–e**) was achieved in excellent yield using chlorosulfonic acid. Condensation of the corresponding sulfonyl chlorides with morpholine followed by reduction of the nitro functionality yielded the substituted aniline derivatives **33a–e**. The target compounds **9–21** were prepared from **33a–e** under classical amide formation methodologies. The synthesis of the 2,3-dihydro-*1H*-indene and 1,2,3,4-tetrahydronaphthalene derivatives, **22** and **23**,

* Corresponding author. Tel.: +1 484 595 1061; fax: +1 484 595 1513.
E-mail address: blebourdonnec@adolor.com (B.L. Bourdonnec).

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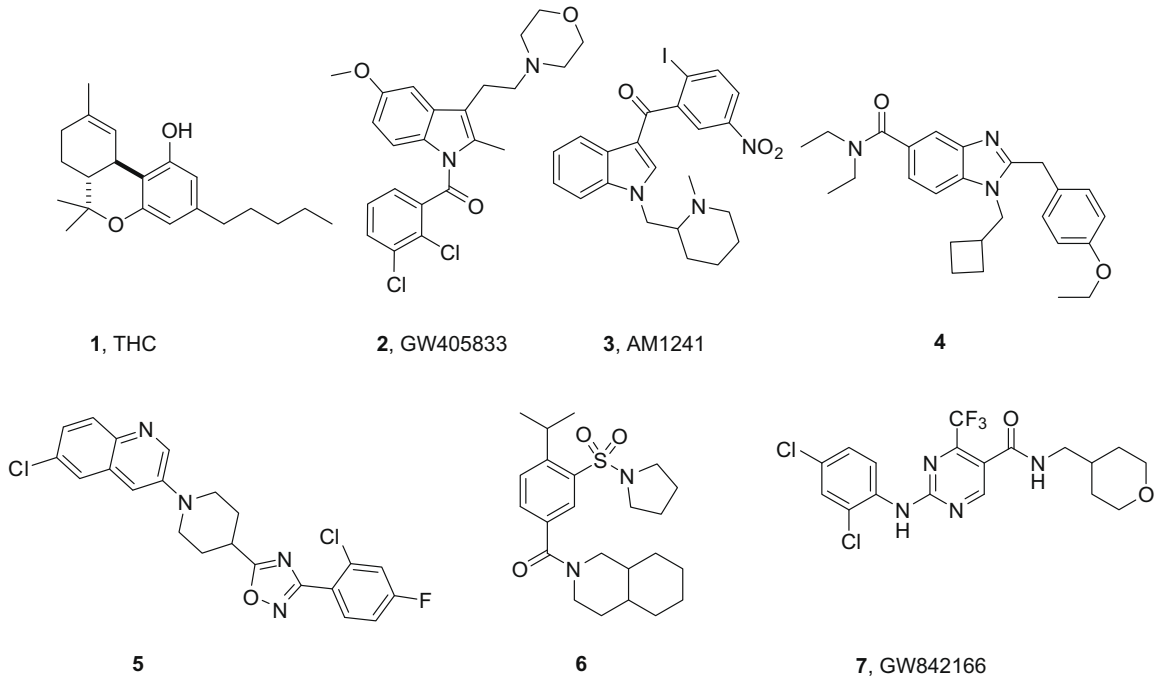
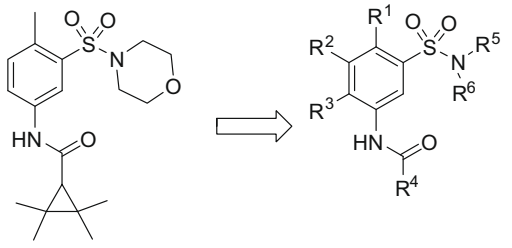
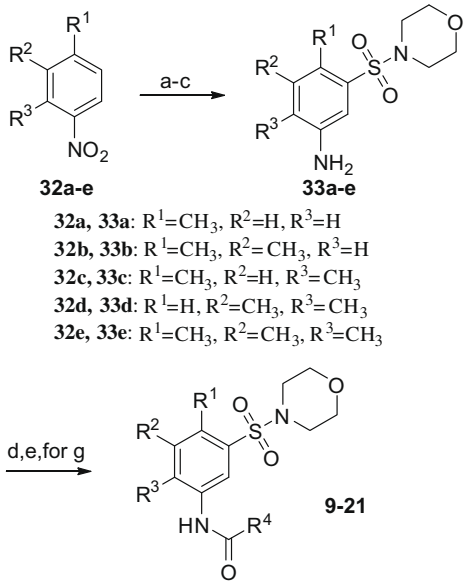


Figure 1. Structures of **1**–**7**.



K_i (CB_2) = 23 nM
K_i (CB_1) = 3400 nM
RLM: 2% remaining @ 30 min
HLM: 1% remaining @ 30 min

Figure 2. Sulfamoyl benzamide class of CB_2 agonists: lead optimization.



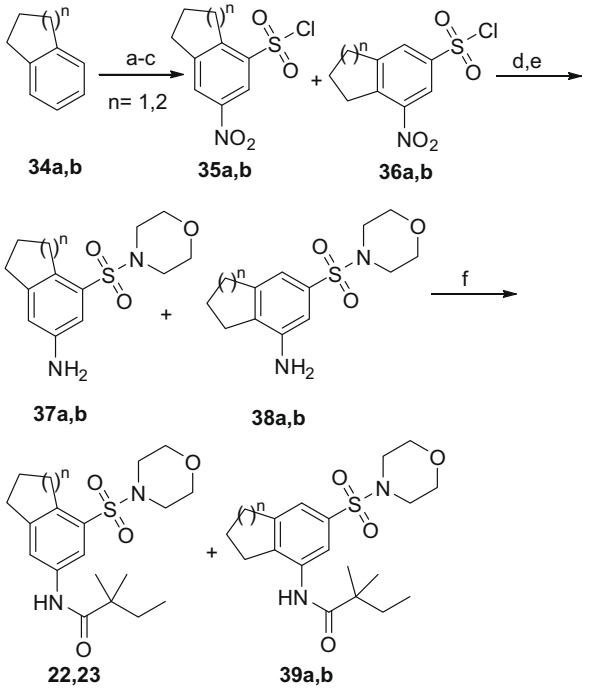
32a, 33a: R^1=CH_3, R^2=H, R^3=H
32b, 33b: R^1=CH_3, R^2=CH_3, R^3=H
32c, 33c: R^1=CH_3, R^2=H, R^3=CH_3
32d, 33d: R^1=H, R^2=CH_3, R^3=CH_3
32e, 33e: R^1=CH_3, R^2=CH_3, R^3=CH_3

Scheme 1. Reagents and conditions: (a) chlorosulfonic acid, 90–94%; (b) morpholine, EtOAc, 94%; (c) Fe, NH_4Cl, EtOH, H_2O, 100%; (d) for synthesis of **12**, **17**: R^4COOH, BOPCl, THF, 16–20%; (e) for synthesis of **9**, **10**, **13**: R^4COOH, TBTU, *i*Pr_2EtN, CH_3CN, 14–46%; (f) for synthesis of **15**, **16**, **18**: R^4COOH, BEP, *i*Pr_2EtN, CH_2Cl_2, 13–37%; (g) for synthesis of **11**, **14**, **19**–**21**: R^4COCl, Et_3N, THF, 52–75%.

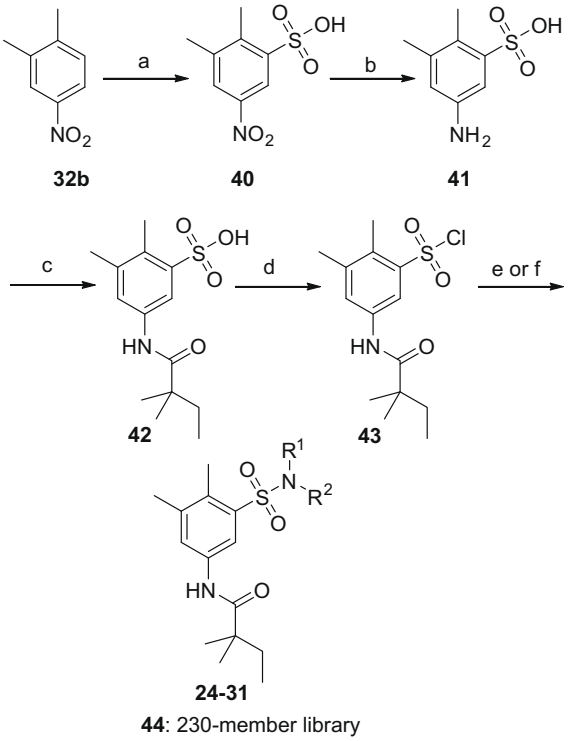
respectively, is outlined in Scheme 2. Nitration of **34a** or **34b** using nitronium tetrafluoroborate, followed by chlorosulfonylation of the resulting nitrobenzene derivatives afforded an inseparable 1:1 mixture of regioisomers (**35a,b** or **36a,b**). These regioisomers were carried through the synthetic scheme as a mixture until the final step where they were separated by column chromatography. Reaction of the sulfonyl chloride (**35a,b** or **36a,b**) with morpholine followed by reduction of the nitro functionality of the corresponding sulfonamides provided the aniline derivatives **37a**,**b** or **38a**,**b**. Treatment of **37a**,**b** or **38a**,**b** with 2,2-dimethylbutyryl chloride in the presence of triethylamine yielded the desired compounds **22** or **23**, respectively.

The 230 member sulfonamide library **44** was synthesized as indicated in Scheme 3. Condensation of commercially available 1,2-dimethyl-4-nitrobenzene (**32b**) with fuming sulfuric acid afforded the sulfonic acid **40** in quantitative yield. Palladium catalyzed reduction of the nitro moiety of **40** provided the corresponding aniline derivative (**41**), which was converted to the amide **42** by treatment with 2,2-dimethylbutyryl chloride in the presence of triethylamine. Treatment of **42** with phosphorus pentachloride afforded the sulfonyl chloride derivative **43**, used for the library synthesis. A set of 230 amines were condensed with **43**, in the presence of polystyrene bound diisopropylethylamine to give the corresponding sulfonamides. Selected compounds (**24–31**) (see discussion) were resynthesized according to Scheme 3.

The target compounds **9**–**31** were evaluated for their affinity toward the cloned human CB_1 and CB_2 receptors, by measuring their ability to displace [^{3}H]CP-55940 in membranes containing CB_1 and



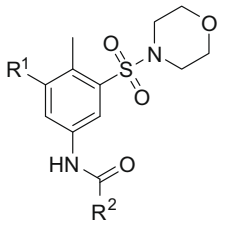
Scheme 2. Reagents and conditions: (a) NO_2BF_4, CH_2Cl_2, 0 °C, 60–65%; (b) fuming H_2SO_4, 99% for n = 1, 2; (c) PCl_5, CH_2Cl_2, 63–69%; (d) morpholine, CH_2Cl_2, 59–84%; (e) Pd/C, H_2, 10 psi, MeOH, 82–88%; (f) 2,2-dimethyl-butyryl chloride, Et_3N, CH_2Cl_2, 10–45% (separation of regioisomers by SiO_2 column chromatography).

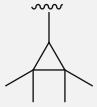
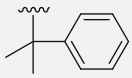
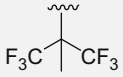
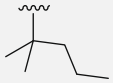
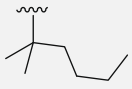
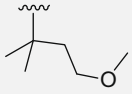


Scheme 3. Reagents and conditions: (a) fuming H_2SO_4, 99%; (b) Pd/C, H_2, 10 psi, CH_3OH, 99%; (c) 2,2-dimethyl-butyryl chloride, Et_3N, CH_2Cl_2, 51%; (d) PCl_5, CH_2Cl_2, 82%; (e) NHR^1R^2, PS-iPr$_2$EtN, THF, 50–90% (library synthesis); (f) Et_3N, CH_2Cl_2, NHR^1R^2, 75–93% (for compounds **24**–**31**).

CB_2 receptors (Tables 1–3).[19] Compounds displaying good affinity (K_i <50 nM) and selectivity [K_i (CB_1)/K_i (CB_2) >50] for the CB_2 receptor, were evaluated for in vitro metabolic stability in rat and human liver microsomes (abbreviated as RLM and HLM, respectively).[20] As indicated previously, compound **8** was identified as a selective CB_2 agonist (K_i CB_2 = 23 nM; K_i CB_1/K_i CB_2 = 150).[20] However, **8** suffered from poor metabolic stability in vitro (2% and 1% remaining of parent compound after 30 min incubation in the presence of RLM and HLM, respectively). We postulated that the rapid metabolism of **8** may be due to hydroxylation of the benzene ring. In order to block this putative metabolic pathway, an additional methyl group was introduced at the 3-position of the benzene template of **8**, to give the xylene analog **9**. As indicated in Table

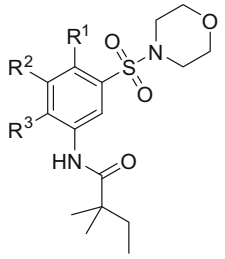
Table 1
Cannabinoid receptor (CB_1 and CB_2) binding data, in vitro agonist activity (CB_2 GTPγS) and in vitro metabolic stability of sulfamoyl benzamide derivatives



Compd	R^1	R^2	K_i CB_2[a] (nM)	EC_{50} CB_2[a] (nM)	K_i CB_1 [a] (nM)	K_i CB_1/ K_i CB_2	RLM[b]	HLM[b]
8	H		23	11	3400	150	2	1
9	CH_3		16	66	1100	69	31	17
10	CH_3		40	90	3100	78	37	52
11	CH_3		120	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
12	CH_3		2080	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
13	CH_3		6.6	11	780	120	22	11
14	CH_3		17	38	2500	150	28	17
15	CH_3		9.7	21	830	86	9	1
16	CH_3		l.l	82	140	130	0	0
17	CH_3		110	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]

[a] Values are the geometric means computed from at least three separate determinations; for assay description see Ref. 21.
[b] % Remaining after 30 min; RLM: rat liver microsomes, HLM: human liver microsomes; for assay description see Ref. 20.
[c] nd: not determined.

Table 2
Cannabinoid receptor (CB_1 and CB_2) binding data, in vitro agonist activity (CB_2 GTPγS) and in vitro metabolic stability of sulfamoyl benzamide derivatives

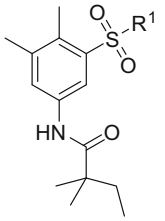


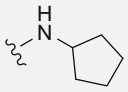
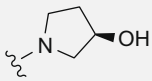
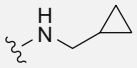
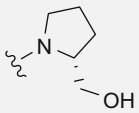
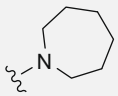
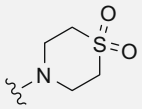
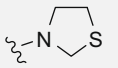
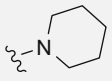
Compd	R^1	R^2	R^3	K_i CB_2[a] (nM)	EC_{50} CB_2[a] (nM)	K_i CB_1[a] (nM)	K_i CB_1/K_i CB_2	RLM[b]	HLM[b]
14	CH_3	CH_3	H	17	38	2500	150	28	17
18	CH_3	H	H	290	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
19	CH_3	H	CH_3	760	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
20	H	CH_3	CH_3	15%[d]	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
21	CH_3	CH_3	CH_3	410	nd[c]	nd[c]	nd[c]	nd[c]	nd[c]
22	–$(CH_2)_3$–		H	12	28	1700	140	9	10
23	–$(CH_2)_4$–		H	14	47	1400	100	2	1

[a] Values are the geometric means computed from at least three separate determinations; for assay description see Ref. 21.
[b] % Remaining after 30 min; RLM: rat liver microsomes, HLM: human liver microsomes; for assay description see Ref. 20.
[c] nd: not determined.
[d] % Inhibition at 10 μM.

1, compound **9** displayed good affinity for the CB_2 receptor and 69-fold selectivity for CB_2 over CB_1. In addition, **9** displayed a much improved metabolic stability profile when compared to its analog **8** (**9**: 31% and 17% remaining of parent in RLM and HLM, respectively). Based on this encouraging result, a series of carboxamide analogs of **9** (compounds **10**–**17**) were prepared in order to increase the CB_1/CB_2 selectivity while maintaining potent affinity for the CB_2 receptor and good in vitro metabolic stability. The in vitro binding and metabolic stability data for compounds **10**–**17** are summarized in Table 1. Substitution of the 2,2,3,3-tetramethylcyclopropanecarboxyl group of **9** with a pivaloyl moiety, as in **10**, led to a slight decrease in the binding affinity toward the CB_2 receptor (**10**: K_i CB_2 = 40 nM). However, this structural modification led to an improvement in the HLM metabolic stability (52% remaining of parent after 30 min incubation). Replacement of the *tert*-butyl group of **10** with a more bulky 2-methyl-2-phenyl propyl group (compound **11**) was accompanied by a threefold decrease in the CB_2 binding affinity, suggesting that the lipophilic pocket in which the *tert*-butyl group of **10** interacts is relatively small. As anticipated, substitution of the *tert*-butyl group of **10** with a more polar 2-hydroxy-2-methyl-propyl moiety led to a 52-fold decrease in the affinity toward the CB_2 receptor. The pivaloyl group of **10** was efficiently replaced by a 3,3,3-trifluoro-2-methyl-2-(trifluoromethyl)propanyl moiety (**13**). Indeed, **13** demonstrated increased CB_2 receptor binding affinity (K_i = 6.6 nM) and improved CB_1/CB_2 receptor selectivity (120-fold), when compared to **10**. However, this modification also led to a decrease in the in vitro metabolic stability. Extending the key *tert*-butyl lipophilic moiety of **10** to a dimethylbutanoyl group (**14**) was beneficial to improve the CB_2 binding affinity and the CB_1/CB_2 selectivity while maintaining moderate metabolic stability. Changing the dimethylbutanoyl group of **14** to a dimethylpentanoyl (**15**) or dimethylhexanoyl (**16**) moieties led to a further increase in the CB_2 affinity. Unfortunately, compounds **15** and **16** also displayed poor metabolic stability, potentially attributed to an increase in the overall lipophilicity and/or flexibility of the molecules. Compound **14**, was then selected for further lead optimization based on its favorable overall in vitro profile. As shown in Table 2, replacement of the methyl substituent at the R^2 position of the phenyl ring of **14** with a hydrogen atom (compound **18**) led to a 17-fold reduction in the CB_2 binding affinity. Similarly, changing the substitution pattern of the dimethyl groups of **14** relative to the benzene ring, as in **19** and **20**, resulted in a significant decrease in the affinity toward the CB_2 receptor. Furthermore, introduction of a third methyl group at the R^3 position of **14** was detrimental to the CB_2 affinity (**21**: K_i = 410 nM). We next investigated the effect of replacing the xylene core of **14** with 2,3-dihydro-*1H*-indene (**22**) and 1,2,3,4-tetrahydronaphthalene templates (**23**). As shown in Table 2, compounds **22** and **23,** exhibited potent affinity at the CB_2 receptor (K_i of 12 and 14 nM, respectively) as well as greater than 1000-fold selectivity for CB_2 versus CB_1. However, neither compound possessed the in vitro metabolic stability sufficient to warrant further investigation.

Table 3
Cannabinoid receptor (CB_1 and CB_2) binding data, in vitro agonist activity (CB_2 GTPγS) and in vitro metabolic stability of sulfamoyl benzamide derivatives



Compd	R^1	K_i CB_2[a] (nM)	CB_2 EC_{50}[a] (nM)	K_i CB_1[a] (nM)	K_i CB_1/K_i CB_2	RLM[c]	HLM[c]
24		94	98	3400	36	nd[d]	nd[d]
25		90	73	6.3%[b]	nd[d]	nd[d]	nd[d]
26		66	135	3800	57	nd[d]	nd[d]
27		43	98	8.3%[b]	nd[d]	nd[d]	nd[d]
28		9.0	22	1800	200	0	0
29		8.4	20	18%[b]	nd[d]	10	1
30		6.9	12	2100	300	0	0
31		2.7	13	650	240	0	0

[a] Values are the geometric means computed from at least three separate determinations; for assay description see Ref. 21.
[b] % Inhibition at 10 μM.
[c] % Remaining after 30 min; RLM: rat liver microsomes, HLM: human liver microsomes; for assay description see Ref. 20.
[d] nd: not determined.

Table 4
Pharmacokinetic parameters of **14** in male Sprague-Dawley rats after iv and po administration

Pharmacokinetic	iv	po
Dose (mg/kg)	1.0	100
CLs (L/h/kg)	4.2 ± 0.2	—
Vdss (L/kg)	2.5 ± 0.7	—
$t_{1/2}$[a] (h)	0.7 ± 0.1	nd[c]
$AUC_{0-\infty}$ (ng * h/mL)	237 ± 13	4805 ± 2051
T_{max}[b] (h)	—	6 (6–8)
C_{max} (ng/mL)	—	1221 ± 450
F (%)	—	22 ± 9

Values represent the mean ± standard deviation of three animals.
[a] Expressed as harmonic mean.
[b] Expressed as median and range.
[c] nd: not determined.

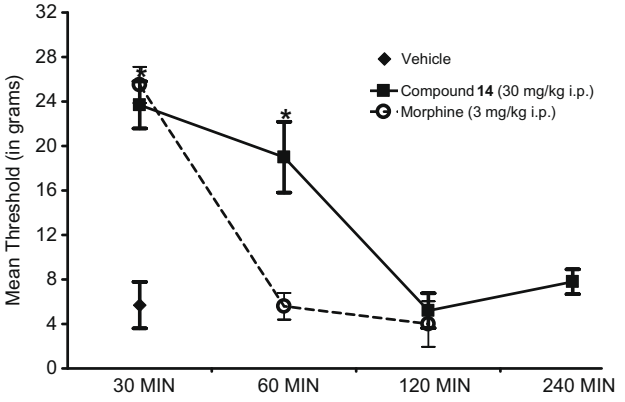


Figure 3. Time course of the in vivo activity of **14** in the rat hindpaw incision model. Mechanical paw withdrawal thresholds for the left hindpaw, compound **14**, a non-sedating dose of morphine and vehicle treatment groups. Data are plotted as the mean (±SEM) withdrawal threshold for the left hindpaw. All statistical analysis was performed with one-way ANOVA followed by post-hoc comparisons (protected *t*-test) among groups. * p <0.05 compared to vehicle-treated, surgery group.

Structure–activity relationships at the sulfonamide moiety of **14** were also studied. A 230 member library of sulfonamides, direct analogs of **14**, was prepared and screened at the CB_2 receptor. Compounds displaying a CB_2 K_i of less than 100 nM were resynthesized and further purified in order to confirm the binding data obtained for the library compounds. The results are summarized in Table 3. We identified from this study several derivatives (e.g., **28**–**31**) displaying +high affinity and selectivity for the CB_2 receptors. However, these compounds all exhibited poor metabolic stability.

Based on its favorable in vitro profile, compound **14** was selected for pharmacokinetic studies. The pharmacokinetic parameters of **14** after iv (1 mg/kg) and po (100 mg/kg) administration to male rats are summarized in Table 4. Compound **14** demonstrates high systemic clearance with a short half-life of 0.7 h (iv). The oral bioavailability of **14** was 22%. Additional in vivo efficacy studies demonstrated that compound **14** (30 mg/kg, ip) exhibited a robust antiallodynic effect in the hindpaw incisional rodent model of post-surgical pain (Fig. 3).

In summary, further SAR investigation in the previously reported sulfonamide series of CB_2 agonists, led to the identification of several compounds (**15**, **16**, **28**–**31**) demonstrating potent affinity for the CB_2 receptor (K_i <10 nM) and improved CB_1/CB_2 receptor selectivity (>200-fold), when compared to the original lead **8**. Compound **14** displayed the best overall in vitro profile and demonstrated robust efficacy in an animal model of post-operative pain. The lipophilic amide moiety of **14** was found to be of crucial importance for potent affinity at the CB_2 receptor. Among the various templates investigated, the xylene moiety of **14** was optimal to obtain good affinity and selectivity for the CB_2 receptor while maintaining moderate in vitro metabolic stability.

References and notes

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