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Sulfamoyl benzamides as novel CB_2 cannabinoid receptor ligands

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Abstract—Sulfamoyl benzamides were identified as a novel series of cannabinoid receptor ligands. Starting from a screening hit **8** that had modest affinity for the cannabinoid CB_2 receptor, a parallel synthesis approach and initial SAR are described, leading to compound **27** with 120-fold functional selectivity for the CB_2 receptor. This compound produced robust antiallodynic activity in rodent models of postoperative pain and neuropathic pain without traditional cannabinergic side effects.

Two cannabinoid receptors, CB_1 and CB_2, have been identified and subsequently cloned. They belong to the family of G-protein coupled receptors and share 44% amino acid sequence homology but differ in anatomical distribution. The CB_1 receptor is expressed mainly in the CNS and to a lesser extent in other tissues. The CB_2 receptor is primarily expressed in peripheral tissues associated with immune functions, including macrophages, B and T cells, as well as in peripheral nerve terminals and on mast cells.[1] Δ^9-Tetrahydrocannabinol (THC, **1**), the main active component of *Cannabis sativa*, and other classical cannabinoids display a wide range of physiological effects including analgesic, anti-inflammatory, anti-convulsive and immunosuppressive activities.[2] Cannabinoid receptor agonists also induce a number of unwanted CNS effects, which are believed to be mediated predominantly by the central distribution pattern of CB_1 receptors.[3]

A separation between therapeutic effects and undesirable CNS side effects could be accomplished either by preventing the cannabinoid from crossing the blood–brain barrier[4] or by increasing the selectivity for the CB_2 receptor over the CB_1 receptor.[5] Several structural classes have displayed selectivity for the CB_2 receptor (Fig. 1).[6] Compound **4** (GW405833) was shown to be antihyperalgesic in rodent models of neuropathic, incisional and chronic inflammatory pain, but had no significant effect in CB_2 knockout mice in the same assays.[7] Compound **5** (AM1241) was reported to reverse carrageenan-induced inflammatory thermal hyperalgesia in rats. This effect was attenuated by a CB_2 selective antagonist, but not a CB_1 selective antagonist.[8] Thus, there is considerable interest in developing new cannabimimetic compounds possessing preferentially high affinity for the CB_2 receptor, which could lead to novel therapeutics for the treatment of inflammation and chronic pain.[9]

During a high-throughput screening campaign[10a] we identified **8** as a compound with modest affinity for the CB_2 receptor (Fig. 2). Initially, SAR was explored via a parallel approach shown in Scheme 1 and Figure 3. Starting from commercially available 4-bromo-3-(chlorosulfonyl)benzoic acid **10a** and amines **11a–h**, we prepared eight 4-bromo-3-sulfamoyl-benzoic acids **12a–h**. A diverse set of 10 amines **13a–j** was attached to aldehyde-based polystyrene resin via reductive amination using sodium triacetoxyborohydride as the reducing agent.[11] The resulting resin-bound amines **14a–j** were then reacted with the sulfamoyl-benzoic acids **12a–h** previously obtained using the coupling reagent bromo-*tris*-pyrrolidinophosphonium hexafluorophosphate (PyBrop) and diisopropylethylamine. After cleavage from solid support with trifluoroacetic acid in

Keywords: Cannabinoid CB_2 receptor; Parallel synthesis; Sulfamoyl benzamides; Structure–activity relationship; Antiallodynic activity.
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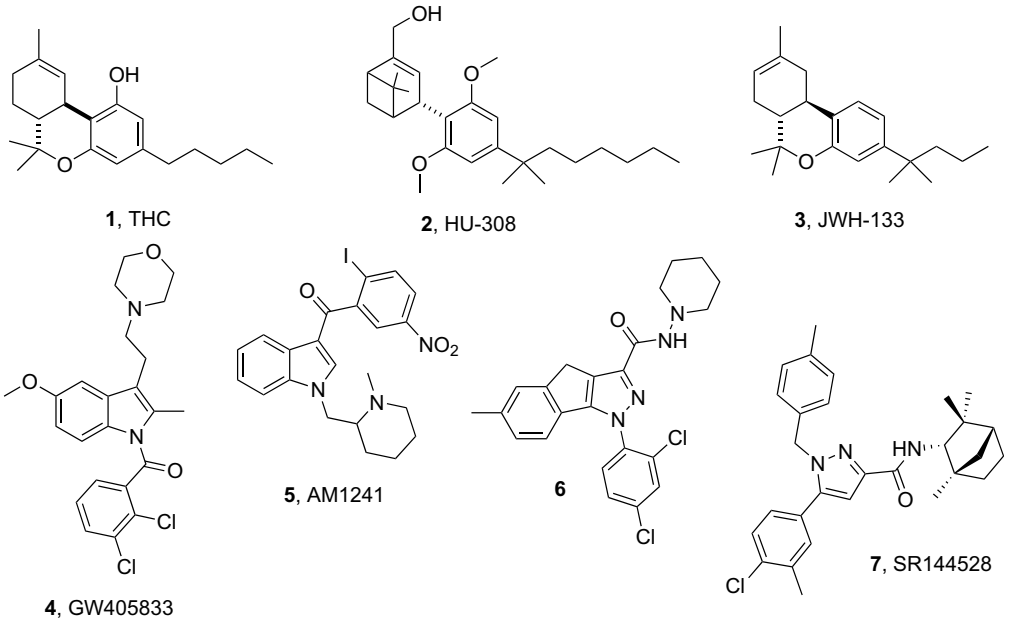


Figure 1. Cannabinoid receptor ligands.

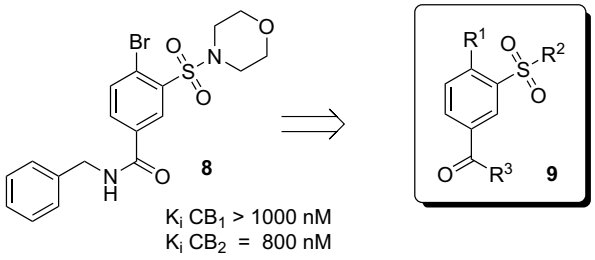
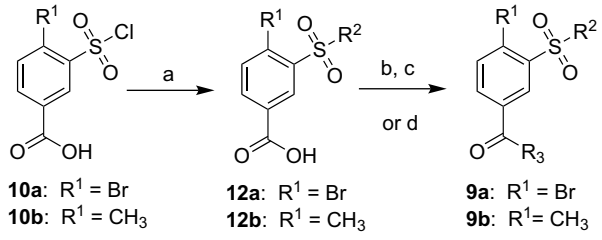


Figure 2. Screening hit.



Scheme 1. Reagents and conditions: (a) R^2-amine **11a**–**h** EtOAc; (b) resin-bound R^3-amine **14a**–**j**, *i*-Pr_2EtN, PyBrop, CH_2Cl_2; (c) TFA/CH_2Cl_2; (d) R^3-amine, TBTU, *i*-Pr_2EtN, ACN.

dichloromethane 80 final compounds, **9a**, were obtained. Almost half of these compounds retained or improved binding affinity for the CB_2 receptor compared to **8**, while the remaining compounds lost affinity for both CB receptors. Representative examples are shown in Table 1. Branched alkyl amines seemed to be preferred as both neopentyl and isobutyl amides yielded combinations with improved binding affinity (K_i CB_2 = 100–450 nM).

Since the selective CB_2 antagonist SR144528[6b] (**7**) also bears a highly branched amine substituent, we attempted to introduce this *S*-fenchyl residue into our system via the solid phase route just described. Due to steric hindrance the coupling of fenchyl amine to the polystyrene solid support failed. Therefore highly branched analogs **23**– **37** were synthesized in solution from respective sulfamoyl-benzoic acids **12a**–**d** utilizing *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetra-methyluronium tetrafluoroborate (TBTU) as the coupling reagent (Table 2).[12,13] All four sulfamoyl-benzoic acids **12a**–**d** yielded analogs with greatly improved binding affinity (**23**– **26**). Morpholine, pyrrolidine, and piperidine in R^2 showed similar profiles, with the morpholine analog **23** being slightly more selective. Methylbenzyl amine in R^2 led to the most selective analog **25** with a binding constant K_i CB_2 = 11 nM and $\geqslant$1000-fold lower binding to the CB_1 receptor (33% inhibition at 10 μM). Compounds **23**– **26** were then evaluated in the [^{35}S]GTPγS functional assay.[10b] Compounds **23**, **24**, and **26** were full agonists, but the most selective compound **25** behaved as an inverse agonist. To exclude possible reactivity with proteins in vivo the bromo substituent in **23** was replaced with a methyl group. Starting the synthesis from 3-(chlorosulfonyl)-4-methylbenzoic acid **10b** we obtained compound **27**, a 31-fold selective agonist with functional activities of EC_{50} CB_2 = 4.6 nM and EC_{50} CB_1 = 550 nM.

In an attempt to further improve selectivity and retain agonist activity, other commercially available branched amines were attached to **12b** (R^2 = morpholino, Table 2, **28**–**37**). Bicyclic amine substituents with branching in the 1 and/or 2 position seem to be preferred. Globular amines like 2-adamantyl, 1-(1-adamantyl)ethylamine, and bornyl amine yielded compounds with binding constants K_i CB_2 $\leqslant$ 10 nM. Analogs containing open chain and monocyclic amides, as well as analog **34** containing the *R*-isomer of fenchyl amine, lost

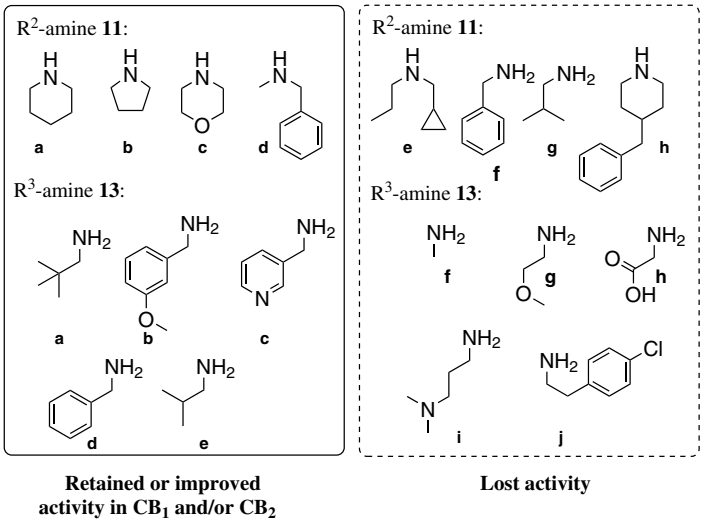
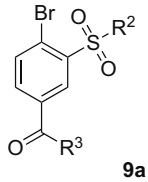
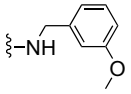
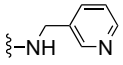
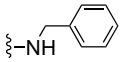
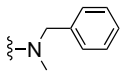
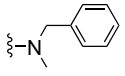


Figure 3. R^2 and R^3 amines used to prepare **9a**.

Table 1. Parallel synthesis approach—example results[a,b]



9a

Compound	R^2	R^3	K_i CB_1 (nM)	K_i CB_2 (nM)	Ratio CB_1/CB_2
14			1300	100	13
15			1400	200	7
16			1800	320	5
17			1100	360	3
18			>1000	410	>2
19			2000	400	5
20			1300	450	3
21			>1000	430	>2
22			>1000	340	>3

[a] Values are the geometric means computed from at least three separate determinations.
[b] For assay description see Ref. 10.

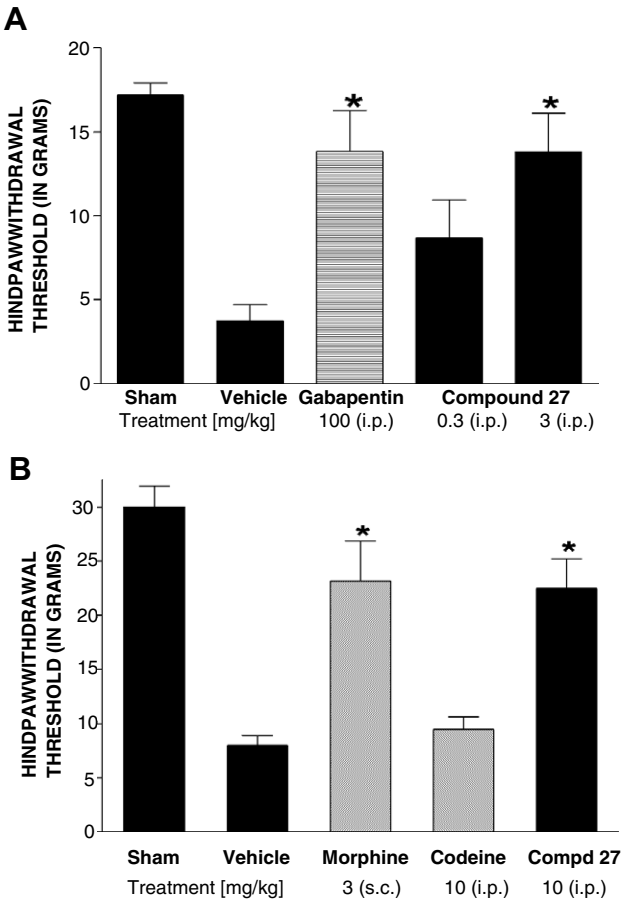


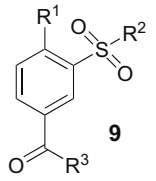
Figure 4. In vivo activity of compound **27** in two rat models: (A) L5 SNL model. Mechanical paw-withdrawal thresholds for the left hindpaw of the sham-operated control group, L5 ligation group, and gabapentin or compound **27** treatment groups. (B) Hindpaw incision model. Mechanical paw-withdrawal thresholds for the left hindpaw of the sham-operated control group, hindpaw incision group, and morphine, codeine and compound **27** treatment groups. Data are plotted as the mean (±SEM) paw-withdrawal threshold of the left hindpaw for each group. All statistical analyses were performed with one-way ANOVA followed by post-hoc comparisons (protected *t*-test) among groups. $^{*}p > 0.05$ compared to vehicle-treated, surgery (L5 SNL or hindpaw incision) group.

at least one order of magnitude in CB_2-binding affinity. No improvement in selectivity could be observed.

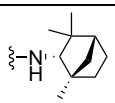
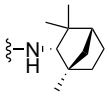
Catalepsy in mice is a behavior that is indicative of central CB_1 receptor activation and predictive of cannabinoid psychoactivity. For example, Pertwee demonstrated a correlation between catalepsy in the ring test in mice and ataxia in the dog static ataxia model.[14] Therefore catalepsy in mice is likely a predictor of central cannabinergic effects in humans.[15] Since compound **27** had a functional selectivity of 120-fold over the CB_1 receptor, and it did not produce catalepsy at doses of 6 and 10 mg/kg ip, it was evaluated for efficacy in two rodent models of nociception, the L5 spinal nerve ligation model (L5 SNL)[16] of neuropathic pain and the hindpaw incision model[17] of postoperative pain (Fig. 4). In the L5 SNL model, compound **27** reversed the nerve injury-induced tactile allodynia at a dose of 3 mg/kg ip. The magnitude of the antiallodynic effect was comparable to the effect produced by a dose of 60 mg/kg ip of gabapentin. In the incisional pain model, a dose of 10 mg/kg ip of compound **27** produced a significant antiallodynic effect that was comparable to the antiallodynic effect that was produced by a dose of 3 mg/kg sc of morphine. There were no overt behavioral side effects that were associated with doses up to 10 mg/kg ip of compound **27**. Administered orally at 30 and 100 mg/kg compound **27** was not active in the incisional pain model, due to low bioavailability.

In summary, the sulfamoyl benzamides represent a new class of ligands that bind to the cannabinoid receptors. Derived from a screening hit with modest affinity to the CB_2 receptor, large lipophilic, branched amide substituents led to improved receptor binding. Increased selectivity for the CB_2 receptor was achieved by the introduction of an *S*-fenchyl residue. Small changes in the sulfonamide part of the molecule produced a switch from full agonist to inverse agonist. Compound **27**, a compound with 120-fold functional selectivity for the CB_2 receptor and devoid of traditional cannabimimetic side effects, dosed ip, showed robust antinociceptive

Table 2. Highly branched sulfamoyl benzamides[a,b]

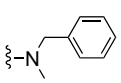
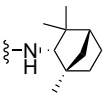
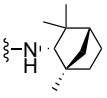
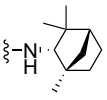
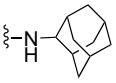
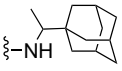
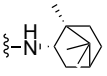
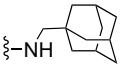
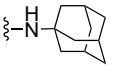
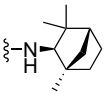
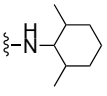


9

Compound	R^1	R^2	R^3	K_i CB_1 (nM)	K_i CB_2 (nM)	Ratio CB_1/CB_2	EC_{50} CB_1	EC_{50} CB_2	Ratio
23	Br			89	3.5	25	560	8.8	64
24	Br			110	9.4	12	220	4.3	51

(*continued on next page*)

Table 2 (*continued*)

Compound	R^1	R^2	R^3	K_i CB_1 (nM)	K_i CB_2 (nM)	Ratio CB_1/CB_2	EC_{50} CB_1	EC_{50} CB_2	Ratio
25	Br			>1000	11	>100	na	na[c]	
26	Br			93	13	7	280	6.6	42
27	CH_3			130	3.9	31	550	4.6	120
28	CH_3			33	5.6	15	270	12	23
29	CH_3			77	6.3	5	290	9.3	31
30	CH_3			50	8.1	7	210	14	15
31	CH_3			91	10	9	210	25	8
32	CH_3			260	19	14	570	48	12
33	CH_3			310	34	9	920	21	44
34	CH_3			540	48	11	880	25	35
35	CH_3			470	92	5	540	44	12
36	CH_3			1300	180	9	2400	125	19
37	CH_3			>1000	390	>3	>1000	1800	nd

[a] Values are the geometric means computed from at least three separate determinations.
[b] For assay description see Ref. 10.
[c] Antagonist with IC_{50} CB_2 = 14 nM.

activity in rodent models of neuropathic and postoperative pain that was comparable to the effect produced by gabapentin and morphine, respectively. Due to their chemical accessibility and pharmacological activity, sulfamoyl benzamides can be considered an attractive lead series for further optimization, with the focus directed

towards improving physicochemical and DMPK properties.

References and notes

1. (a) Howlett, A. C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, P.; Devane, W. A.; Felder, C. C.; Herkenham, M.; Mackie, K.; Martin, B. R.; Mechoulam, R.; Pertwee, R. G. *Pharmacol. Rev.* **2002**, *54*, 161; (b) Pertwee, R. G. *Prog. Neurobiol.* **2001**, *63*, 569.
2. (a) Farquhar-Smith, W. P. *Pain Rev.* **2002**, *9*, 41; (b) Klein, T. W. *Nat. Rev. Immunol.* **2005**, *5*, 400; (c) Walter, L.; Stella, N. *Br. J. Pharmacol.* **2004**, *141*, 775; (d) Smith, P. F. *Curr. Opin. Investig. Drugs* **2005**, *6*, 680.
3. Rice, A. S. *Curr. Opin. Investig. Drugs* **2001**, *2*, 399.
4. Worm, K.; Zhou, Q. J.; Stabley, G. J.; DeHaven, R. N.; Conway-James, N.; LaBuda, C. J.; Koblish, M.; Little, P. J.; Dolle, R. E. *Abstracts of Papers*, 234th National Meeting of the American Chemical Society, Boston, MA, August 19–23, 2007; MEDI 030.
5. Malan, T. P.; Ibrahim, M. M.; Lai, J.; Vanderah, T. W.; Makriyannis, A.; Porreca, F. *Curr. Opin. Pharmacol.* **2003**, *3*, 62.
6. (a) Huffman, J. W. *Mini Rev. Med. Chem.* **2005**, *5*, 641; (b) Rinaldi Carmona, M.; Barth, F.; Millan, J.; Derocq, J.; Casellas, P.; Congy, C.; Oustric, D.; Sarran, M.; Bouaboula, M.; Calandra, B.; Portier, M.; Shire, D.; Breliere, J.; Le Fur, G. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 644.
7. Valenzano, K. J.; Tafesse, L.; Lee, G.; Harrison, J. E.; Boulet, J. M.; Gottschall, S. L.; Mark, L.; Pearson, M. S.; Miller, W.; Shan, S.; Rabadi, L.; Rotshteyn, Y.; Chaffer, S. M.; Turchin, P. I.; Elsemore, D. A.; Toth, M.; Koetzner, L.; Whiteside, G. T. *Neuropharmacology* **2005**, *48*, 658.
8. (a) Quartilho, A.; Mata, H. P.; Ibrahim, M. M.; Vanderah, T. W.; Porreca, F.; Makriyannis, A.; Malan, T. P. *Anesthesiology* **2003**, *99*, 955; (b) Ibrahim, M. M.; Deng, H.; Zvonok, A.; Cockayne, D. A.; Kwan, J.; Mata, H. P.; Vanderah, T. W.; Lai, J.; Porreca, F.; Makriyannis, A.; Malan, T. P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10529.
9. Whiteside, G. T.; Lee, G. P.; Valenzano, K. J. *Curr. Med. Chem.* **2007**, *14*, 917.
10. (a) Binding assays were performed by modification of the method of Pinto, J. C.; Potie, F.; Rice, K. C.; Boring, D.; Johnson, M. R.; Evans, D. M.; Wilken, G. H.; Cantrell, C. H.; Howlett A. *Mol. Pharmacol.* **1994**, *46*, 516: Receptor binding assays were performed by incubating 0.2–0.6 nM [^{3}H]CP55940 with membranes prepared from cells expressing cloned human CB_1 or CB_2 receptors in buffer consisting of 50 mM Tris–HCl, pH 7.0, 5.0 mM $MgCl_2$, 1.0 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 1.0 mg/mL fatty acid free bovine serum albumin. After incubation for 60 min at room temperature for CB_2 binding or 120 min at 30 °C for CB_1 binding, the assay mixtures were filtered through GF/C filters that had been pre-soaked overnight in 0.5% (w/v) poly(ethyleneimine) and 0.1% BSA in water. The filters were rinsed six times with 1 mL each of cold assay buffer, 30 μL of MicroScint 20 (Perkin-Elmer) was added to each filter and the radioactivity on the filters was determined by scintillation spectroscopy in a TopCount (Perkin-Elmer). Nonspecific binding was determined in the presence of 10 μM WIN55212-2.; (b) The [^{35}S]GTPγS binding method is a major modification of the method by Selley, D. E.; Stark, S.; Sim, L. J.; Childers, S. R. *Life Sci.* **1996**, *59*, 659: CB_2-mediated stimulation of [^{35}S]GTPγS binding was measured in a mixture containing 100–150 pM [^{35}S]GTPγS, 150 mM NaCl, 45 mM $MgCl_2$, 3 μM GDP, 0.4 mM dithiothreitol, 1.0 mM EGTA, 1.0 mg/mL fatty acid free bovine serum albumin, 25 μg of membrane protein, and agonist in a total volume of 250 μL of 50 mM Tris–HCl buffer, pH 7.0 in 96-well Basic FlashPlates (Perkin-Elmer). After incubation at room temperature for 6 h, the plates were centrifuged at 800 *g* at 4 °C for 5 min and the radioactivity bound to the membranes was determined by scintillation spectrometry using a TopCount (Perkin-Elmer). The extent of stimulation over basal [^{35}S]GTPγS binding was calculated as a percentage of the stimulation by 10 μM WIN55212-2. Basal [^{35}S]GTPγS binding was determined in the absence of agonist. Generally, the stimulation by 10 μM WIN55212-2 was between 50% and 100% over basal binding. Full agonists stimulate binding to the same maximal extent as WIN55212-2.
11. *General procedure for preparing resin-bound amines **14a–j**:* The polystyrene resin with aldehyde linker (1.6 mmol/g, 500 mg, 0.8 mmol) was placed in a vessel, 5 mL of solvent dichloroethane were added, followed by 4 mmol (5 equiv) of respective amine **13a–j**. The mixture was shaken for 2 h at rt, then the reducing agent $NaB(OAc)_3H$ (4 mmol, 5 equiv, 848 mg) was added and shaken overnight. The resin was filtered, washed six times, alternating between dichloromethane and MeOH and used for the next step.
12. Compounds were fully characterized by ^{1}H NMR and LC/MS.
13. *Compound **27**:* ^{1}H NMR ($CDCl_3$), d 0.86 (s, 3H), 1.11 (s, 3H), 1.19 (s, 3H), 1.29 (m, 3H), 1.52 (m, 1H), 1.72 (m, 2H), 1.82 (t, J = 2 Hz, 1H), 2.69 (s, 3H), 3.20 (t, J = 5 Hz, 4H), 3.73 (t, J = 5 Hz, 4H), 3.83 (dd, J = 9 Hz and 2 Hz, 1H), 6.07 (d, J = 9 Hz, 1H), 7.43 (d, J = 8 Hz, 1H), 7.86 (dd, J = 8 Hz and 2 Hz, 1H), 8.26 (d, J = 2 Hz, 1H). MS *m/z* 421 ($M+H^+$). Anal. Calcd ($C_{22}H_{32}N_2O_4S$): C, 62.83; H, 7.67; N, 6.66. Found: C, 62.84; H, 7.73; N 6.66.
14. Pertwee, R. G. *Br. J. Pharmacol.* **1972**, *46*, 753.
15. (a) Compton, D. R. *Marijuana: Int. Res. Rep.* **1987**, *7*, 213; (b) Gill, E. W.; Jones, G. *Biochem. Pharmacol.* **1972**, *21*, 2237; (c) Gill, E. W.; Paton, W. D. M.; Pertwee, R. G. *Nature* **1970**, *228*, 134.
16. LaBuda, C. J.; Little, P. J. *J. Neurosci. Methods* **2005**, *144*, 175.
17. Brennan, T. J.; Vandermeulen, E. P.; Gebhart, G. F. *Pain* **1996**, *64*, 493.