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Synthesis and evaluation of 2-amido-3-carboxamide thiophene CB_2 receptor agonists for pain management

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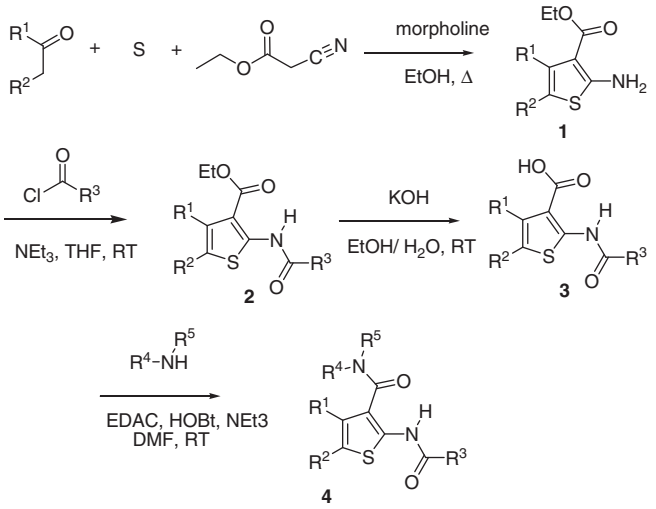
ABSTRACT

SAR studies on a series of thiophene amide derivatives provided CB_2 receptor agonists. The activity of the compounds was characterized by radioligand binding determination, multiple functional assays, ADME, and pharmacokinetic studies. A representative compound with selectivity for CB_2 over CB_1 effectively produced analgesia in behavioral models of neuropathic, inflammatory, and postsurgical pain. Control experiments using a CB_2 antagonist demonstrated the efficacy in the pain models resulted from CB_2 agonism.

The cannabinoid G-protein coupled receptor (GPCR) family consists of two well-characterized subtypes.[1] Cannabinoid 1 (CB_1) receptors exist primarily in the central nervous system (CNS) and also in peripheral tissues.[2] Activation of CB_1 receptors produces a variety of effects, including sedation, euphoria, and appetite stimulation.[3] Cannabinoid 2 (CB_2) receptors are expressed peripherally in tissues associated with the immune system.[4] Cannabinoid receptors couple primarily to $G_{i/o}$ proteins and activation inhibits cyclic adenosine monophosphate (cAMP) production,[5] but the mechanism and site of action in pain states remain under investigation.[6] Activation of CB_1 or CB_2 receptors can produce analgesia,[7] but agonists that lack selectivity for CB_2 over CB_1 cause the undesirable effects associated with Δ^9-tetrahydrocannabinol (Δ^9-THC).[8] Abbott initiated a program to pursue CB_2-selective agonists for pain management,[9] and this report describes advances in one series of CB_2 agonists.

Selective CB_2 agonists have been reported, including Δ^9-THC mimetics, indole derivatives, and compounds from several other structural classes.[10] A high throughput-screen (HTS) of the Abbott compound collection using a single point functional assay identified several thiophene bisamide compounds (**4**) as CB_2 agonists. Structure–activity relationship (SAR) studies around the screening hits revealed trends between CB_2 agonism and the peripheral functionality of the thiophene bisamide core.[11] Lipophilic hydrocarbon moieties increased ligand affinity and function at CB_2 receptors, but the resulting compounds lacked physicochemical properties appropriate for drug-like molecules. In addition, many of the initial nonpolar compounds lacked selectivity for CB_2 over CB_1 which limited their utility in evaluating CB_2 as a target for pain therapy.

The present SAR studies around the thiophene bisamide core incorporated polar functionality in the periphery of the structure to increase solubility and address instability toward oxidative metabolism. The general synthetic scheme for the synthesis of



Scheme 1. Synthetic route for preparation of **4**.

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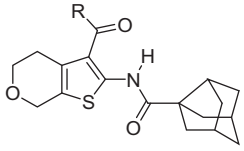
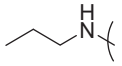
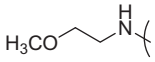
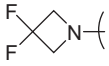
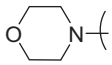
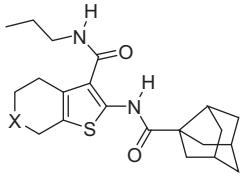
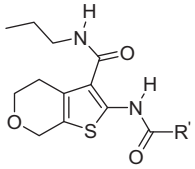
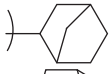
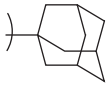
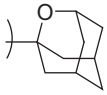
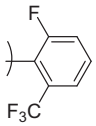
the thiophene bisamides is shown in Scheme 1. The Gewald reaction[12] (Scheme 1, Step 1) afforded the thiophene core with substitution appropriate for further functionalization. Standard acylation procedures using acid chlorides generated the C(2) amido group. Saponification, followed by amide formation using carbodiimide coupling reagents, installed the C(3) carboxamide.

Compounds were evaluated using a panel of in vitro assays. Competitive radioligand binding assays measured the displacement of [^{3}H]CP-55,940 from membrane preparations obtained from HEK 293 cells stably transfected with recombinant human (h) or rat (r) CB_2 receptors and CHO cells stably transfected with human or rat CB_1 receptors. Two different assays evaluated functional activity. Fluorescence imaging plate reader (FLIPR) technology measured the evoked response in HEK 293 cells stably expressing the chimeric $G_{aq/o}$ protein and the appropriate CB receptor. In the FLIPR assays, net peak responses were compared to peak responses from CP-55,940. Adenylyl cyclase (cAMP) assays complemented the FLIPR assays. The cAMP assays used HEK 293

Table 1
Radioligand binding data for selected thiophenes[a]

4		hCB_2 binding pK_i[b]	rCB_2 binding pK_i[b]	hCB_1 binding pK_i[b]	rCB_1 binding pK_i[b]
					
a		6.67	6.53	<5	
b		7.68	7.47[c]	5.81[c]	6.52[c]
c		6.92	6.58[c]	<5	
d		8.25	7.33	5.47	7.18
e		7.04	<5	<5	<5
					
f	X = –CH_2–	8.71	8.45	5.72	7.33
g	–NH–	<6			
h	–N(CH_3)–	7.04[c]	8.23[c]	<5	
					
i		7.04[c]	8.36[c]	<5	6.72[c]
j		6.98	6.57	<5	
k		7.47	7.70[c]	<5	6.28[c]
l		6.59[c]	6.31[c]	<5	
m		6.98	6.48	<5	

[a] Number of determinations ≥2 (duplicate of duplicate).
[b] Error (±SEM ≤0.10).
[c] Error (0.10 > ±SEM >0.27).

cells stably expressing the appropriate CB receptor in the presence of a fixed concentration of forskolin. ELISA methods measured the cAMP levels. Receptor activation by test ligands is reported as a percent response of the control CP-55,940.

Table 1 contains the radioligand binding data for a series of compounds with structural variation in the C(2) and C(3) amide groups as well as in the ring fused to the thiophene core. Small changes in the alkyl group of the C(3) amide impacted CB_2 ligand affinity. The propyl amide provided greater potency (**4a** vs **4b**). The CB_2 binding site tolerated both secondary and cyclic tertiary amides (Examples **4d**, **4e**). Heteroatoms were tolerated in the C(3) moiety, but ligand affinity decreased as polarity increased (**4c**, **4e**). Discrepancies occurred between affinity at the human and rat CB_2 receptors (**4d**, **4e**), and selectivity for CB_2 over CB_1 varied upon modification of the C(3) amide (**4b**, **4d**). Compound **4b** demonstrated acceptable ligand affinity at both human and rat CB_2 with selectivity over CB_1 ranging from ~10-50x.

Additional trends in binding and selectivity emerged for compounds with varied ring systems fused to C(4)/C(5) of the thiophene. The tetrahydrobenzothiophene core (**4f**) provided the greatest CB_2 ligand affinity, although a substantial discrepancy in selectivity existed between human and rat. The tetrahydropyridine-fused thiophene derivative (**4g)** did not bind to the CB_2 receptor. The methylated derivative (**4h)** regained ligand affinity at CB_2 with poor CB_1 binding. The fused thienodihydropyran core provided a balance of ligand affinity, selectivity, and polarity.

Table 1 also contains radioligand binding data for a series of compounds bearing modified acyl substituents in the C(2) amido group. Examples **4i–k** illustrate that bulky hydrocarbon moieties can afforded high levels of ligand affinity. Selectivity for CB_2 over CB_1 varied significantly, and the ratio of CB_1/CB_2 binding was higher for the human receptors. The decrease in the ligand affinity of oxaadamantane derivative **4l** compared to its hydrocarbon counterpart **4k** illustrates the sensitivity of the binding site to the presence of heteroatoms. Fluorinated benzamide **4m** bound to the CB_2 receptors with affinities similar to analogs containing rigid polycyclic groups, suggesting that the bulky hydrocarbon moieties could be replaced.

Evaluation of the function of several potent cannabinoid ligands from Table 1 allowed identification of candidates for advancement to in vivo studies. The FLIPR assay using recombinant human CB_2 receptor established whether a compound functioned as an agonist. The full complement of FLIPR assays was not utilized due to technical difficulties, but rat CB_1 FLIPR data provided a quick and reliable assessment of selectivity. The lower throughput cyclase assays afforded more rigorous measurement of function and selectivity. Functional data for selected thiophene bisamides are listed in Table 2. Compound **4b** functioned as a full agonist in the FLIPR and cyclase assays at both the CB_2 and CB_1 receptors across species. The functional selectivity parallels the binding selectivity. The amide derived from difluoroazetine (**4d**) provided potency and full agonist function at the human receptor, but the cyclase data indicate reduced selectivity for the CB_2 receptor. Compound **4f** produced partial efficacy at hCB_2 in both the FLIPR and cyclase assays despite strong ligand affinity. The methylated tetrahydropyridine derivative **4h** did not function as an agonist in the FLIPR assay. The steric bulk of the tetramethylcyclopropyl (**4i**) and adamantyl (**4k**) derivatives provided agonist potency and efficacy in human CB_2 FLIPR and cyclase assays, but neither compound maintained agonist function in the rat CB_2 cyclase assay.

The in vitro profile of compound **4b** describes a potent CB_2 agonist with selectivity over CB_1 ranging from 10–1000. The data are consistent between binding and functional assays, and minor discrepancies exist between species. The ADME data for **4b** were obtained. Plasma protein binding for this compound was high (97% rat, 98% human), but many of the more lipophilic derivatives such as **4g** and **4k** bound to plasma proteins more strongly (>99%). Metabolic stability of **4b**, as measured by incubation of the compound with rat microsomes, was below optimal levels with 9% and 5% remaining after 30 min for human and rat, respectively. However, when **4b** advanced to pharmacokinetic studies in rats, the compound exhibited excellent bioavailability from intraperitoneal administration (96% *F*) with a half-life of 6.7 h (Fig. 1). The compound afforded modest oral bioavailability (11% *F*). The half-life from oral administration was shorter (1.7 h) and comparable to that from intravenous administration. The potency, selectivity, and high level of bioavailability upon intraperitoneal administration allowed compound **4b** to advance to behavioral pain models.

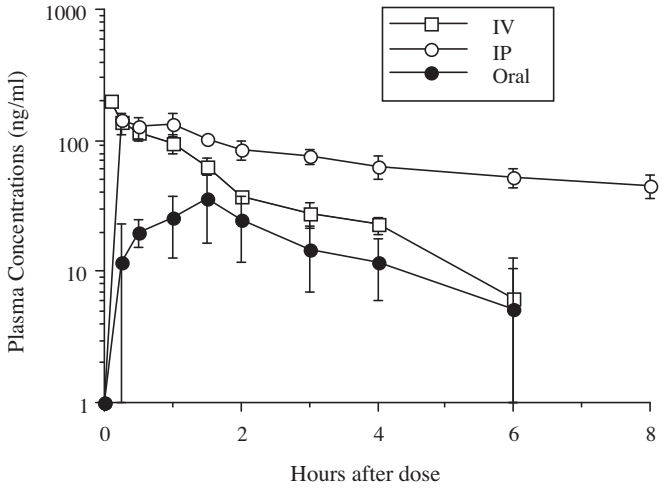
The compound was tested in two different models of neuropathic pain, and significant efficacy was observed in each model. In the spinal nerve ligation (Chung) model,[13] intraperitoneal administration of **4b** two weeks post-surgery and 30 min prior to testing produced a dose-related reduction in mechanical allodynia in rats (Fig. 2). The reduction in paw withdrawal threshold reached statistical significance at a dose of 10 μmol/kg ip with a maximum response of 60% at 30 μmol/kg. Similar efficacy was observed in the chronic constriction injury (Bennett) model[14] of neuropathic pain. With ip administration 30 min prior to testing, compound **4b** reduced mechanical allodynia with a maximum response of 74% of 30 μmol/kg. Compound **4b** was also evaluated in the skin incision model[15] of postoperative pain. In these experiments the compound was administered 2 h after the surgical insult and 30 min prior to testing. The CB_2 agonist produced a dose-related reduction in mechanical allodynia manifested by a 74% reduction in paw withdrawal threshold at 30 μmol/kg ip similar to that observed in the models of neuropathic pain.

Finally, compound **4b** was evaluated in the Complete Freund's Adjuvant (CFA) model[16] with oral administration. Despite the substantial difference between intraperitoneal and oral bioavailability, **4b** produced a dose-related related reduction in thermal hyperalgesia when administered 48 h after injection and 90 min before testing. The determination of the paw withdrawal threshold in this

Table 2
Functional data for selected thiophenes[a]

4	hCB_2 FLIPR pIC_{50}[b] (% max)[d]	rCB_1 FLIPR pIC_{50}[b] (% max)[d]	hCB2 cAMP *p* IC_{50}[b] (% max)[d]	hCB_1 cAMP pIC_{50}[b] (% max)[d]	rCB_2 cAMP pIC_{50}[b] (% max)[d]	rCB_1 cAMP pIC_{50}[b] (% max)[d]
b	7.15 (88%)	5.81 (79%)	8.71[c] (97%)	5.46[c](108%)	8.03[c] (72%)	5.85 (67%)
d	8.25 (124%)		9.70(105%)	6.26 (110%)	8.02[c] (53%)	6.64[c] (131%)
f	7.08 (42%)		8.01[c] (57%)			
h	<5					
i	7.28 (78%)		9.00 (88%)		<5	
k	7.37[c](68%)		7.95 [c](86%)		<5	

[a] Number of determinations ≥2 (duplicate of duplicate).
[b] Error (±SEM ≤0.10).
[c] Error (0.10 > ±SEM >0.23).
[d] Error ±15% (SD).



route	V_β (L/kg)	Cl_p (L/hr·kg)	C_{max} (ng/mL)	T_{max} (hr)	AUC (μg·hr/L)	$t_{1/2}$ (hr)	F(%)
IV	8.7	3.8			320	1.5	
IP			150	0.6	1020	6.7	96
PO			39	1.2	123	1.6	11

Animals administered doses of 3 μmol/kg IV, 10 μmol/kg IP or PO in 10% DMSO/90% PEG.

Figure 1. Pharmacokinetic profile of **4b** in rats.

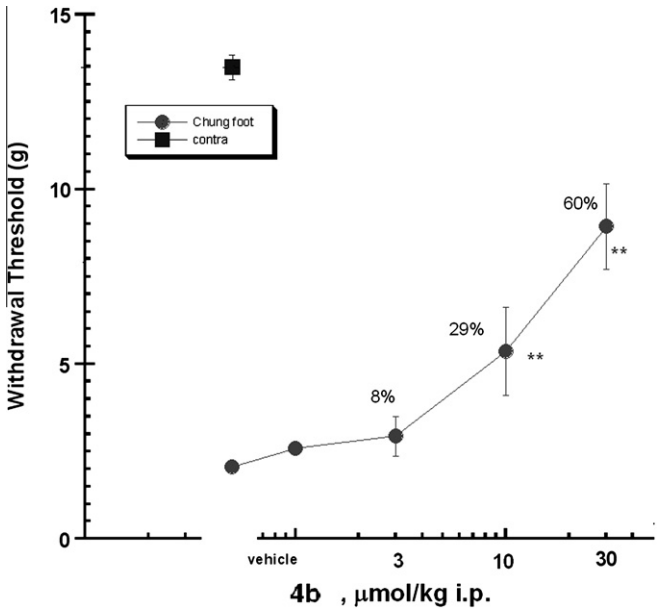


Figure 2. Dose–response curve for the spinal nerve ligation model of neuropathic pain.

series of experiments was conducted at 90 min post compound administration to coincide with the C_{max} identified from the PK experiment (Fig. 1). Efficacy in the CFA model with oral administration reached a statistically significant level of 58% at a dose of 100 μmol/kg. The dose response curve for oral administration of **4b** in the CFA model represents a significant decrease in potency compared to the curve obtained using ip administration in the same model. With ip administration of **4b** in the CFA model, the reduction in thermal hyperalgesia reached statistical significance at a dose of 3 μmol/kg and reached 74% at a dose of 10 μmol/kg.

The efficacy of **4b** in the CFA model results from agonism of the CB_2 receptor as demonstrated by a set of control experiments in which **4b** and the CB_2 antagonist SR-144528[17] were administered individually and combined (Fig. 3). Antagonist SR-144528 independently produced no significant reversal of the thermal hyperalgesia with ip administration of a dose of 10 μmol/kg. Compound **4b** reduced the thermal hyperalgesia by 91% which is consistent with the results in the full dose–response study. When **4b** was coadministered with SR-144528, the analgesic effects of **4b** were effectively

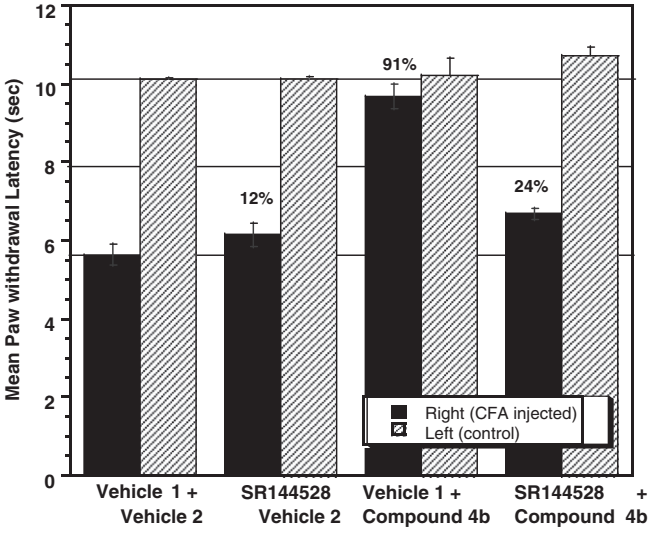


Figure 3. Blocking experiment demonstrating the effects of **4b** in the CFA model are blocked by CB_2 antagonist SR144528.

blocked and only 24% reduction of the thermal hyperlagesia occurred.

The results summarized in Figure 3 indicate that the analgesic activity observed for **4b** result from CB_2 agonism. The selectivity profile (Tables 1 and 2) indicates **4b** functions as a full agonist of the CB_1 receptor, although less potent at CB_1 than at CB_2. Agonists of the CB_1 receptor produce behaviors such as sedation and decreased coordination that could complicate the interpretation of the results from the behavioral pain models. Compound **4b** was evaluated using the locomotor assay to determine if the analgesic effects could be separated from CB_1-mediated side effects. In the locomotor assay, the compound was administered intraperitoneally 30 min prior to testing. A dose-related decrease in horizontal locomotor activity that reached statistical significance (83%) at a dose of 100 μmol/kg was observed. The significant decrease in activity occurred at a dose ~30 fold higher than the approximate EC(50) determined for **4b** in the CFA model using ip dosing.

In conclusion, several examples of a new class of CB_2 agonists have been synthesized and evaluated. Based on the radioligand binding and functional profile, one example (**4b**) from this series was studied extensively. The compound binds well to the CB_2 receptor and functions as a full agonist. The selectivity of **4b** for CB_2 over CB_1 was modest and depended on the nature of the assay. The pharmacokinetic profile indicated excellent bioavailablity with ip administration but limited bioavailability upon oral administration. The compound effectively reduced mechanical allodynia and thermal hyperalgesia in several different behavioral pain models. In spite of its pharmacokinetic profile, **4b** effectively produced analgesic effects with oral administration. While compound **4b** decreased motor coordination, the analgesic effects were blocked effectively by a CB_2 antagonist. Potential ancillary pharmacology of **4b** was assessed by evaluating the compound against the CEREP panel, but no significant effect of >50% at 10 μM was determined for any of the neuroscience targets.

The CB_2 agonists in this series of compounds have not been optimized. Modification to enhance selectivity of CB_2 over CB_1, increase metabolic stability, and improve pharmacokinetic profile is required. Efforts to address the limitations of these compounds will be disclosed in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.121.

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