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Decahydroquinoline amides as highly selective CB2 agonists: Role of selectivity on in vivo efficacy in a rodent model of analgesia

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ABSTRACT

A novel series of decahydroquinoline CB2 agonists is described. Optimization of the amide substituent led to improvements in CB2/CB1 selectivity as well as physical properties. Two key compounds were examined in the rat CFA model of acute inflammatory pain. A moderately selective CB2 agonist was active in this model. A CB2 agonist lacking functional CB1 activity was inactive in this model despite high in vivo exposure both peripherally and centrally.

The cannabinoid receptors, CB1 and CB2, are well characterized members of the G-protein coupled receptor (GPCR) family of receptors and are a part of the endocannabinoid system. Non-selective ligands for these receptors exhibit a number of biological properties, one of which is analgesia. In addition to this desirable effect these molecules also exhibit undesired neurological effects linked to central CB1 agonist activity[1,2] which, in turn, could limit their usefulness in the treatment of pain. Many labs in both academia and industry have postulated that a selective CB2 agonist might be therapeutically useful in the treatment of pain without the undesired neurological effects of the non-selective cannabinoids.

The literature contains a number of reports describing the design, synthesis, and in vivo evaluation of selective CB2 agonists in the pursuit of new treatments for pain.[3] The vast majority of these studies, however, involve molecules which retain some measurable in vitro CB1 agonist activity. In the previous Letter[4] Trotter et al. described a novel class of imidazopyridine CB2 agonists. The authors synthesized and evaluated a collection of potent imidazopyridine CB2 agonists with a range of selectivity profiles and physical properties. Compounds which were selective for CB2 but retained some CB1 agonist activity in vitro elicited a positive response in the rat CFA hyperalgesia model similar to other reports in the literature. However, compounds which lacked measurable in vitro CB1 agonist activity failed to affect pain responses in the same rat model despite high peripheral and central exposures. This result was rationalized by previous reports of the existence of a large CB1 receptor reserve in the CNS and the ability of compounds with weak CB1 binding to elicit substantial agonist responses.[5] The authors concluded that CB2 agonist activity alone was not sufficient to alleviate pain in a rat model of acute inflammatory pain.

In order to interrogate this hypothesis further our group sought out compounds with unique structures and similar in vitro profiles to those of the imidazopyridines. Compounds were required to have a range of selectivity profiles for CB2/CB1 and physical properties appropriate for in vivo evaluation. High-throughput screening of the Merck sample collection resulted in the identification of lead **1** having undetermined stereochemistry (Fig. 1).

A two-pronged approach was taken to investigate the structural requirements around the decahydroquinoline core. In order to determine the optimal stereochemical arrangement about carbons 1, 2, and 3, two large, diverse libraries of amides derived from racemic amines **2** and **3** were synthesized. Analysis of in vitro CB2 and

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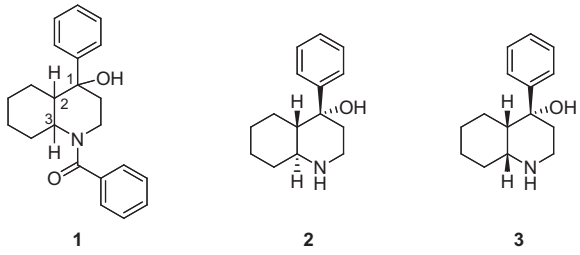


Figure 1.

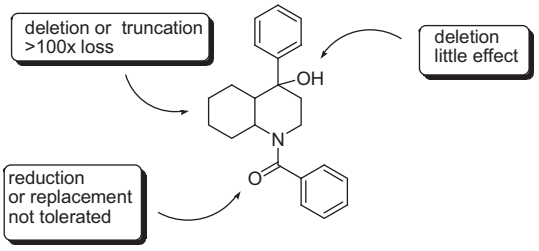


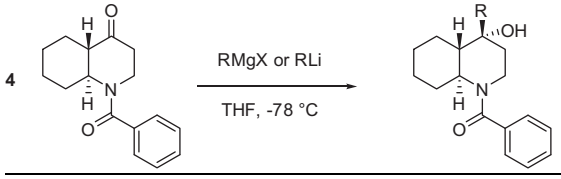
Figure 2.

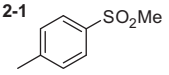
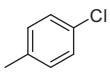
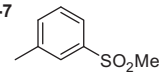
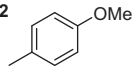
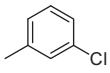
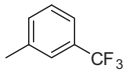
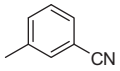
CB1 agonist activity[6] revealed a clear correlation between decahydroquinoline ring stereochemistry and CB2 agonist potency. The *cis* stereochemistry of amine **3**, in which the two ring protons and the aryl group are all on the same face of the molecule, was crucial for CB2 agonist activity over a wide range of structures (Table 1).

Simultaneous work on the decahydroquinoline core itself revealed that deletion or truncation of the unsubstituted cyclohexane ring resulted in a 100-fold or greater loss of CB2 agonist activity. Deletion of the tertiary alcohol in the trans-ring system (amine **2**) had little effect on CB2 agonist activity (83 nM vs 45 nM); this hydroxyl group was therefore retained in the *cis*-ring system for ease of synthesis and improved physical properties. Altering the connectivity about the amide nitrogen, either by reducing the carbonyl to the corresponding tertiary amine or replacing the carbonyl with either a sulfonamide or urea linkage, was generally not tolerated (Fig. 2).

In analogy to the previous Letter[4] we sought to increase polarity in our series in order to lower the log *P* and protein binding of our lead structures. Initially this was attempted by varying the aryl ring at carbon 1. Ketone **4** was synthesized according to the methods of Prost.[7] Grignards or organolithiums were added to **4** in THF at low temperature to give the desired alcohols (Table 2) in most cases. When the addition did not occur in sufficient yield the use

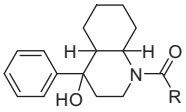
Table 2
Aryl substituents: hCB2 cAMP IC_{50} nM (% E_{max})[a]

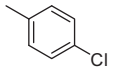
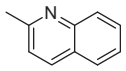
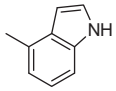
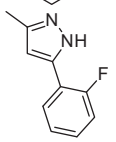


2-1 	**2-4** 	**2-7** 
106 (56)	155 (88)	338 (52)
2-2 	**2-5** 	**2-8** 
358 (66)	493 (69)	810 (-16)
2-3 	**2-6** 	**2-9** 
938 (86)	1045 (85)	1357 (65)

[a] See Ref. 6.

Table 1
Representative structures from diverse amide libraries



Entry	R	*Trans*-fused amine **2**		*Cis*-fused amine **3**	
		hCB2 cAMP IC_{50} nM (% E_{max})[a]	hCB1 AMP IC_{50} nM (% E_{max})[a]	hCB2 cAMP IC_{50} nM (% E_{max})[a]	hCB1 cAMP IC_{50} nM (% E_{max})[a]
1		45 (99)	>17,000 (14)	4.2 (100)	4543 (76)
2		24 (90)	963 (72)	0.14 (90)	312 (79)
3		41 (98)	2022 (84)	0.82 (98)	188 (98)
4		22 (94)	>17,000 (31)	0.62 (100)	>17,000 (64)
5		1286 (81)	>17,000 (~56)	21 (99)	>17,000 (46)
6		>17,000 (34)	>17,000 (34)	5.9 (102)	>17,000 (6)

[a] See Ref. 6.

of $CeCl_3$ was employed to give the desired products.[8] While some substitution on the phenyl ring was moderately tolerated (**2-1**, **2-4**) polar heterocycles (**2-3**, **2-9**) were not. Ultimately the unsubstituted phenyl ring was determined to be optimal.

With the pharmacophore around the decahydroquinoline core well defined, efforts were focused on optimizing the amide substituent for agonist activity, selectivity, and physical properties. This was achieved using an iterative library approach based on the acylation of racemic amine **3**. The synthesis of amine **3** was initiated by modifying the procedures of Prost.[7] First, elaboration of 1-acetylcyclohexene (**I**) with paraformaldehyde and dimethylamine hydrochloride under Mannich conditions gave aminoketone **II** in 60% yield. **II** was treated with concentrated aqueous ammonia in 1,4-dioxane at 120 °C in a stainless steel pressure vessel to give decahydroquinolinone **III**. After work-up crude **III** was acylated with benzyloxycarbonyl chloride to give a primarily *trans* mixture of **IV**. Isomerization of the center adjacent to the ketone was achieved by treatment of **IV** with K_2CO_3 in MeOH to give *cis*-**V** in 30% isolated yield over three steps. Addition of PhMgBr to **V** at low temperature gave alcohol **VI** in 74% yield. Deprotection of **VI** by hydrogenolysis gave racemic **3** in quantitative yield. Acylation of **3** was achieved using standard peptide coupling conditions (Scheme 1).

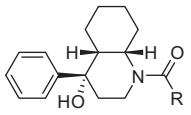
Fused heterocycles, derived from the coupling of 6,5- and 6,6-fused heterocyclic carboxylic acids and racemic amine **3**, were generally potent CB2 agonists (Table 3). While only moderate selectivity was achieved for most heterocycles tested, a few exceptions were observed. Indazole **3-6** was a potent CB2 agonist with no measurable functional CB1 activity in the human cAMP assay. **3-6** exhibited a reasonable plasma free fraction (4.8%) in rats but displayed poor pharmacokinetic behavior (2 mpk iv; Cl = 70 mL/min/kg, $t_{1/2}$ = 0.43 h) and was thus unsuitable for evaluation in in vivo pain models.

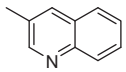
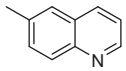
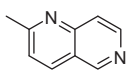
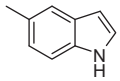
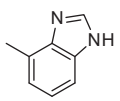
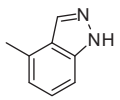
Acylation of racemic amine **3** with either aromatic (Table 4) or saturated (Table 5) monocyclic heterocyclic carboxylic acids was achieved in a similar manner to that of the fused heterocycles. In general both of these classes of compounds had no measurable CB1 agonist activity in the human cAMP assay (CB1 IC_{50} >17,000 nM) across a broad range of structures and CB2 agonist activities.

Substituted pyridines emerged as promising candidates for further evaluation from the aromatic heterocycle class of compounds. The 6-methyl-3-pyridyl analog **4-13** exhibited good overall physical properties (log *P* 2.26) and high plasma free fraction (15%) in rats. The single enantiomer **4-13a**[9] maintained CB2 agonist activity and was not a substrate for rat P-glycoprotein efflux[10] (B–A/A–B = 1.3, P_{app} 36 × 10e^{-6} cm/s). **4-13a** did exhibit some erosion in selectivity over CB1 in both the human and rat cAMP assays (Table 6). *N*-Methyl-morpholine **5-2** stood out in the saturated heterocycles class of compounds, displaying good overall physical properties (log *P* 2.28) and very high free fraction (34%) in rats. Like **4-13a**, the single enantiomer **5-2a**[9] was not a substrate for rat P-glycoprotein efflux[10] (B–A/A–B = 1.3, P_{app} 39 10e^{-6} cm/s). **5-2a** maintained its in vitro selectivity profile and exhibited only a slight loss in CB2 agonist activity in the rat cAMP assay relative to the human data (Table 6).

4-13a and **5-2a** were selected for evaluation in the rat CFA hyperalgesia model.[11] Compound levels were measured in the plasma, brain homogenate (brain), and cerebrospinal fluid (CSF)[12] in all experiments to ensure adequate exposure for CB2 activity. When dosed subcutaneously (10, 30, and 100 mpk) **4-13a** exhibited a dose dependent reversal of paw withdrawal threshold (28%, 53%, and 76% respectively) at the 60 min timepoint (Fig. 3). At the minimum dose needed to achieve naproxen-like efficacy (30 mpk) **4-13a** reached levels in the plasma, brain, and CSF of 4200, 3200, and 223 nM respectively. In contrast, dosing **5-2a** subcutaneously at 30 mpk failed to elicit a response in the rat CFA hyperalgesia model (Fig. 4) despite exposure in plasma, brain, and CSF (6600, 9800, and 1500 nM respectively) well above its CB2 IC_{50}.

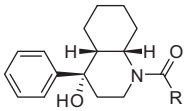
Table 3
6,5- and 6,6-fused heterocyclic amides

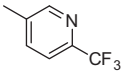
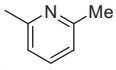
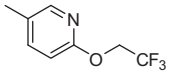
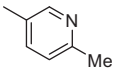
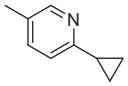
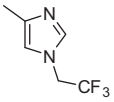


Entry	R	hCB2 cAMP IC_{50} nM (% E_{max})[a]	hCB1 cAMP IC_{50} nM (% E_{max})[a]	Selectivity hCB2/hCB1
3-1		3.38 (96)	1528 (69)	452
3-2		0.53 (87)	261 (95)	492
3-3		5.5 (99)	3441 (69)	626
3-4		1.4 (104)	1002 (91)	716
3-5		35 (101)	>17,000 (~53)	>486
3-6		4.0 (102)	>17,000 (15)	>4000

[a] See Ref. 6.

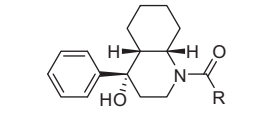
Table 4
Monocyclic aromatic heterocyclic amides

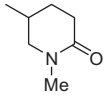
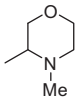
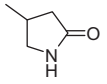
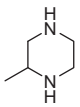
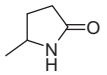
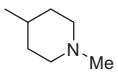
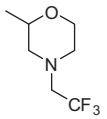
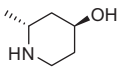


Entry	R	hCB2 cAMP IC_{50} nM (% E_{max})[a,b]	Entry	R	hCB2 cAMP IC_{50} nM (% E_{max})[a,b]
4-1		11 (102)	**4-8**		41 (101)
4-2		6.3 (99)	**4-9**		29 (101)
4-3		124 (100)	**4-10**		12 (96)
4-4		6.1 (99)	**4-11**		140 (103)
4-5		16 (100)	**4-12**		156 (98)[c]
4-6		282 (101)	**4-13**		14 (99)
4-7		12 (96)	**4-14**		26 (104)

[a] See Ref. 6.
[b] Except where noted, compounds exhibited hCB1 IC_{50} >17,000 nM.
[c] hCB1 IC_{50} = 3445 nM (50%).

Table 5
Monocyclic saturated heterocyclic amides



Entry	R	hCB2 cAMP IC_{50} nM (% E_{max})[a,b]	Entry	R	hCB2 cAMP IC_{50} nM (% E_{max})[a,b]
5-1		56 (100)	**5-6**		135 (99)
5-2		30 (100)	**5-7**		3471 (89)
5-3		62 (91)	**5-8**		193 (102)
5-4		395 (98)	**5-9**		30 (99)[c]
5-5		2175 (87)			

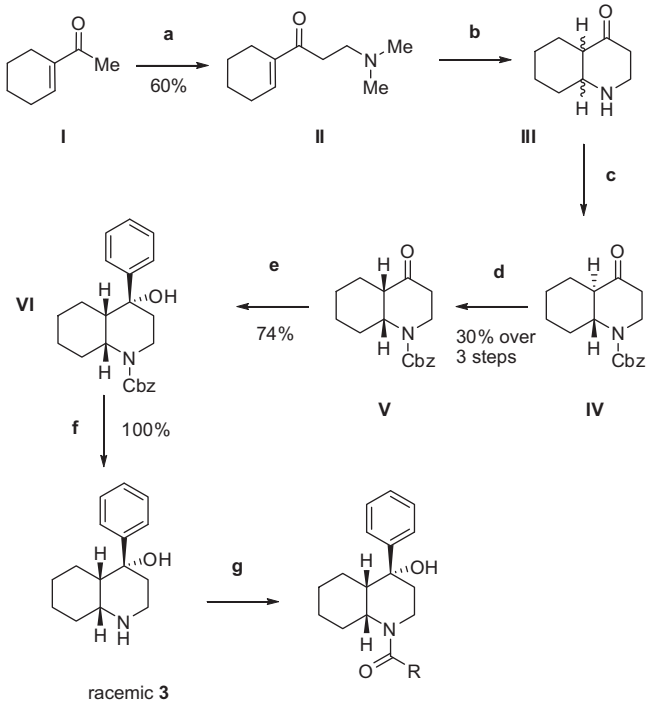
[a] See Ref. 6.
[b] Except where noted, compounds exhibited hCB1 IC_{50} >17,000 nM.
[c] hCB1 IC_{50} = 4044 nM (96%).

Table 6
Properties of **4-13a** and **5-2a**

	4-13a	**5-2a**
hCB2 cAMP IC_{50} nM (% E_{max})[a]	7.3 (103)	16 (101)
hCB1 cAMP IC_{50} nM (% E_{max})[a]	5020 (78)	>17,000 (11)
rCB2 cAMP IC_{50} nM (% E_{max})[a]	6.3 (119)	81 (98)
rCB1 cAMP IC_{50} nM (% E_{max})[a]	1887 (94)	>17,000 (8)
Rat plasma free fraction (%)	15	34

[a] See Ref. 6.

In this and the previous Letter[4] we have demonstrated that two structurally novel and moderately selective CB2 agonists show a dose dependent effect on analgesia in a rat model of acute inflammatory pain. The in vivo results of **4-13a** and imidazopyridne **6a**[4] are similar to other moderately selective CB2 agonists in the \literature.[13,14] The observation of Gifford et al.[5] that a large CB1 receptor reserve exists in the CNS and that compounds with weak CB1 binding exhibit substantial agonist responses led us to search for structurally similar compounds which were functionally CB2 specific in vitro. Improving the functional selectivity of our compounds for CB2 over CB1, as in **5-2a** and imidazopyridine **17b**,[4] resulted in abolishment of the previously observed antinociceptive effects in rats despite high central and peripheral exposure. These results are consistent with Sain et al.'s[15] recent demonstration using transgenic mice and a non-selective CB2/CB1 agonist that



Scheme 1. Conditions: (a) dimethylamine hydrochloride, paraformaldehyde, HCl, EtOH, reflux; (b) concentrated NH_4OH, 1,4-dioxane, 120 °C; (c) CBz-Cl, TEA, CH_2Cl_2, rt; (d) K_2CO_3, MeOH, rt; (e) phenylmagnesium bromide, THF, −78 °C; (f) H_2, 10% Pd/C, EtOH; (g) carboxylic acid, EDC, HOAt, TEA, DMF.

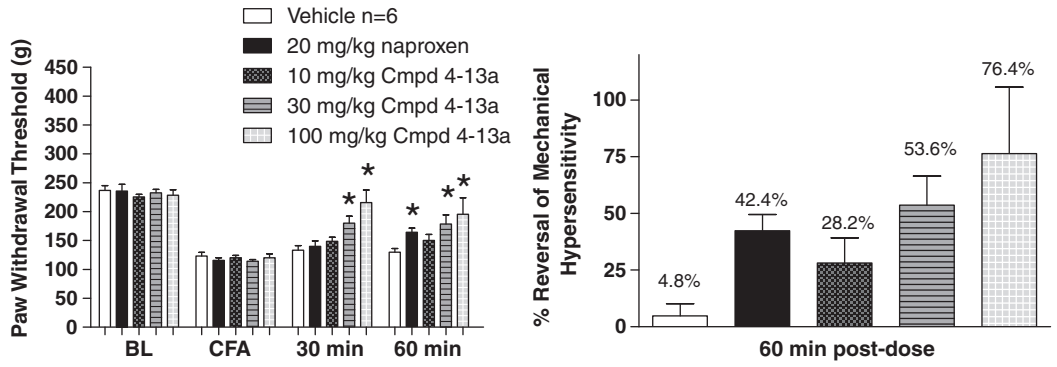


Figure 3. Effects of **4-13a** on paw withdrawal threshold in rat CFA model.

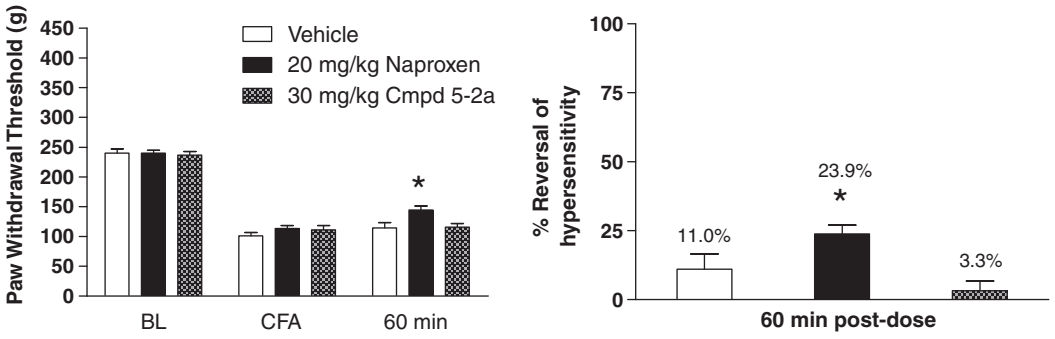


Figure 4. Effects of **5-2a** on paw withdrawal threshold in rat CFA model.

CB1 receptor activation is necessary for antinociception in animal models.

Acknowledgments

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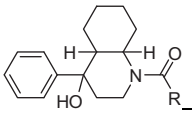
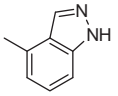
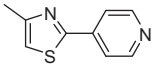
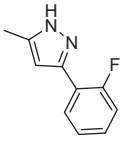
References and notes

1. Graham, E. S.; Ashton, J. C.; Glass, M. *Front. Biosci.* **2009**, *14*, 944.
2. Beltramo, M. *Mini-Rev. Med. Chem.* **2009**, *9*, 11.
3. Thakur, G. A.; Tichkule, R.; Bajaj, S.; Makriyannis, A. *Expert Opin. Ther. Pat.* **2009**, *19*, 1647.
4. Trotter, B. W.; Nanda, K. K.; Burgey, C. B.; Potteiger, C. M.; Deng, J. Z.; Green, A. I.; Hartnett, J. C.; Kett, N. R.; Wu, Z.; Henze, D. A.; Della Penna, K.; Desai, R.; Leitl, M. D.; Lemaire, W.; White, R. B.; Yeh, S.; Urban, M. O.; Kane, S. A.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2011**, in press.
5. Gifford, A. N.; Bruneus, M.; Gatley, S. J.; Lan, R. X.; Makriyannis, A.; Volkow, N. D. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 478.
6. Compounds were added to a Greiner black 384 well low volume assay plate. 1000 CHO-K1 cells (expressing human or rat CB1 or CB2) were added to each well of the assay plate containing compound, then incubated at room temperature for 15 min. Then, forskolin at EC_{70} was added and incubated at room temperature for an additional 30 min. Detection of cAMP was performed using Cisbio's HRTF cAMP dynamic 2 kit following the manufacturer's protocol for cAMP detection. After adding d2-cAMP and anti-cAMP-cryptate to all the wells, there was a final incubation at room temperature for 1 h. Plates were then read on an EnVision plate reader (Perkin Elmer). CV values calculated for a positive control compound (>250 replicates) were 59% and 70%, respectively, for CB1 and CB2 IP values.
7. Prost, M.; Urbain, M.; Schumer, A.; Houben, C.; Van Meerbeeck, C. *Helv. Chim. Acta* **1975**, *58*, 40.
8. Dimitrov, V.; Kostova, K.; Genov, M. *Tetrahedron Lett.* **1996**, *37*, 6787.
9. Racemic amine **3** was resolved into its enantiomers (**3a** and **3b**) by preparative chiral HPLC (Chiracel-OD, 20% IPA in hexanes containing 0.1% DEA). **3a** and **3b** were coupled with a number of different carboxylic acids to probe the effects of absolute stereochemistry on CB2 agonist activity. In every example compounds derived from **3a** were significantly more potent than those derived from **3b**.

	Enantiomer **3a**		Enantiomer **3b**	
R	hCB2 cAMP IC_{50} nM (E_{max})[a]	hCB1 cAMP IC_{50} nM (E_{max})[a]	hCB2 cAMP IC_{50} nM (E_{max})[a]	hCB1 cAMP IC_{50} nM (E_{max})[a]
	1.6 (100)	>17,000 (28)	388 (97)	>17,000 (51)
	4.9 (100)	4247 (95)	5221 (77)	>17,000 (10)
	0.55 (100)	>17,000 (12)	597 (89)	>17,000 (3)
	7.9 (98)	>17,000 (18)	>17,000 (19)	930 (46)

[a] See Ref. 6.

10. Miller, D. S.; Bauer, B.; Hartz, A. M. S. *Pharmacol. Rev.* **2008**, *60*, 196.
11. Nagakura, Y.; Okada, M.; Kohara, A.; Kiso, T.; Toya, T.; Iwai, A.; Wanibuchi, F.; Yamaguchi, T. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 490.
12. Liu, X. R.; Smith, B. J.; Chen, C.; Callegari, E.; Becker, S. L.; Chen, X.; Cianfrogna, J.; Doran, A. C.; Doran, S. D.; Gibbs, J. P.; Hosea, N.; Liu, J. H.; Nelson, F. R.; Szewc, M. A.; Van Deusen, J. *Drug Metab. Dispos.* **2006**, *34*(9), 1443.
13. GSK CB2 agonist clinical candidate GW-842166X is unique in that it is reported to produce analgesia in preclinical models despite exceedingly weak CB1 agonist activity. However, investigators at Abbott (Ref. 13) did observe measurable rat CB1 agonist activity for this compound. Giblin, G. M. P.; O'Shaughnessy, C. T.; Naylor, A.; Mitchell, W. L.; Eatherton, A. J.; Slingsby, B. P.; Rawlings, D. A.; Goldsmith, P.; Brown, A. J.; Haslam, C. P.; Clayton, N. M.; Wilson, A. W.; Chessell, I. P.; Wittington, A. R.; Green, R. *J. Med. Chem.* **2007**, *50*, 2597.
14. Yao, B. B.; Hsieh, G. C.; Frost, J. M.; Fan, Y.; Garrison, T. R.; Daza, A. V.; Grayson, G. K.; Zhu, C. Z.; Pai, M.; Chandran, P.; Salyers, A. K.; Wensink, E. J.; Honore, P.; Sullivan, J. P.; Dart, M. J.; Meyer, M. D. *Br. J. Pharmacol.* **2008**, *153*, 390.
15. Sain, N. M. H.; Liang, A.; Kane, S. A.; Urban, M. O. *Neuropharmacology* **2009**, *57*, 235.