Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3287–3291

Novel inhibitors of fatty acid amide hydrolase

S. Y. Sit,[a,*] Charlie Conway,[b] Robert Bertekap,[b] Kai Xie,[a] Clotilde Bourin,[b] Kevin Burris[c] and Hongfeng Deng[d]

[a]*Department of Chemistry, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492-7660, USA*
[b]*Department of Neurosciences, The Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492-7660, USA*
[c]*Palatin Technologies, Inc. 4-C Cedar Brook Drive, Cedar Brook Corporate Center, Cranbury, NJ 08512, USA*
[d]*GlaxoSmithKline, 830 Winter Street, Waltham, MA 02451, USA*

Received 11 January 2007; revised 3 April 2007; accepted 4 April 2007
Available online 10 April 2007

Abstract—A class of bisarylimidazole derivatives are identified as potent inhibitors of the enzyme fatty acid amide hydrolase (FAAH). Compound **17** (IC_{50} = 2 nM) dose-dependently (0.1–10 mg/kg, iv) potentiates the effects of exogenous anandamide (1 mg/kg, iv) in a rat thermal escape test (Hargreaves test), and shows robust antinociceptive activity in animal models of persistent (formalin test) and neuropathic (Chung model) pain. Compound **17** (20 mg/kg, iv) demonstrates activity in the formalin test that is comparable to morphine (3 mg/kg, iv), and is dose-dependently inhibited by the CB1 antagonist SR141716A. In the Chung model, compound **17** shows antineuropathic effects similar to high-dose (100 mg/kg) gabapentin. FAAH inhibition shows potential utility for the clinical treatment of persistent and neuropathic pain.

The cloning of cannabinoid (CB) receptors and the recent discovery of endogenous agonists has resulted in considerable interest in the cannabinoid system as a target for drug discovery.[1] The cannabinoid system has been implicated in regulating numerous physiological and pathological conditions including pain and inflammation.[2] Development of agonists at central CB1 receptors has been dampened by the inability to separate potential therapeutic benefits from side-effects such as ataxia, hypothermia, and potential for abuse.

Endogenous cannabinoids, like anandamide, are released from cells in a stimulus-dependent manner (event driven) and rapidly inactivated by enzymes such as fatty acid amide hydrolase (FAAH).[3,4] Recently, mice that lack FAAH (FAAH knock-out, KO mice) were developed by researchers at The Scripps Research Institute.[5] These FAAH KO mice reportedly have increased brain levels of fatty acid amides, including anandamide. However, these mice do not display all of the typical signs of cannabinoid agonist activation (i.e., hypothermia, catalepsy, decreased locomotor activity) suggesting that inhibition of FAAH is not the functional equivalent of widespread CB1 receptor activation. These FAAH KO mice do, however, demonstrate decreased thermal pain sensitivity that is blocked by a CB1 receptor antagonist. Inhibitors of FAAH may provide selective prolongation of endogenous cannabinoid activity at local sites of release and present a potentially useful strategy for maximizing beneficial effects such as analgesia.[6] Here we identify a novel series of FAAH inhibitors that potentiate the effects of exogenous anandamide and demonstrate robust activity in animal models of persistent and neuropathic pain.

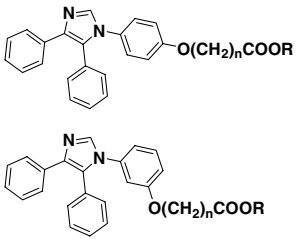
Utilizing a high-throughput screen (HTS) on a targeted subset of Bristol-Myers Squibb's proprietary collection of compounds, a class of bisarylazole derivatives emerged as potent inhibitors of the enzyme FAAH [Table 1].

The compounds chosen for the initial screen were originally designed as nonprostanoid prostacyclin mimetics,

Keywords: Fatty acid amide hydrolase; FAAH; Enzyme; Inhibitor; Bisarylimidazole; Pain; SR141716A; Anandamide; Hargreaves test; Chung model; Formalin test; Persistent; Neuropathic; Antineuropathic; Gabapentin; Morphine; Cannabinoid receptor.
* Corresponding author. Tel.: +1 203 677 6678; fax: +1 203 677 5775; e-mail addresses: singyuen.sit@bms.com; kburris@palatin.com; hongfeng.x.deng@gsk.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.009

Table 1. Compounds identified from HTS



#	n	R	IC50 (nM)
1	1	Me	670*
2	3	Et	64±25**
3	4	Et	>10000
#	n	R	IC50 (nM)
4	1	Me	6500*
5	3	Et	91±2**

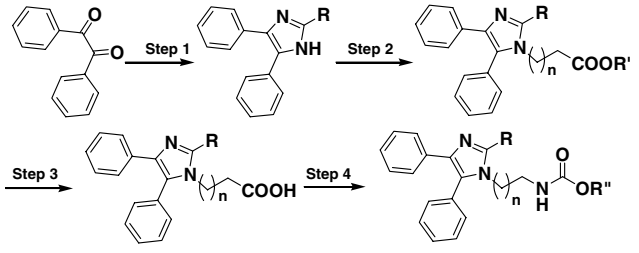
(**replicates, *single readings)

reflecting a common arachidonic acid lineage, but were only weakly active as inhibitors of blood platelet aggregation.[7] Initial in vitro findings suggested that the active chemotype possessed very well-defined structural requirements for expression of potent FAAH inhibition. One of the key objectives of the study was to validate the fundamental concept that blocking the degradation of the endocannabinoid signaling agents such as anandamide in vivo could precipitate an 'event-triggered' and localized analgesic response that is qualitatively different from a centrally administered cannabinoid agonist.[8]

The active hits from the HTS were resynthesized and their in vitro activity confirmed [Table 1]. The SAR was established by chemical modifications of the original hits, as depicted in Scheme 1, that resulted in novel and potent analogs with IC_{50} values in the low single digit nM range [Table 2].[9]

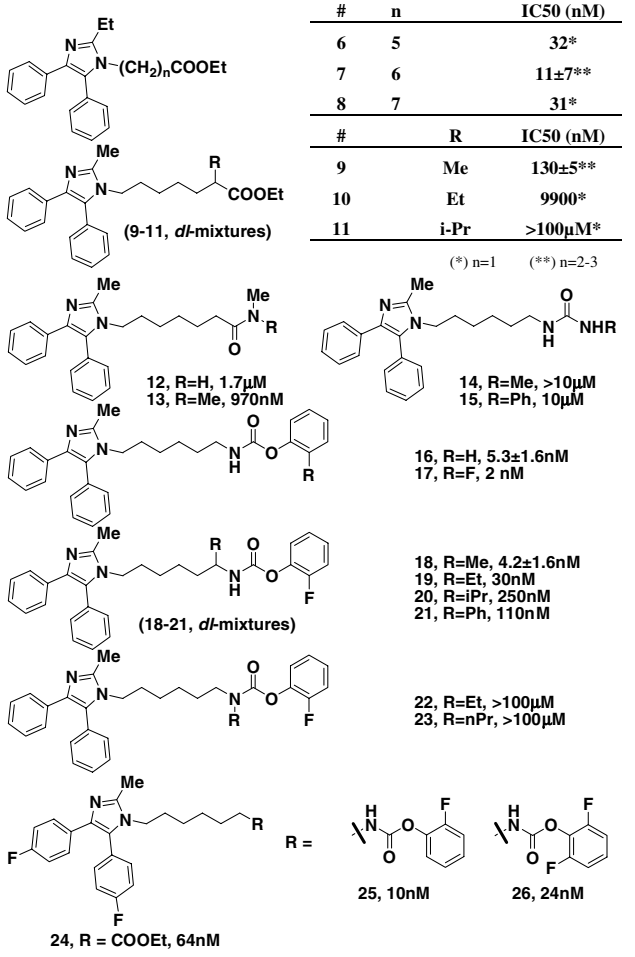
Determination of FAAH activity. Homogenates of crude membranes were prepared from H4 cells that express transfected human FAAH (H4-FAAH cells).[9] Activity of FAAH was measured using a modification of the method described by Omeir et al.[10] IC_{50} values were determined using a four-parameter logistic equation for dose–response curves.

Preliminary in vitro SAR work focused on utilizing alkyl chains to replace the substituted phenyl ring. This exercise revealed that the inhibitory potency is highly sensitive to the chain length separating the bisarylimidazole core and the terminal functionality (**6**–**8**). It was initially considered that this class of inhibitors would behave like other typical serine hydrolase inhibitors that act by way of an electrophilic functional group interacting with the



Scheme 1. Reagents and conditions: (1) Alkyl aldehyde, ammonium acetate in acetic acid at 100 °C; (2) aliphatic halide, NaH in DMF at rt; (3) NaOH–EtOH at rt; (4) $(PhO)_2P(O)N_3$, Et_3N in toluene at 105 °C, followed by R″OH.

Table 2. Novel inhibitors of FAAH



#	n		IC50 (nM)
6	5		32*
7	6		11±7**
8	7		31*
#		R	IC50 (nM)
9		Me	130±5**
10		Et	9900*
11		i-Pr	>100μM*

(*) n=1 (**) n=2-3

catalytic triad to produce a covalent intermediate. Indeed, substitution at the α-position of **7** exerted a significant negative effect on the enzyme inhibitory activity, as shown by compounds **9**–**11**. Replacing the ester moiety with an amide (**12**, **13**), or urea moiety (**14**, **15**), rendered the compounds inactive. Further optimization with the primary goal of increasing inhibitory potency showed that the carbamate terminal group provided the most potent compounds within this series.[11] Thus, both phenyl carbamates **16** and **17** showed very potent enzyme inhibitory activity toward FAAH with IC_{50} values in the low single digit nM. By way of contrast, the corresponding alkyl carbamates were found to be less active (data not shown). It is interesting to note that substitution α- to the N atom in the carbamate series (**18**–**20**) does not affect IC_{50} values as much as in the ester series, presumably reflecting the increased separation from the carbonyl moiety. However, substituting the carbamate N resulted in a complete loss of inhibitory activity, reinforcing the importance of a sterically unencumbered carbonyl moiety in this series (**22**, **23**). A consequence that is consistent with carbamate functional group participation in the inhibition of FAAH. Finally the effects of aryl ring modification toward inhibitory activity were explored in a single structural context. The bis(4-fluor-

ophenyl) substituted imidazole derivatives showed reduced activity (~5×) compared to the parent unsubstituted compounds. It is conceivable that fluoro substitution could affect the overall recognition of the inhibitor by FAAH in a subtle fashion.

The initial goal was to demonstrate proof of concept in vivo for FAAH inhibition, followed by assessment in multiple animal models of persistent and neuropathic pain. Compound **17** (IC_{50} = 2 nM) from the carbamate series was selected for in vivo testing. In the Hargreaves thermal escape test, **17** was shown to transform an otherwise *inactive* dose of exogenous anandamide into an agent with robust, morphine-like analgesic properties [Fig. 1].[12]

Briefly, compound **17** (0.1, 1, and 10 mg/kg, iv) given 15 min prior to exogenous anandamide (1 mg/kg, iv) produced a significant dose-dependent antinociceptive effect from 3 to 30 min. Neither anandamide nor 10 mg/kg compound **17** was active if given alone, but together their effect was comparable to the positive control morphine sulfate (3 mg/kg, iv). This combination of two inactive doses yielding an robust antinociceptive effect provides a plausible, in vivo, proof of concept for inhibition of FAAH.[13]

Carbamate **17** was examined in a model of persistent pain where the test animals were challenged with subcutaneous paw injection of formalin.[14] The goal was to determine the effect of **17** upon the spontaneous behavior as measured by the frequency of 'paw flinch' responses.

In this model, **17** (20 mg/kg, iv) produced a significant ($p < 0.01$) suppression of formalin-induced paw flinches that was comparable to morphine (3 mg/kg, iv) [Fig. 2]. The CB1 antagonist SR141716A (**SR**, 3 and 10 mg/kg, ip) completely reversed the effect of **17** (20 mg/kg, iv) in the persistent pain model (Phase II) [Fig. 3b], and partly reversed in the model of acute pain (Phase I) [Fig. 3a].

Finally, compound **17** was studied in a neuropathic pain model (Chung model) where nerve injured rats (surgical

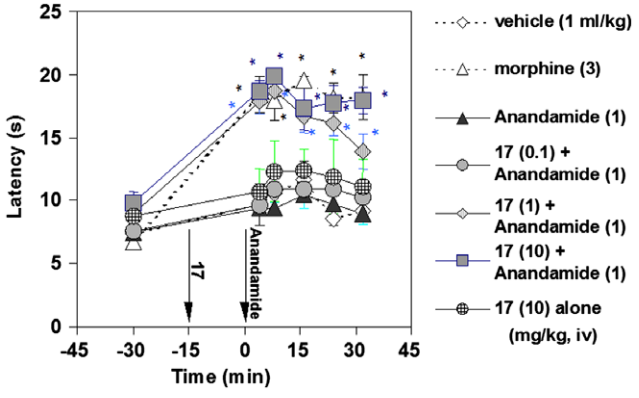


Figure 1. Hargreaves test—compound **17** Potentiates the Antinociceptive Effects of Exogenous Anandamide. (n = 5–8 per group; $^{*}p < 0.01$ vs vehicle group).

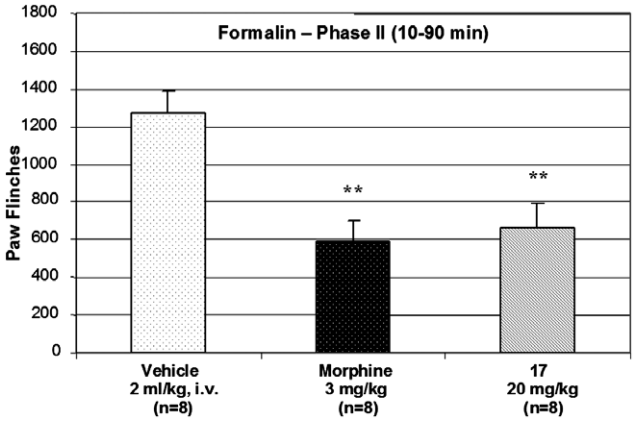


Figure 2. 17 Elicits morphine-like activity against persistent pain. $^{**}p < 0.01$ versus vehicle group (Dunnett's test); iv, jugular catheter.

L5/6 spinal nerve ligation) exhibit pain-escape responses (tactile allodynia) to light touch that is normally an innocuous stimulus [Fig. 4]. Compound **17** (20 mg/kg, iv) produced a significant ($p < 0.01$) reversal of tactile allodynia (neuropathic pain behavior) that was comparable to gabapentin (100 mg/kg, iv).[15]

In conclusion, novel inhibitors of the enzyme FAAH responsible for the signal termination of the endocannabinoids, like anandamide, were identified. Further optimization yielded potent inhibitors suitable for a proof of concept study. In a battery of in vivo whole animal pain models, the FAAH inhibitor **17** demonstrated potentiation of the antinociceptive effect of an ineffective dose of anandamide. High-dose exogenous anandamide can produce strong analgesia, and potentiation of an inactive dose is consistent with the actions of an FAAH inhibitor. In the formalin test, compound **17** showed robust antinociception comparable to that of morphine in Phase II (persistent pain). The specific CB1 antagonist SR141716A blocks completely the antinociceptive effect in the persistent phase (II); and partially in the acute phase (I). These results support the mode of action was via the endocannabinoid signaling pathway. The effect of **17** in the Chung model was particularly striking. Compound **17** demonstrated significant effect in reversing mechanical allodynia (neuropathic pain behavior) comparable to the clinically active reference agent gabapentin. Taken together, we have identified a novel class of inhibitors that block the metabolic degradation of endocannabinoid signaling agents. While it is not possible to specifically pinpoint the precise mode of action of these compounds in vivo, the implications of blocking FAAH are clearly demonstrated by **17** in a series of in vivo whole animal studies.

Additional enzyme inhibition studies showed that **17** was only weakly active in cytosolic phospholipase A2, (cPLA2, $K_i > 15$ μM) thus precluding mechanisms typically associated with the cPLA2 triggered inflammation processes.[16] Furthermore, the compound itself showed very little affinity to CB1/CB2 (K_i = 23/28 μM, respectively).[17] Collectively, the results from the in vitro and in whole animal studies strongly support the relevance

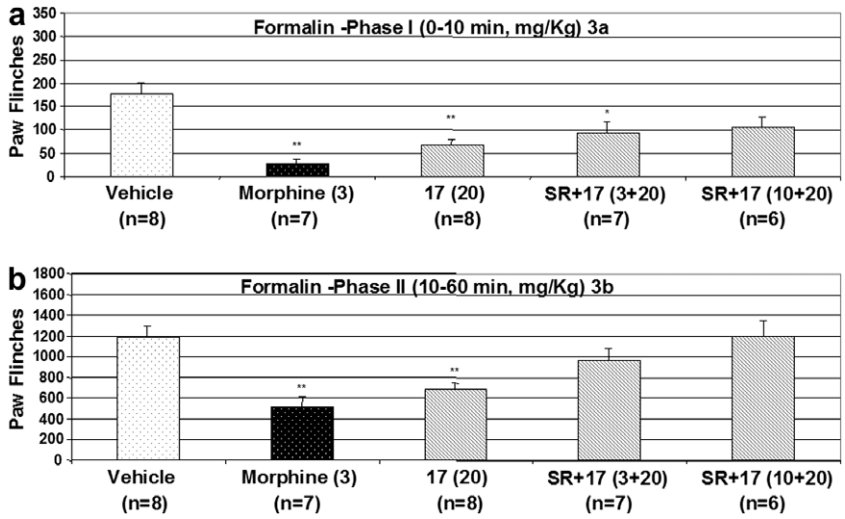


Figure 3. 3a, **3b** CB1 antagonist shows dose-dependent reversal of **17** action in formalin test. −30 min: CB1 antagonist SR141716A given ip; −15 min: **17** given iv; 0 min: formalin; $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle (Dunnett's test); iv, jugular catheter.

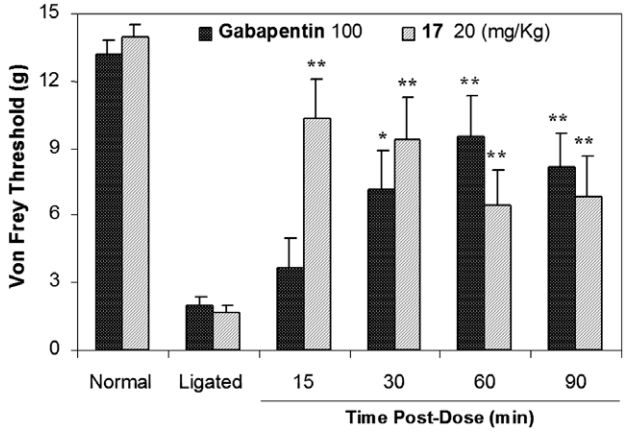


Figure 4. Compound **17** reverses neuropathic pain behavior like high-dose gabapentin. $^{*}p < 0.05$, $^{**}p < 0.01$ versus baseline ligated (Dunnett's test); iv, tail vein.

of blocking the endocannabinoid signaling by **17** in the modulation of pain responses.

References and notes

1. For general reviews: (a) Pacher, P.; Batkai, S.; Kunos, G. *Pharmacol. Rev.* **2006**, *58*, 389; (b) Cravatt, B. F.; Lichtman, A. H. *Curr. Opin. Chem. Biol.* **2003**, *7*, 469; (c) Boger, D. L.; Henriksen, S. J.; Cravatt, B. F. *Curr. Pharm. Des.* **1998**, *44*, 303.
2. (a) Pertwee, R. G. *Prog. Neurobiol.* **2001**, *63*, 569; (b) Cravatt, B. F.; Lichtman, A. H. *J. Neurobiol.* **2004**, *61*, 149; (c) Lu, D.; Vemuri, V.; Kiran, D.; Richard, I., Jr.; Makriyannis, A. *Curr. Top. Med. Chem.* **2006**, *6*, 1401; (d) Guindon, J.; LoVerme, J.; De Lean, A.; Piomelli, D.; Beaulieu, P. *Eur. J. Pharmacol.* **2006**, *550*, 68.
3. (a) Elphick, M. R.; Egertova, M. *Philos. Trans. R. Soc. London Ser. B* **2001**, *356*, 381; (b) Cravatt, B. F.; Lichtman, A. H. *Chem. Phys. Lipids* **2002**, *121*, 135; (c) Walker, J. M.; Huang, S. M.; Strangman, N. M.; Tsou, K.; Sanudo-Pena, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12198.
4. (a) Desarnaud, F.; Cadas, H.; Piomelli, D. *J. Biol. Chem.* **1995**, *270*, 6030; (b) Piomelli, D.; Beltramo, M.; Giuffrida, A.; Stella, N. *Neurobiol. Dis.* **1998**, *5*, 462.
5. Cravatt, B. F.; Demarest, K.; Patricelli, M. P.; Bracey, M. H.; Giang, D. K.; Martin, B. R.; Lichtman, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9371.
6. (a) Boger, D. L.; Miyauchi, H.; Du, W.; Hardouin, C.; Fecik, R. A.; Cheng, H.; Hwang, I.; Hedrick, M. P.; Leung, D.; Acevedo, O.; Guimaraes, C. R. W.; Jorgensen, W. L.; Cravatt, B. F. *J. Med. Chem.* **2005**, *48*, 1849; (b) Leung, D.; Du, W.; Hardouin, C.; Cheng, H.; Hwang, I.; Cravatt, B. F.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1423; (c) Guimaraes, C. R. W.; Boger, D. L.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2005**, *127*, 17377; (d) Michaux, C.; Muccioli, G. G.; Lambert, D. M.; Wouters, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4772; (e) Piomelli, D.; Tarzia, G.; Duranti, A.; Tontini, A.; Mor, M.; Compton, T. R.; Dasse, O.; Monaghan, E. P.; Parrott, J. A.; Putman, D. *CNS Drug Reviews* **2006**, *12*, 21.
7. (a) Seiler, S. M.; Brassard, C. L.; Federici, M. E.; Romine, J.; Meanwell, N. A. *Prostaglandins* **1997**, *53*, 21; (b) Meanwell, N. A.; Romine, J. L.; Seiler, S. M. *Drugs Fut.* **1994**, *19*, 361; (c) Meanwell, N. A.; Rosenfeld, M. J.; Trehan, A. K.; Romine, J. L.; Kim, Wright J. J.; Brassard, C. L.; Buchanan, J. O.; Federici, M. E.; Fleming, J. S.; Gamberdella, M.; Seiler, S. M. *J. Med. Chem.* **1992**, *35*, 3498.
8. Fox, A.; Bevan, S. *Expert Opin. Invest. Drugs* **2005**, *14*, 695.
9. Sit, S. Y.; Xie, K. U.S. Patent 6,562,846, 2003; preliminary report disclosed as a Poster Presentation, Sit et al. 39th National Organic Chemistry symposium, Number D30, Salt Lake City, Utah, June 12–16, 2005; American Chemical Society, Division of Organic Chemistry.
10. Omeir, R. L.; Chin, S.; Hong, Y.; Ahern, D. G.; Deutsch, D. G. *Life Sci.* **1995**, *56*, 1999.
11. Carbamate functionality is uniquely present in many FAAH inhibitors, see reference [1](b), and Alexander, J. P.; Cravatt, B. F. *Chem. Biol. (Cambridge, MA, U.S.)* **2005**, *12*, 1179.
12. Hargreaves, K.; Dubner, R.; Brown, F.; Flores, C.; Joris, J. *Pain* **1988**, *32*, 77.
13. Comparable, in vivo, analgesic properties have been demonstrated previously, for example, see Refs. 6a–e.
14. Hunskaar, S.; Hole, K. *Pain* **1987**, *30*, 103.

15. (a) Honore, P. *Drug Dev. Res.* **2006**, *67*, 302; (b) Joshi, S. K.; Honore, P. *Expert Opin. Drug Discovery* **2006**, *14*, 323.
16. Lee, K. L.; Foley, M. A.; Chen, L.; Behnke, M. L.; Lovering, F. E.; Kirincich, S. J.; Wang, W.; Shim, J.; Tam, S.; Shen, M. W. H.; Khor, S.; Xu, X.; Goodwin, D. G.; Ramarao, M. K.; Nickerson-Nutter, C.; Donahue, F.; Ku, M. S.; Clark, J. D.; McKew, J. C. *J. Med. Chem.* **2007**, *50*, 1380.
17. CB1/CB2 binding competition of [^{3}H]-CP55,940 in HEK-CB1 and CHO-CB2 preparations.