Bioorganic & Medicinal Chemistry Letters 19 (2009) 6793–6796

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Mining biologically-active molecules for inhibitors of fatty acid amide hydrolase (FAAH): Identification of phenmedipham and amperozide as FAAH inhibitors

Fabien Vincent *, Margaret T. Nguyen, Daniel E. Emerling, Michael G. Kelly, Matthew A. J. Duncton *

Renovis, Inc. (a wholly-owned subsidiary of Evotec AG), Two Corporate Drive, South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 27 July 2009
Revised 18 September 2009
Accepted 22 September 2009
Available online 30 September 2009

Keywords:
Fatty acid amide hydrolase
FAAH
Inhibitor
Screen
Marketed compound
Herbicide
Drug
Phenmedipham
Amperozide

ABSTRACT

The screening of known medicinal agents against new biological targets has been shown to be a valuable approach for revealing new pharmacology of marketed compounds. Recently, carbamate, urea and ketone inhibitors of fatty acid amide hydrolase (FAAH) have been described as promising treatments for pain, anxiety, depression and other CNS-related conditions. In order to find novel FAAH inhibitors, a focused screen of molecules containing potentially reactive moieties or having in vivo effects that are possibly relevant to the biology of FAAH was conducted. These studies revealed phenmedipham **13** and amperozide **14** to be inhibitors of human FAAH, with an IC_{50} of 377 nM and 1.34 μM, respectively.

The endocannabinoid system is composed of the cannabinoid receptors CB_1 and CB_2, their cognate ligands anandamide (AEA, **1**) and arachidonoyl glycerol (2-AG) and the enzymes that respectively regulate these ligands' levels through hydrolysis, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL).[1] While CB_1 agonism has been clinically validated for several indications, psychotropic side effects and sociopolitical issues have prevented the full therapeutic exploitation of this pathway.[2] On the basis of numerous animal model studies, inhibition of FAAH-mediated degradation of AEA appears to hold promise in harnessing these therapeutic benefits, whilst avoiding unwanted side effects.[3] Pioneering work on FAAH as a medicinal target has led to the identification of numerous inhibitors, mostly based around activated ketones, carbamates and ureas (e.g., compounds **2**–**6**, Fig. 1).[4–9] Importantly, mechanistic studies have revealed that inhibitors of each chemical class, including ureas, covalently attach to the catalytic serine S241 of FAAH.

In recent years, detailed investigations have revealed new pharmacological insights for existing medications.[10] For instance, Thalidomide **7**, a molecule with a tragic history of teratogenicity, has found new therapeutic uses treating multiple myeloma and erythema nodosum leprosum (Fig. 2).[11] Additionally, more detailed understandings of Mefloquine[12] **8**, Clofazimine[13] **9**, Itraconazole[14] **10**, Astemizole[15] **11** and Digoxin[16] **12** have provided new starting points for medicinal chemistry campaigns, or potential new applications for these approved drugs. Accordingly, we applied a similar

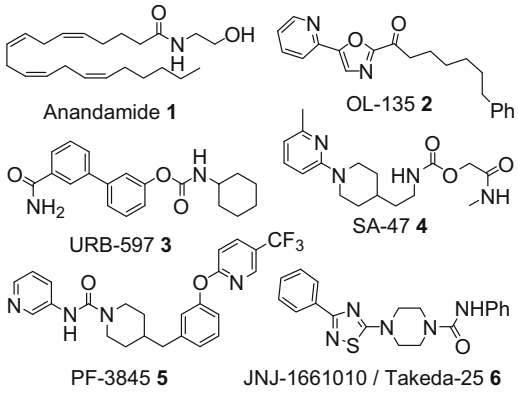


Figure 1. Anandamide and representative inhibitors of FAAH.

* Corresponding authors. Tel.: +1 650 619 0722 (F.V.).
E-mail addresses: fabien_vincent_us@yahoo.com (F. Vincent), mattduncton@yahoo.com (M.A.J. Duncton).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.09.086

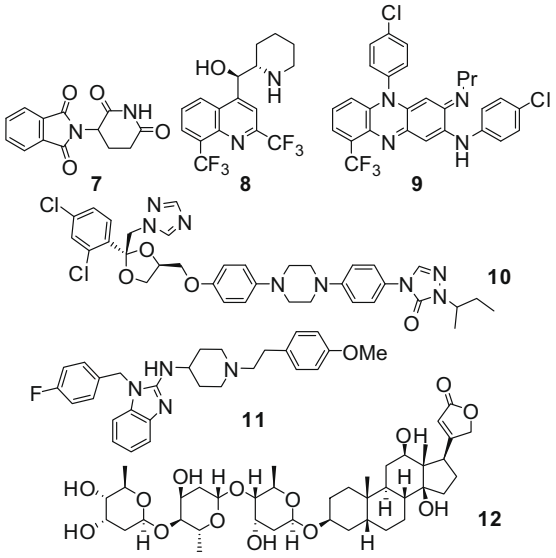


Figure 2. Examples of marketed drugs with recently discovered pharmacological properties.

biology- and structure-based mining strategy to identify molecules that may inhibit FAAH. Herein, we present the results of this focused screen, and reveal carbamate phenmedipham **13**, and piperazinyl urea amperozide **14**, to be inhibitors of FAAH.

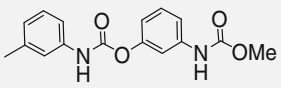
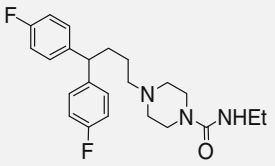
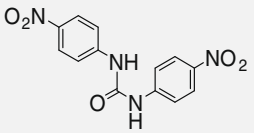
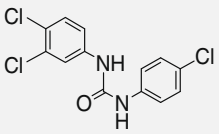
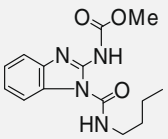
The goal of this study was to uncover molecules extensively characterized in animals and/or humans as novel inhibitors of FAAH. We reasoned that positive results might suggest additional applications for a given compound, or shed new light on the effects of in vivo inhibition of FAAH. As noted before, nearly all known inhibitors of FAAH covalently, and often irreversibly, react with the enzyme. While such inhibitors are over-represented in cases of idiosyncratic toxicity, the advantages they present in terms of receptor occupancy are equally well known.[17] Specifically, inhibitor binding is not subject to competition from the substrate, recovery of enzymatic activity may require protein re-synthesis, and long incubation times can allow a compound with poor binding affinity to completely inhibit the enzyme.[7,18]

Compound selection was conducted by choosing some representative examples containing carbamate, urea, ketone, aldehyde and amide (including Weinreb amide) substructures.[19] The pool of molecules against which these searches were conducted included marketed drugs, herbicides and pesticides, as well as molecules in clinical trials for indications for which FAAH inhibition had shown efficacy in preclinical models. A special emphasis was placed on molecules with an uncertain molecular target, as it was reasoned that some of these compounds could be exerting their pharmacological effect through inhibition of the FAAH enzyme. In total, about 100 compounds were selected for this focused screen.[20]

Several groups have reported a rather high hit rate in their screening campaigns against FAAH, in agreement with our own results outside of this study, although reactive impurities might explain at least part of the activities observed.[21] Since we only wanted to uncover inhibition with a reasonable likelihood of physiological relevance, we conducted the screening of this focused library at a compound concentration of 1 μM, using a 30 min pre-incubation time. A fluorescent readout was used to quantify enzyme inhibition.[22]

Table 1 details the activity of the most potent molecules evaluated in this study.[20] Several interesting features from the screening results were noted. First, the low number of compounds that were found to inhibit FAAH was somewhat surprising. At the outset of

Table 1
Percent inhibition of select compounds versus hFAAH

Compound	Structure	% Inhibition of hFAAH ([drug] = 1 μM)[a]
13 (Phenmedipham)		90
14 (Amperozide)		54
15 (Component of nicarbazin)		51
16 (Triclocarbam)		35
17 (Benomyl)		34

[a] Compounds with >30% inhibition of hFAAH at 1 μM are shown. URB-597 **3** (100% inhibition at 1 μM) was included as a positive control.

these studies, we postulated that multiple molecules might show themselves to be potent inhibitors of FAAH, particularly those with carbamate or urea substructures. We adopted this thinking because FAAH has been reported to be a particularly efficient amidase, due to the presence of a novel catalytic triad within the active site.[23] Indeed, the enzyme is capable of efficiently reacting with substrates containing esters, amides (by definition), carbamates and even ureas. The reactivity with ureas is rather unusual, and highlights the extraordinary ability of the FAAH enzyme to organize and activate substrates so that they are rendered open to nucleophilic attack.[7,8b,24,30a] Thus, what emerges from this set of results is that the FAAH enzyme appears to have a high degree of discrimination in selecting a reacting partner, in contrast to some previous observations.[21,25]

The IC_{50} of phenmedipham **13** and amperozide **14** were measured against human and rat FAAH (Fig. 3).[22] Phenmedipham **13** inhibited hFAAH and rFAAH, with an average IC_{50} of 377 nM and 77 nM, respectively. Amperozide **14** had an average IC_{50} of 1.34 μM against hFAAH, and inhibited rFAAH by approximately 50 at 10 μM. Species difference in potency for **13** and **14** may be due to documented amino acid variations in the enzyme's active site.[7,26] Both compounds were also tested in a radioactive ^{3}H-AEA hydrolysis assay with a much longer pre-incubation time of 2 h.[22] Predictably, lower IC_{50} values were obtained against hFAAH under these conditions (phenmedipham IC_{50} = 152 nM; amperozide IC_{50} = 455 nM).

Additionally, we examined the enzyme's kinetics upon inhibition by **13** (Fig. 4). A significant lowering of V_{max} was observed upon inhibitor treatment, accompanied by a comparatively smaller shift towards higher K_M values. Lineweaver–Burk analysis of this

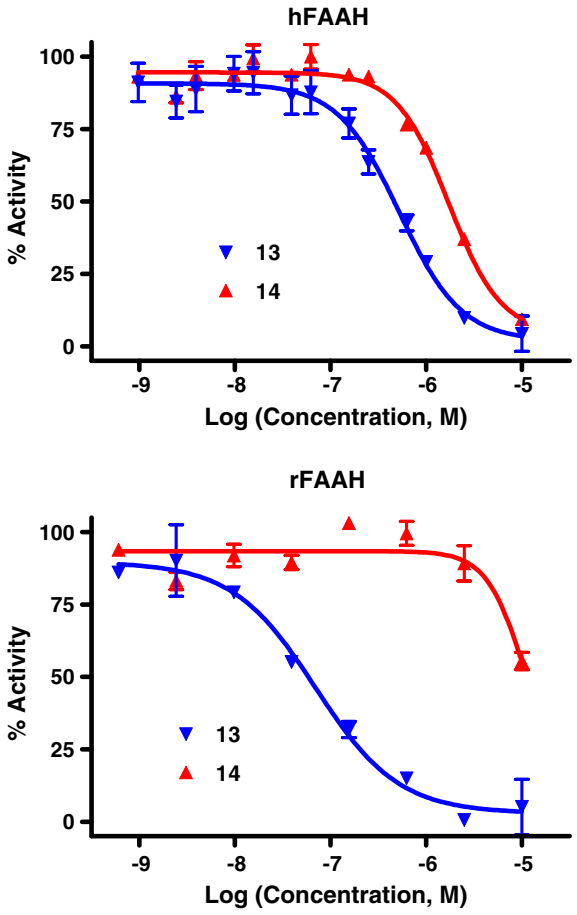


Figure 3. Representative IC_{50} curves for phenmedipham **13** and amperozide **14** against hFAAH (upper panel) and rFAAH (lower panel).

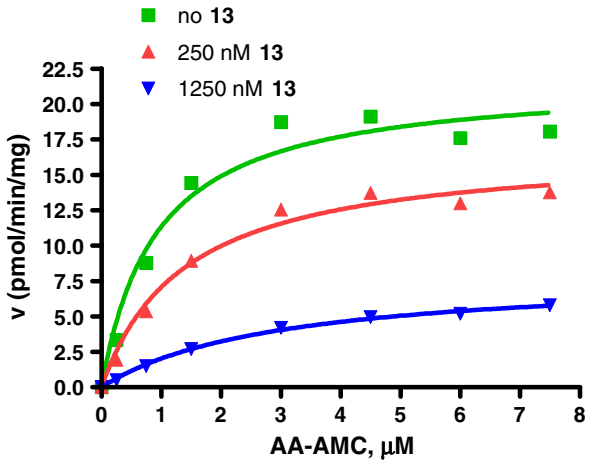


Figure 4. Enzyme kinetics study of **13** against hFAAH. V_{max} and K_M determination in the presence of increasing concentrations of **13**.

data revealed a non-competitive inhibition pattern. Similar results were obtained for control carbamate URB-597 (data not shown). Importantly, irreversible inhibitors, including those binding at the active site, are expected to display such a non-competitive pattern.[18]

Phenmedipham **13** is an herbicide used in sugar beet and spinach culture. Its herbicidal activity is exerted through disruption of photosynthesis.[27] While no behavioural studies appear to have been conducted, a wealth of rodent and dog toxicity data is available.[28] When dosed orally, **13** displayed an LD_{50} of >8000 mg/kg in rats. No genotoxicity, or developmental toxicity, were observed. However, chronic dosing resulted in a reproducible, and dose-dependent, reduction in body weight.[28] Interestingly, a similar weight loss has been reported for rats treated with URB-597.[29]

The second most potent inhibitor revealed by this screen was the piperazine urea, amperozide **14**. Related piperazine and piperidine ureas have been described by numerous research groups studying FAAH.[7,8,24,30] Indeed, several compounds from this chemical class have shown promising results in advanced preclinical testing.[7,24,30a,b] Although amperozide is active against a range of CNS-mediated conditions in vivo,[31] it is highly debatable whether this behaviour is mediated through inhibition of FAAH, since amperozide is a potent antagonist of other CNS targets such as 5-HT_{2a}, 5-HT_6 and D_2 receptors.[32] After being pursued as an atypical antipsychotic, its development for human indications appears to have been stopped.[32,33] Future studies examining both brain AEA levels and FAAH ex vivo activity after steady state in vivo exposure to amperozide and phenmedipham would clarify the ability of these compounds to respectively inhibit FAAH in vivo.[7,30b]

In conclusion, the general principle of screening known medicinal agents against new biological targets, in this case FAAH, was applied to a focused library of potentially reactive substrates and molecules with interesting in vivo biological profiles. Although few hits were obtained, suggesting that FAAH is a discriminating enzyme, phenmedipham and amperozide were identified as new inhibitors. The wealth of animal and human data associated with these compounds could potentially prove helpful in the clinical development of FAAH inhibitors. Overall, this study adds to the literature describing new biological properties for older remedies, and supports the examination of a larger pool of historical drugs against the FAAH enzyme.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.086.

References and notes

1. Di Marzo, V. *Nat. Rev. Drug Disc.* **2008**, *7*, 438.
2. Kumar, R. N.; Chambers, W. A.; Pertwee, R. G. *Anaesthesia* **2001**, *56*, 1059.
3. Ahn, K.; McKinney, M. K.; Cravatt, B. F. *Chem. Rev.* **2008**, *108*, 1687.
4. (a) Boger, D. L.; Sato, H.; Lerner, A. E.; Hedrick, M. P.; Fecik, R. A.; Miyauchi, H.; Wilkie, G. D.; Austin, B. J.; Patricelli, M. P.; Cravatt, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5044; (b) 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.
5. Kathuria, S.; Gaetani, S.; Fegley, D.; Valino, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La Rana, G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. *Nat. Med.* **2003**, *9*, 76.
6. Abouabdellah, A.; Burnier, P.; Hoornaert, C.; Jeunesse, J.; Puech, F. Patent application US20060089344, 2006.
7. Ahn, K.; Johnson, D. S.; Mileni, M.; Beidler, D.; Long, J. Z.; McKinney, M. K.; Weerapana, E.; Sadagopan, N.; Liimatta, M.; Smith, S. E.; Lazerwith, S.; Stiff, C.; Kamtekar, S.; Bhattacharya, K.; Zhang, Y.; Swaney, S.; Van Becelaere, K.; Stevens, R. C.; Cravatt, B. F. *Chem. Biol.* **2009**, *16*, 411.
8. (a) Matsumoto, T.; Kori, M.; Miyazaki, J.; Kiyota, Y. Patent application WO 2006054652, 2006.; (b) Keith, J. M.; Apodaca, R.; Xiao, W.; Seierstad, M.; Pattabiraman, K.; Wu, J.; Webb, M.; Karbarz, M. J.; Brown, S.; Wilson, S.; Scott, B.; Tham, C.-S.; Luo, L.; Palmer, J.; Wennerholm, M.; Chaplan, S.; Breitenbucher, J. G. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4838.
9. (a) Muccioli, G. G.; Fazio, N.; Scriba, G. K. E.; Poppitz, W.; Cannata, F.; Poupaert, J. H.; Wouters, J.; Lambert, D. M. *J. Med. Chem.* **2006**, *49*, 417; (b) Adams, J.; Behnke, M. L.; Castro, A. C.; Evans, C. A.; Grenier, L.; Grogan, M. J.; Liu, T.; Snyder, D. A.; Tibbits, T. T. Patent application WO2008063300, 2008.; (c) Minkkila, A.; Saario, S. M.; Kasnanen, H.; Leppanen, J.; Poso, A.; Nevalainen, T. . *J. Med. Chem.* **2008**, *51*, 7057.
10. Chong, C. R.; Sullivan, D. J. *Nature* **2007**, *448/449*, 645.
11. (a) Matthews, S. J.; McCoy, C. *Clin. Ther.* **2003**, *25*, 342; (b) Joglekar, S.; Levin, M. *Drugs Today* **2004**, *40*, 197; (c) Laffitte, E.; Revuz, J. *Exp. Opin. Drug Safety* **2004**, *3*, 4; (d) Abdel-Razeq, H. N.; Mousa, S. A. *Drugs Future* **2004**, *29*, 1059.
12. (a) Weiss, S. A.; Benwell, K.; Cliffe, I. A.; Gillespie, R. J.; Knight, A. R.; Lerpiniere, J.; Misra, A.; Pratt, R. M.; Revell, D.; Upton, R.; Dourish, C. T. *Neurology* **2003**, *61*, S101; (b) Gillespie, R. J.; Adams, D. R.; Bebbington, D.; Benwell, K.; Cliffe, I. A.; Dawson, C. E.; Dourish, C. T.; Fletcher, A.; Gaur, S.; Giles, P. R.; Jordan, A. M.; Knight, A. R.; Knutsen, L. J. S.; Lawrence, A.; Lerpiniere, J.; Misra, A.; Porter, R. H. P.; Pratt, R. M.; Shepherd, R.; Upton, R.; Ward, S. E.; Weiss, S. M.; Williamson, D. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2916; (c) Gillespie, R. J.; Bamford, S. J.; Botting, R.; Comer, M.; Denny, S.; Gaur, S.; Griffin, M.; Jordan, A. M.; Knight, A. R.; Lerpiniere, J.; Leonardi, S.; Lightowler, S.; McAteer, S.; Merrett, A.; Misra, A.; Padfield, A.; Reece, M.; Saadi, M.; Selwood, D. L.; Stratton, G. C.; Surry, D.; Todd, R.; Tong, X.; Ruston, V.; Upton, R.; Weiss, S. M. *J. Med. Chem.* **2009**, *52*, 33.
13. Ren, Y. R.; Pan, F.; Parvez, S.; Fleig, A.; Chong, C. R.; Xu, J.; Dang, Y.; Zhang, J.; Jiang, H.; Penner, R.; Liu, J. O. *PLoS One* **2008**, *3*, E4009.
14. Chong, C. R.; Xu, J.; Lu, J.; Bhat, S.; Sullivan, D. J.; Liu, J. O. *ACS Chem. Biol.* **2007**, *2*, 263.
15. Chong, C. R.; Chen, X.; Shi, L.; Liu, J. O.; Sullivan, D. J. *Nat. Chem. Biol.* **2006**, *2*, 415.
16. Zhang, H.; Qian, D. Z.; Tan, Y. S.; Lee, K.; Gao, P.; Ren, Y. R.; Rey, S.; Hammers, H.; Chang, D.; Pili, R.; Dang, C. V.; Liu, J. O.; Semenza, G. L. *Proc. Nat. Acad. Sci. U.S.A.* **2008**, *105*, 19579.
17. Swinney, D. C. *Nat. Rev. Drug Disc.* **2004**, *3*, 801.
18. (a) Copeland, R. A. In *Evaluation of Enzyme Inhibitors in Drug Discovery: A Guide for Medicinal Chemists and Pharmacologists*; Wiley: New York, 2005; (b) Copeland, R. A. In *Enzymes. A Practical Introduction to Structure, Mechanism and Data Analysis*, 2nd ed.; Wiley: New York, 2000.
19. Carbamates, ureas, amides, ketones and other compounds possessing an interesting biological profile were selected by searching the Merck Index, SciFinder and DiscoveryGate in substructure mode. Representative compounds were chosen, based upon the bias of the individual conducting the search and commercial availability of the compounds.
20. See Supplementary data for a full list of compounds evaluated in this study, together with the activity observed at the human FAAH enzyme at 1 μM.
21. (a) Wang, Y.; Ramirez, F.; Krishnamurthy, G.; Gilbert, A.; Kadakia, N.; Xu, J.; Kalgaonkar, G.; Ramarao, M. K.; Edris, W.; Rogers, K. E.; Jones, P. G. *J. Biomol. Screen.* **2006**, *11*, 519; (b) Kage, K. L.; Richardson, P. L.; Traphagen, L.; Severin, J.; Pereda-Lopez, A.; Lubben, T.; Davis-Taber, R.; Vos, M. H.; Bartley, D.; Walter, K.; Harlan, J.; Solomon, L.; Warrior, U.; Holzman, T. F.; Faltynek, C.; Surowy, C. S.; Scott, V. E. *J. Neurosci. Methods* **2007**, *161*, 47.
22. Ramarao, M. K.; Murphy, E. A.; Shen, M. W.; Wang, Y.; Bushell, K. N.; Huang, N.; Pan, N.; Williams, C.; Clarck, J. D. *Anal. Biochem.* **2005**, *343*, 143.
23. McKinney, M. K.; Cravatt, B. F. *J. Biol. Chem.* **2003**, *278*, 37393.
24. Ahn, K.; Johnson, D. S.; Fitzgerald, L. R.; Liimatta, M.; Arendse, A.; Stevenson, T.; Lund, E. T.; Nugent, R. A.; Nomanbhoy, T. K.; Alexander, J. P.; Cravatt, B. F. *Biochemistry* **2007**, *46*, 13019.
25. Boger, D. L.; Fecik, R. A.; Patterson, J. E.; Miyauchi, H.; Patricelli, M. P.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2613.
26. (a) Bracey, M. H.; Hanson, M. A.; Masuda, K. R.; Stevens, R. C.; Cravatt, B. F. *Science* **2002**, *298*, 1793; (b) Mileni, M.; Johnson, D. S.; Wang, Z.; Everdeen, D. S.; Liimatta, M.; Pabst, B.; Bhattacharya, K.; Nugent, R. A.; Kamtekar, S.; Cravatt, B. F.; Ahn, K.; Stevens, R. C. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 12820.
27. Tisher, W.; Strotman, H. *Biochim. Biophys. Acta* **1977**, *460*, 113.
28. Environmental Protection Agency (EPA) report on *Phenmedipham. Human health risk assessment*, 2005.
29. Parrott, J. A.; Compton, T. R.; Dasse, O.; Putman, D. Patent application WO2008042892, 2008.
30. (a) Johnson, D. S.; Ahn, K.; Kesten, S.; Lazerwith, S. E.; Song, Y.; Morris, M.; Fay, L.; Gregory, T.; Stiff, C.; Dunbar, J. B.; Liimatta, M.; Beidler, D.; Smith, S.; Nomanbhoy, T. K.; Cravatt, B. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2865; (b) Kabarz, M. J.; Luo, L.; Chang, L.; Tham, C.-S.; Palmer, J. A.; Wilson, S. J.; Wennerholm, M. L.; Brown, S. M.; Scott, B. P.; Apodaca, R. L.; Keith, J. M.; Wu, J.; Breitenbucher, J. G.; Chaplan, S. R.; Webb, M. *Anaesth. Analg.* **2009**, *108*, 316; (c) Ishii, T.; Sugane, T.; Maeda, J.; Narazaki, F.; Kakefuda, A.; Sato, K.; Takahashi, T.; Kanayama, T.; Saitoh, C.; Suzuki, J.; Kanai, C. Patent application WO 2006088075, 2006.
31. For representative examples of in vivo activity in CNS-mediated disorders with amperozide see: (a) Price, I. V.; Gorzalka, B. B.; White, S. J.; Arkinstall, K. H. *Neuropsychobiology* **1998**, *37*, 155; (b) Myers, R. D.; Lankford, M.; Bjoerk, A. *Pharmacol., Biochem. Behav.* **1992**, *43*, 661; (c) Gustafsson, B.; Christensson, E. *Pharmacol. Toxicol.* **1990**, *66*, 34; (d) Tung, C.-S.; Wu, W.-H.; Tseng, C.-J.; Yin, T.-H. *Eur. J. Pharmacol.* **1994**, *256*, 193; (e) Bjork, A.; Olsson, N. G.; Christensson, E.; Martinsson, K.; Olsson, O. *J. Animal Sci.* **1988**, *66*, 669.
32. Svartengren, J.; Pettersson, E.; Björk, A. *Biol. Psychiatry* **1997**, *42*, 247.
33. Arnt, J.; Skarsfelt, T. *Neuropsychopharmacology* **1998**, *18*, 63.