DOI: 10.1002/cmdc.200900472

Enol Carbamates as Inhibitors of Fatty Acid Amide Hydrolase (FAAH) Endowed with High Selectivity for FAAH over the Other Targets of the Endocannabinoid System

Sonia Gattinoni,[a] Chiara De Simone,[b, c] Sabrina Dallavalle,[a] Filomena Fezza,[b, c] Raffaella Nannei,[a] Daniele Amadio,[b, c] Patrizia Minetti,[d] Gianandrea Quattrociocchi,[d] Antonio Caprioli,[d] Franco Borsini,[d] Walter Cabri,[d] Sergio Penco,[d] Lucio Merlini,*[a] and Mauro Maccarrone*[c]

Fatty acid amide hydrolase (FAAH) is an enzyme bound to intracellular membranes, mainly of the endoplasmic reticulum,[1] which regulates the cellular level and activity of fatty acid amides.[2] These substances belong to the endogenous cannabinoids ("endocannabinoids"), a family of lipid signals that exert several biological activities, including anticancer, anti-ischemic, anti-inflammatory, antidepressant, analgesic, anxiolytic, anorectic and bone-stimulant actions, to name just a few.[3] Endocannabinoids include the neurotransmitter *N*-arachidonoylethanolamine (anandamide, AEA),[4] the anti-inflammatory factor *N*-palmitoylethanolamine,[5] the sleep-inducing compound oleoylamide,[6] and the satiating signal *N*-oleoylethanolamine.[7] Some of the effects of endocannabinoids are mediated via the type-1 (CB1R) or type-2 (CB2R) cannabinoid receptors, while others may involve additional targets like the transient receptor potential vanilloid-1 (TRPV1)[3] and peroxisome proliferator-activated receptors (PPARs).[8]

Among different metabolic pathways that can modulate the endogenous levels of endocannabinoids, and hence their biological actions,[9] FAAH has emerged as a key player. In fact, FAAH inactivation by either genetic ablation of the *faah* gene[10] or chemical inhibition[11] increases the level of fatty acid amides, and consequently their central and peripheral activities. However, unlike direct agonists of CB1R, FAAH inhibitors do not cause classical effects triggered by this receptor (e.g., catalepsy, hypothermia, and hyperphagia), nor are they accompanied by undesirable side effects typically due to CB1R activation, like impairment of cognition and motor control. Therefore, chemical inhibition of FAAH is considered a useful therapeutic approach for the treatment of cancer, eating disorders, inflammation, pain, anxiety, depression, sleep disturbances, and other central nervous system (CNS) disorders.[12, 13]

Of course, it is also important that the beneficial effects of increasing the endogenous level of AEA through FAAH inhibition are not offset by unwanted modulation of other biological targets that recognize this lipid, such as the cannabinoid and vanilloid receptors, or the proteins that synthesize *N*-acylphosphatidylethanolamide-phospholipase D (NAPE-PLD) and allegedly transport it (anandamide membrane transporter, AMT). These proteins form the endocannabinoid system (ECS), which includes other well-characterized elements, such as the enzymes responsible for synthesis (diacylglycerol lipase, DAGL) or degradation (monoacylglycerol lipase, MAGL) of 2-arachidonoylglycerol.[14] It is also noteworthy that AEA can have contrary biological effects when acting at different receptors (e.g., CBRs vs TRPV1), and a different activity compared to that of 2-arachidonoylglycerol.[15] Therefore, it is not surprising that interaction of a drug, targeted towards a particular ECS enzyme, with other ECS components could lead to reduced therapeutic efficacy, for example in the treatment of cancer,[14] or of neurodegenerative/inflammatory diseases.[15] For instance, the therapeutic advantage of a popular AMT inhibitor AM404 has been reconsidered when it was shown to also activate TRPV1 receptors.[16] Similarly, the TRPV1 agonist olvanil has been shown to inhibit AMT,[17] and the putative AMT blocker LY2183240 is known to also inhibit FAAH and MAGL.[18] Overall, the promiscuity of such drugs, intended to target a single ECS component but able to interact with others, has raised concerns about their true efficacy as therapeutics. On this basis, a careful evaluation of the selectivity of inhibitors for FAAH over other targets of the ECS seems a prerequisite for effective drug development.

FAAH belongs to an unusual class of serine hydrolases that utilizes a serine–serine–lysine catalytic triad,[2] and therefore potent electrophiles, such as fluorophosphonates and fluorosulfonates, are important tools for evaluating the biochemistry of FAAH.[19] Starting from activated ketones designed as putative active-site traps, and including chloroketones, α-ketoamides, α-ketoesters, trifluoromethylketones and diazoketones,[12] past development of FAAH inhibitors has led to the recent discovery of several classes of α-ketoheterocycles as potent and highly selective blockers of FAAH, compared to other serine hydrolases.[13, 20] However, the systematic study of specificity towards FAAH over all the other well-defined ECS elements has been investigated only rarely (e.g., in the case of

[a] *Dr. S. Gattinoni,[+] Dr. S. Dallavalle, Dr. R. Nannei, Prof. L. Merlini*
DISMA, Università degli Studi di Milano, Via Celoria 2, 20133 Milano (Italy)
Fax: (+39) 02-50316801
E-mail: lucio.merlini@unimi.it

[b] *Dr. C. De Simone,[+] Dr. F. Fezza, Dr. D. Amadio*
Dipartimento di Medicina Sperimentale e Scienze Biochimiche
Università degli Studi di Roma "Tor Vergata", 00133 Rome (Italy)

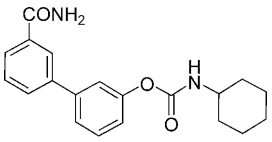
[c] *Dr. C. De Simone,[+] Dr. F. Fezza, Dr. D. Amadio, Prof. Dr. M. Maccarrone*
Dipartimento di Scienze Biomediche, Università degli Studi di Teramo
Piazza A. Moro 45, 64100 Teramo, Italy, & Centro Europeo di Ricerca sul Cervello (CERC)/IRCCS Fondazione Santa Lucia, 00143 Rome (Italy)
Fax: (+39) 0861-266877
E-mail: mmaccarrone@unite.it

[d] *Dr. P. Minetti, Dr. G. Quattrociocchi, Dr. A. Caprioli, Dr. F. Borsini, Dr. W. Cabri, Dr. S. Penco*
Sigma-Tau R&D, 00040 Pomezia (Italy)

[+] *These authors contributed equally to the study.*

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200900472.

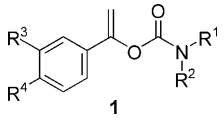
OL-135 only with respect to CB1R, CB2R and TRPV1).[21] Another class of FAAH inhibitors currently under investigation are the carbamate-based compounds.[12] Modification of the initial hit compounds and molecular modeling based on the X-ray co-crystal structure of methoxy-arachidonoyl-fluorophosphonate (MAFP) bound to FAAH[22] led Kathuria and co-workers to develop URB597, an *O*-aryl carbamate endowed with potent FAAH inhibition, with analgesic and anti-inflammatory activity.[23] Mass spectral studies indicated that the structure–activity relationships (SAR) within the carbamate-based series might be driven by the ability of the phenol group to leave the molecule.[24]



URB597

Other carbamates have been reported by Sanofi–Aventis[25–29] and Bristol–Myers Squibb.[30,31] More recently, aryl ureas were found to be remarkably selective FAAH inhibitors.[32–34] In this context, it should be stressed that both carbamates and aryl ureas covalently bind to FAAH, and generally act as irreversible inhibitors. However, it has been suggested that reversible inhibitors might offer advantages over irreversible blockers as lead compounds for drug design.[35] However, only a few reversible FAAH inhibitors have been described in the literature, like the α-ketoheterocycle OL-135,[36] and the covalent but slowly reversible piperazine urea JNJ-1661010 developed by Johnson & Johnson.[33]

Herein, we report the synthesis of a new series of enol carbamates (**1**), the most potent of which appear to reversibly inhibit FAAH. These novel compounds were designed based on the hypothesis that FAAH would catalyze the hydrolysis of the carbamate group to release an enol, which would immediately convert to the tautomeric keto form, thus shifting the equilibrium towards the products of hydrolysis. To the best of our knowledge, there are no other examples of use of this class of compounds in medicinal chemistry.

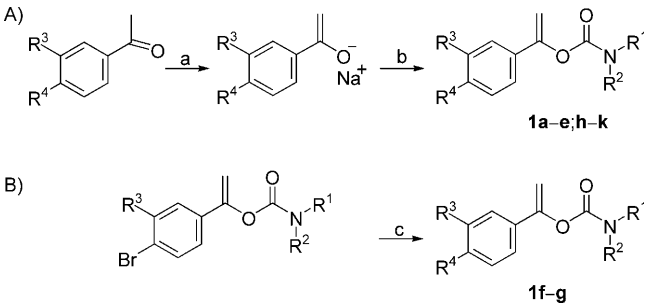


1

Compounds **1** were prepared by reacting the appropriate ketone with a strong base, such as NaH, usually in dimethylsulfoxide, followed by treatment with the corresponding carbamoyl chloride (route A, Scheme 1),[38] or by reaction of a bromophenylvinylcarbamate with an arylboronic acid via a Suzuki–Miyaura reaction (route B, Scheme 1).

Compounds **1** were evaluated for their ability to inhibit FAAH. The IC_{50} values were calculated as detailed in the Supporting Information, and are summarized in Table 1. In addition, we investigated the effect of the selected FAAH inhibitors towards other components of the ECS; the assays were carried out as previously described,[37] (for details see Supporting Information).

Two compounds (**1b**, **1j**) were found to be potent inhibitors of FAAH, with IC_{50} values in the low nanomolar range (Table 1). Therefore, these inhibitors were selected for further evaluation along with the less potent FAAH inhibitors **1h** and **1i**, selected because of their structural similarities with compound **1b**; we wished to ascertain whether changes in the position of the substituents could affect the mechanism-of-action or the selectivity of the compound. The position of the phenyl group with respect to the enol carbamate group differs in compounds **1b** (*para*, R^4) and **1i** (*meta*, R^3), while the substitution of the carbamate nitrogen differs in compounds **1h** (dimethyl) and **1i** (cyclic amine structure). Initial SAR showed that dimethyl substitution of the carbamate nitrogen (compounds **1a** and **1h**) leads to weaker activity than analogues with a bulkier (e.g., piperidine **1b**,**e**–**g, i, j**) but not aromatic (**1k**) cyclic amines. In addition, the presence of substituents on the distal phenyl ring can modulate the activity (**1j** vs **1i** and **1f**,**g** vs **1b**).

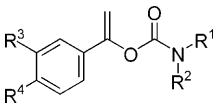


1a–**e**;**h**–**k**

1f–**g**

Scheme 1. Synthesis of compounds **1**. *Reagents and conditions*: a) NaH, DMSO, RT, 1 h, 18–57%; b) ClCON(R^2 R^1), DMSO, RT, 3 h, 18–57%; c) $R^4B(OH)_2$, $NaHCO_3$, Pd(tetrakis), MeOH, reflux, 7 h, Ar, or MW, 140 °C, 30 min (15–66%).

Table 1. Inhibition of FAAH by compounds **1**.



Compd	R^1	R^2	R^3	R^4	IC_{50} [nM]
1a	CH_3	CH_3	H	Ph	3930 ± 500
1b	$(CH_2)_5$		H	Ph	9 ± 1
1c	$(CH_2)_2O(CH_2)_2$		H	Ph	292 ± 30
1d	CH_3	Ph	H	Ph	4600 ± 920
1e	$(CH_2)_5$		H	2-thienyl	40 ± 5
1f	$(CH_2)_5$		H	3′-NH_2COPh	116 ± 12
1g	$(CH_2)_5$		H	2′-NH_2COPh	167 ± 17
1h	CH_3	CH_3	Ph	H	1743 ± 180
1i	$(CH_2)_5$		Ph	H	133 ± 14
1j	$(CH_2)_5$		3′-CNPh	H	13 ± 3
1k	CH_3	Ph	Ph	H	340 ± 20

Compounds **1b, 1h, 1i, 1j** were found to inhibit FAAH reversibly. At least 80% of enzyme activity was recovered after dialysis of the enzyme–inhibitor complexes for 18 h (see Supporting Information figure S1). In contrast, dialysis of FAAH–URB597 complexes under similar conditions has shown that this agent is an irreversible inhibitor of the enzyme,[39] due to carbamoylation of Ser241 in the active site.[40] Furthermore, nonlinear regression analysis of the Michaelis–Menten curves demonstrated that all of the selected compounds were non-competitive inhibitors of FAAH with respect to the natural substrate AEA (see Supporting Information figure S2A). In fact, compounds **1** markedly reduced the maximal velocity (V_{max}) without affecting the Michaelis–Menten constant (K_m), a typical effect of canonical noncompetitive inhibitors.[40] The same phe-

nomenon can also be visualized by Lineweaver–Burk analysis of double-reciprocal plots of the kinetic data (see Supporting Information figure S2 B).

Noncompetitive inhibition seems in keeping with the differences in structures between compounds **1** and AEA. In fact, it should be recalled that by definition, a noncompetitive inhibitor binds reversibly and equally well to the enzyme alone and to the enzyme–substrate complex, and therefore it does not affect (nor is affected by) the binding of the substrate at the active site.[41] On the other hand, compounds **1 b, 1 h, 1 i, 1 j** might lead to carbonylation of Ser 241, that is the alternative mode of FAAH inhibition by carbamate blockers,[40] thus forming hydrolysable (and hence reversible) intermediates. The actual mechanism by which our compounds operate can be ascertained using mass spectrometry as described by Cravatt et al.,[40] an experiment that is planned for the future.

Another unanswered question is the binding mode of our compounds to FAAH. Their structural similarity with URB597 would suggest the occupation of the cytosolic access (CA) channel by the biphenylyl moiety, with the N-terminal moiety positioned in the acyl-chain-binding (ACB) channel of the enzyme.[42] In the absence of a crystal structure, further SAR data and molecular modeling studies are needed to support this binding mode or suggest alternative. In addition, it has recently been demonstrated that compound JNJ-1661010 inhibits FAAH by carbamoylation followed by hydrolysis, thus acting as a covalent but slowly reversible blocker.[33] Therefore, the possibility that compounds **1 b, 1 h, 1 i, 1 j** also inhibit FAAH through reversible carbamoylation can not be ruled out. On a general note, it seems noteworthy that the ability of carbamate compounds to inhibit FAAH in a noncompetitive manner is not unprecedented,[43] and that clinically used carbamate drugs (i.e., rivastigmine) are known to act through a covalent carbamoylation of acetylcholinesterase,[44] that leads to a reversible and noncompetitive inhibition.[45,46] In any case, compounds **1 b, 1 h, 1 i, 1 j** appear to be the first reversible enol carbamate inhibitors of FAAH described to date.

To further support this conclusion, we performed time-dependent inhibition experiments. The most potent compounds (**1 b** and **1 j**) were preincubated with FAAH for different time periods and the substrate turnover was measured.[47] The results show that inhibition by either compound was not time-dependent (Supporting Information table S1), confirming that **1 b** and **1 j** are indeed reversible inhibitors of FAAH. URB597 was also tested under the same experimental conditions and shown to inhibit FAAH in a time-dependent manner, which is in accordance with a previous report.[47] This result corroborates the hypothesis that URB597 acts as a covalent and irreversible inhibitor of the enzyme.[39,40]

The apparent K_i values of compounds **1 b, 1 h, 1 i, 1 j** towards FAAH are shown in Table 2. In a second set of experiments, we analyzed the interaction of compounds **1 b, 1 h, 1 i, 1 j** with the other targets of the ECS, in order to calculate selectivity index values compared with FAAH (see Table 2). From these results it can be concluded that compounds **1 b** and **1 j**, in addition to yielding potent FAAH inhibition, also show a high degree of selectivity towards FAAH over any other ECS target, with IC_{50} values ~1000-fold higher than those shown towards FAAH. Incidentally, it should be stressed that the ability of compounds **1 b** and **1 j** to inhibit the AEA membrane transporter AMT (Table 2) could simply reflect the contribution of FAAH to the transport process. In fact, hydrolysis by FAAH drives AEA uptake by creating and maintaining a concentration gradient across the plasma membrane.[2,13,18]

Table 2. Apparent K_i values towards FAAH, and inhibition (IC_{50}) of the activity of the ECS elements by selected compounds **1**. Selectivity index values are reported in square brackets.

Compd	K_i [nM]	IC_{50} [nM]						
		CB1R	CB2R	TRPV1	AMT	NAPE-PLD	MAGL	DAGL
1 b	54 ± 4	> 10000 [1111]	> 10000 [1111]	> 10000 [1111]	100 [111]	> 10000 [1111]	> 10000 [1111]	> 10000 [1111]
1 h	2259 ± 356	> 10000 [6]	> 10000 [6]	> 10000 [6]	> 10000 [6]	> 10000 [6]	> 10000 [6]	> 10000 [6]
1 i	274 ± 93	> 10000 [75]	10000 [75]	> 10000 [75]	10000 [75]	> 10000 [75]	> 10000 [75]	> 10000 [75]
1 j	46 ± 6	> 10000 [769]	> 1000 [769]	> 10000 [769]	100 [77]	> 10000 [769]	> 10000 [769]	> 10000 [769]

Details of the methods used to perform dialysis, to determine the type of inhibition, and to calculate the K_i values and selectivity index values are reported in the Supporting Information.

Overall, compound **1 b** showed the best combination of inhibitory potency towards FAAH and selectivity for the target over the other ECS components. Therefore, further experiments were performed to confirm the stability of **1 b** at 37 °C at pH 1.5 and pH 8.4, mimicking the stomach and intestine environments, respectively (Supporting Information table S2).[48] Moreover, compound **1 b** was not at all hydrolyzed by esterases from mouse or rat plasma after 1 h of incubation at 37 °C, and was only slightly (~20%) degraded by esterases from human plasma (Supporting Information table 2). In addition, compound **1 b** was subjected to in vitro pharmacology experiments in order to ascertain its possible interference with different off-targets (i.e., 45 receptors, 5 ion channels and 2 neurotransmitter transporters). The data, shown in the Supporting Information (table S3), demonstrate that compound **1 b** had no effect on any of the off-targets tested, yielding IC_{50} values > 10000 nM in all cases. Finally, oral administration of compound **1 b** to mice (at 30 mg kg^{-1}) produced anxiolytic-like effects in the elevated plus maze model, where an increase in time spent in open arms was observed, in a same manner as the reference compound, diazepam (Supporting Information, table S2). These in vivo data support the pharmacological efficacy of enol carbamates as promising anxiolytic therapeutics.[49,50]

In conclusion, a new class of enol carbamates have been identified as potent reversible inhibitors of FAAH endowed with high selectivity towards FAAH over the other targets of the endocannabinoid system. Notably, our systematic investigation into this selectivity opens the avenue to their further evaluation in clinical trials for the treatment of FAAH-related human pathologies.[2, 13, 15, 27, 29–31, 46]

Experimental Section

Representative and general experimental procedures, compound characterization data (^{1}H and ^{13}C NMR, HRMS, elemental analysis), and details of the biochemical assays and the associated data are available in the Supporting Information.

Male CD1 mice were used for all the experiments. Animals were housed, four per cage, on a 12 h light/dark cycle with lights on at 06:00. All efforts were made to minimize animal suffering and to reduce the number of mice used, in accordance with the European Communities Council Directive of 24 November, 198 (86/609/EEC).

Keywords: endocannabinoids · enol carbamates · FAAH · hydrolases · inhibitors

[1] F. Fezza, C. De Simone, D. Amadio, M. Maccarrone, *Subcell. Biochem.* **2008**, *49*, 101 – 132.
[2] M. K. McKinney, B. F. Cravatt, *Annu. Rev. Biochem.* **2005**, *74*, 411 – 432.
[3] V. Di Marzo, *Nat. Rev. Drug Discovery* **2008**, *7*, 438 – 455.
[4] K. Ahn, M. K. McKinney, B. F. Cravatt, *Chem. Rev.* **2008**, *108*, 1687 – 1707.
[5] W. A. Devane, L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, *Science* **1992**, *258*, 1946 – 1949.
[6] D. M. Lambert, S. Vandevoorde, K. O. Jonsson, C. J. Fowler, *Curr. Med. Chem.* **2002**, *9*, 663 – 674.
[7] B. F. Cravatt, O. Prospero-Garcia, G. Siuzdak, N. B. Gilula, S. J. Henriksen, D. L. Boger, R. A. Lerner, *Science* **1995**, *268*, 1506 – 1509.
[8] J. Fu, S. Gaetani, F. Oveisi, J. Lo Verme, A. Serrano, F. Rodríguez De Fonseca, A. Rosengarth, H. Luecke, B. Di Giacomo, G. Tarzia, D. Piomelli, *Nature* **2003**, *425*, 90 – 93.
[9] Y. Okamoto, K. Tsuboi, N. Ueda, *Vitam. Horm.* **2009**, *81*, 1 – 24.
[10] F. Rodríguez de Fonseca, M. Navarro, R. Gómez, L. Escuredo, F. Nava, J. Fu, E. Murillo-Rodríguez, A. Giuffrida, J. LoVerme, S. Gaetani, S. Kathuria, C. Gall, D. Piomelli, *Nature* **2001**, *414*, 209 – 212.
[11] B. F. Cravatt, K. Demarest, M. P. Patricelli, M. H. Bracey, D. K. Giang, B. R. Martin, A. H. Lichtman, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9371 – 9376.
[12] M. Seierstad, J. G. Breitenbucher, *J. Med. Chem.* **2008**, *51*, 7327 – 7343.
[13] K. Ahn, D. S. Johnson, B. F. Cravatt, *Expert Opin. Drug Discovery* **2009**, *4*, 763 – 784.
[14] M. Maccarrone in *Endocannabinoids: The Brain and Body's Marijuana and Beyond*, (Ed.: E. S Onaivi), CRC Press, Boca Raton, **2006**, pp. 451 – 466.
[15] D. Centonze, A. Finazzi-Agrò, G. Bernardi, M. Maccarrone, *Trends Pharmacol. Sci.* **2007**, *28*, 180 – 187.
[16] P. M. Zygmunt, H. Chuang, P. Movahed, D. Julius, E. D. Högestätt, *Eur. J. Pharmacol.* **2000**, *396*, 39 – 42.
[17] M. Beltramo, D. Piomelli, *Eur. J. Pharmacol.* **1999**, *364*, 75 – 78.
[18] J. P. Alexander, B. F. Cravatt, *J. Am. Chem. Soc.* **2006**, *128*, 9699 – 9704.
[19] D. M. Lambert, C. J. Fowler, *J. Med. Chem.* **2005**, *48*, 5059 – 5087.
[20] J. K. DeMartino, J. Garfunkle, D. G. Hochstatter, B. F. Cravatt, D. L. Boger, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5842 – 5846 and refs. quoted therein.
[21] V. Di Marzo, G. Griffin, L. De Petrocellis, I. Brandi, T. Bisogno, W. Williams, M. C. Grier, S. Kulasegram, A. Mahadevan, R. K. Razdan, B. R. Martin, *J. Pharmacol. Exp. Ther.* **2002**, *300*, 984 – 991.
[22] M. H. Bracey, M. A. Hanson, K. R. Masuda, R. C. Stevens, B. F. Cravatt, *Science* **2002**, *298*, 1793 – 1796.
[23] S. Kathuria, S. Gaetani, D. Fegley, F. Valiño, A. Duranti, A. Tontini, M. Mor, G. Tarzia, G. La Rana, A. Calignano, A. Giustino, M. Tattoli, M. Palmery, V. Cuomo, D. Piomelli, *Nat. Med.* **2003**, *9*, 76 – 81.
[24] E. Basso, A. Duranti, M. Mor, D. Piomelli, A. Tontini, G. Tarzia, P. Traldi, *J. Mass Spectrom.* **2004**, *39*, 1450 – 1455.
[25] A. Abouabdellah, R. Bartsch-Li, C. Hoornaert, A. Ravet, (Sanofi–Aventis, Paris, France), WO/2005/090292, **2005**.
[26] A. Abouabdellah, A. Almario Garcia, C. Hoornaert, A. T. Li, (Sanofi–Aventis, Paris, France), WO/2005/090322, **2005**.
[27] A. Abouabdellah, A. Almario Garcia, C. Hoornaert, P. Lardenois, F. Marguet, (Sanofi–Aventis, Paris, France), WO/2005/090347, **2005**.
[28] A. Abouabdellah, A. Almario Garcia, J. Froissant, C. Hoornaert, (Sanofi–Aventis, Paris, France), WO/2005/077898, **2005**.
[29] A. Abouabdellah, M. Bas, G. Dargazanli, C. Hoornaert, A. T. Li, F. Medaisko, (Sanofi–Aventis, Paris, France), WO/2004/020430, **2004**.
[30] S.-Y. Sit, K. Xie, (Bristol–Meyers Squibb, Princeton, NJ, USA), WO/2002/087569, **2002**.
[31] S.-Y. Sit, K. Xie, H. Deng, (Bristol–Meyers Squibb, Princeton, NJ, USA), US/6949574, **2005**.
[32] K. Ahn, D. S. Johnson, L. R. Fitzgerald, M. Liimatta, A. Arendse, T. Stevenson, E. T. Lund, R. A. Nugent. T. K. Nomanbhoy, J. P. Alexander, B. F. Cravatt, *Biochemistry* **2007**, *46*, 13019 – 13030.
[33] J. M. Keith, R. Apodaca, W. Xiao, M. Seierstad, K. Pattabiraman, J. Wu, M. Webb, M. J. Karbarz, S. Brown, S. Wilson, B. Scott, C.-S. Tham, L. Luo, J. Palmer, J.;M. Wennerholm, S. Chaplan, J. G. Breitenbucher, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4838 – 4843.
[34] K. Ahn, D. S. Johnson, M. Mileni, D. Beidler, D. J. Long, M. K. McKinney, E. Weerapana, N. Sadagopan, M. Liimatta, S. E. Smith, S. Lazerwith, C. Stiff, S. Kamtekar, K. Bhattacharya, Y. Zhang, S. Swaney, K. Van Becelaere, R. C. Stevens, B. F. Cravatt, *Chem. Biol.* **2009**, *16*, 411 – 420.
[35] D. Leung, C. Hardouin, D. L. Boger, B. F. Cravatt, *Nat. Biotechnol.* **2003**, *21*, 687 – 691.
[36] A. H. Lichtman, D. Leung, C. C. Shelton, A. Saghatelian, C. Hardouin, D. L. Boger, B. F. Cravatt, *J. Pharmacol. Exp. Ther.* **2004**, *311*, 441 – 448.
[37] M. Maccarrone, S. Rossi, M. Bari, V. De Chiara, F. Fezza, A. Musella, V. Gasperi, C. Prosperetti, G. Bernardi, A. Finazzi-Agrò, B. F. Cravatt, D. Centonze, *Nat. Neurosci.* **2008**, *11*, 152 – 159.
[38] L. Panella, B. L. Feringa, J. G. de Vries, A. J. Minnaard, *Org. Lett.* **2005**, *7*, 4177 – 4180.
[39] M. J. Karbarz, L. Luo, L. Chang, C. S. Tham, J. A. Palmer, S. J. Wilson, M. L. Wennerholm, S. M. Brown, B. P. Scott, R. L. Apodaca, J. M. Keith, J. Wu, J. G. Breitenbucher, S. R. Chaplan, M. Webb, *Anesth. Analg.* **2009**, *108*, 316 – 329.
[40] J. P. Alexander, B. F. Cravatt, *Chem. Biol.* **2005**, *12*, 1179 – 1187.
[41] A. Fersht, *Enzyme structure and mechanism*, W. H. Freeman & Company, New York, **1977**.
[42] J. R. Clapper, F. Vacondio, A. R. King, A. Duranti, A. Tontini, C. Silva, S. Sanchini, G. Tarzia, M. Mor, D. Piomelli, *ChemMedChem* **2009**, *4*, 1505 – 1513.
[43] G. Tarzia, A. Duranti, A. Tontini, G. Piersanti, M. Mor, S. Rivara, P. V. Plazzi, C. Park, S. Kathuria, D. Piomelli, *J. Med. Chem.* **2003**, *46*, 2352 – 2360.
[44] M. H. Potashman, M. E. Duggan, *J. Med. Chem.* **2009**, *52*, 1231 – 1246.
[45] A. Enz, H. Boddeke, J. Gray, R. Spiegel, *Ann. N. Y. Acad. Sci.* **1991**, *640*, 272 – 275.
[46] B. Ibach, E. Haen, *Curr. Pharm. Des.* **2004**, *10*, 231 – 251.
[47] X. Wang, K. Sarris, K. Kage, D. Zhang, S. P. Brown, T. Kolasa, C. Surowy, O. F. El Kouhen, S. W. Muchmore, J. D. Brioni, A. O. Stewart, *J. Med. Chem.* **2009**, *52*, 170 – 180.
[48] L. Di, E. H. Kerns, G. T. Carter, *Curr. Pharm. Des.* **2009**, *15*, 2184 – 2194.
[49] S. Patel, C. J. Hillard, *J. Pharmacol. Exp. Ther.* **2006**, *318*, 304 – 311.
[50] F. A. Moreira, N. Kaiser, K. Monory, B. Lutz, *Neuropharmacology* **2008**, *54*, 141 – 150.

Received: November 17, 2009
Revised: January 12, 2010
Published online on January 28, 2010

