Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5959–5963
Bioorganic & Medicinal Chemistry Letters

Conformationally constrained analogues of 2-arachidonoylglycerol

Subramanian K. Vadivel, Sundararaman Vardarajan, Richard I. Duclos, Jr., JodiAnne T. Wood, Jianxin Guo and Alexandros Makriyannis*

Center for Drug Discovery, Northeastern University, 116 Mugar Hall, 360 Huntington Avenue, Boston, MA 02115, USA

Received 15 June 2007; revised 18 July 2007; accepted 19 July 2007
Available online 21 August 2007

Abstract—Novel monocyclic analogues of 2-arachidonoylglycerol (2-AG) were designed in order to explore the pharmacophoric conformations of this endocannabinoid ligand at the key cannabinergic proteins. All 2-arachidonoyl esters of 1,2,3-cyclohexanetriol [*meso*-**7** (AM5504), (±)-**8** (AM5503), and *meso*-**9** (AM5505)] were synthesized by regioselective acylation of 2,3-dihydroxycyclohexanone followed by selective reductions. The optically active isomers (+)-**8** (AM4434) and (−)-**8** (AM4435) were synthesized from (2*S*,3*S*)- and (2*R*,3*R*)-2,3-dihydroxycyclohexanone, respectively, via a chemoenzymatic route. These head group constrained and conformationally restricted analogues of 2-AG as well as the 1-keto precursors were evaluated as substrates for the endocannabinoid deactivating hydrolytic enzymes monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH), and also were tested for their affinities for CB1 and CB2 cannabinoid receptors. The observed biochemical differences between these ligands can help define the conformational requirements for 2-AG activity at each of the above endocannabinoid protein targets.

2-Arachidonoylglycerol (2-AG, **1**) (Fig. 1) is a monoacylglycerol identified as an endogenous ligand which binds to both CB1 and CB2 cannabinoid receptors.[1,2] The other key endocannabinoid is *N*-arachidonoylethanolamine (AEA, **2**), although it has been postulated that 2-AG is the primary endocannabinoid agonist ligand for CB1[3,4] as well as CB2[5] receptors. For example, 2-AG binds to the CB1 cannabinoid receptors on presynaptic axons during the duration of its existence in the extracellular space and functions as a retrograde synaptic neurotransmitter,[6,7] where it elicits a variety of cannabinergic effects in vitro and in vivo.[8] 2-AG is primarily inactivated by an efficient transporter system-mediated cellular uptake followed by intracellular enzymatic hydrolysis to arachidonic acid and glycerol by monoacylglycerol lipase (MGL).[9–12] In addition to the hydrolysis of this metabolically labile molecule by MGL, hydrolysis by fatty acid amide hydrolase (FAAH),[10,12] phosphorylation,[13] as well as metabolism by lipoxogenases (LOX)[14] and cyclooxygenase 2 (COX 2)[15] also occur, although the biological relevance of these other mechanisms for 2-AG deactivation has not yet been fully established. Thus, 2-AG interacts not only with the CB receptors, but with a transporter system, intracellular MGL and FAAH, as well as other enzymes. We postulated that the conformations of 2-AG required for interactions with each of these targets may be different and that probing its bioactive conformation in each target could lead to information useful in the design of selective inhibitors which have the potential to be therapeutic drugs.[16–18]

Our approach for the design of conformationally defined 2-AG analogues involved constraining the conformation of the glycerol moiety by incorporation of its key pharmacophoric features into a six-membered carbocyclic ring system. This series of analogues includes all the 2-arachidonoyl esters of 1,2,3-cyclohexanetriol as well as the corresponding keto analogues. These ligands were synthesized and assayed for their affinities for the two known cannabinoid receptors and evaluated as substrates for MGL and FAAH, as we have been particularly interested in identifying the structural features of the 2-AG molecule which can discriminate between these two endocannabinoid deactivating enzymes.

As shown in Scheme 1, 2,3-dihydroxycyclohexanone (**4**) was an intermediate in the synthesis of all possible isomeric 2-AG analogues. Commercially available cyclohexenone (**3**) was treated with osmium tetroxide and *N*-methylmorpholine *N*-oxide (NMO) to give the

Keywords: 2-Arachidonoylglycerol, 2-AG; Monoacylglycerol lipase, MGL; Fatty acid amide hydrolase, FAAH; Cannabinoid, Endocannabinoid.
* Corresponding author. Tel.: +1 617 373 4200; fax: +1 617 373 7493; e-mail: a.makriyannis@neu.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.07.064

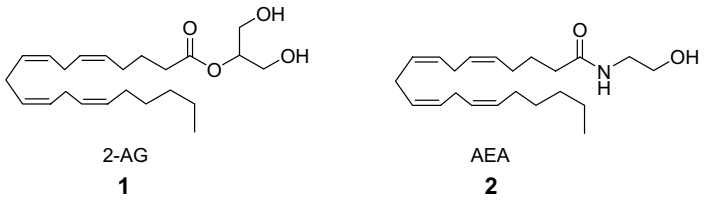
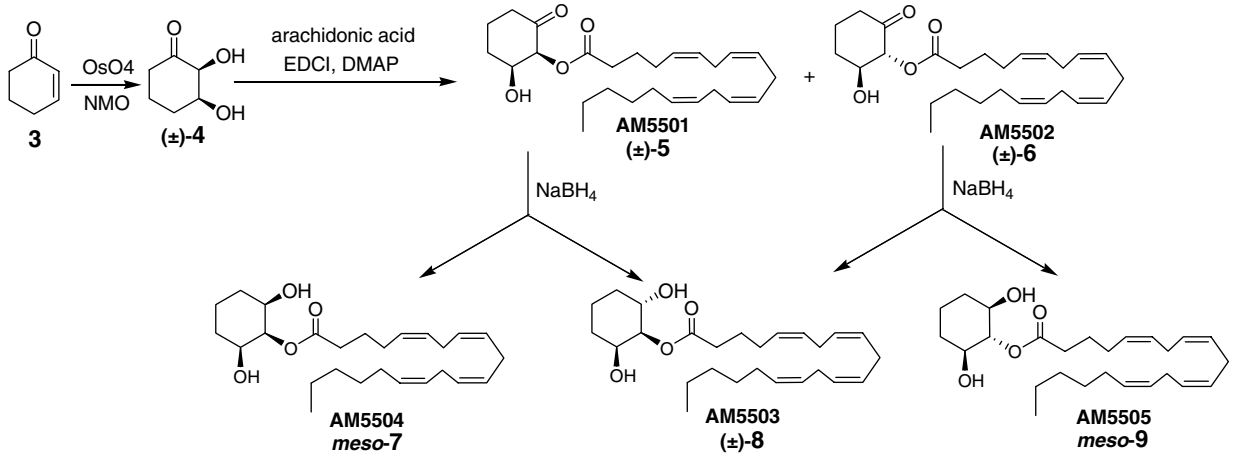


Figure 1. Endocannabinoids 2-arachidonoylglycerol (2-AG, **1**) and *N*-arachidonoylethanolamine (AEA, **2**).

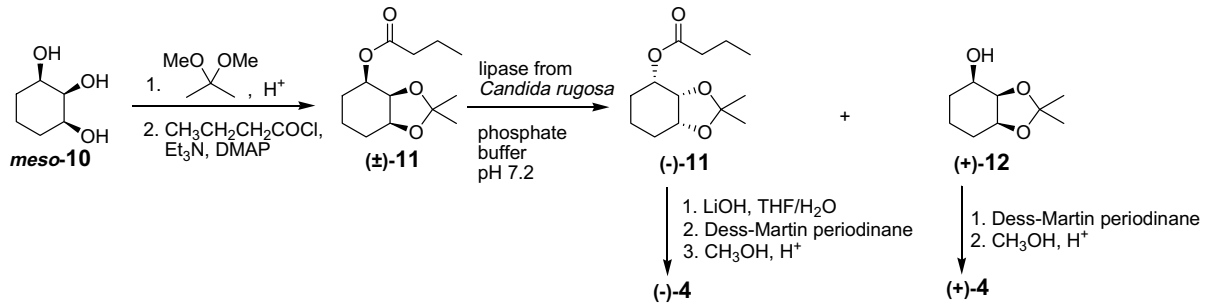


Scheme 1. Syntheses of 1,2,3-cyclohexanetriol ester analogues of 2-AG.

racemic ketodiol (±)-**4**, which was acylated at the more acidic α-hydroxyl group using arachidonic acid in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP) at −20 °C to give (±)-**5** (AM5501) and a minor amount of the epimerized byproduct (±)-**6** (AM5502). Both (±)-**5** and (±)-**6** were reduced with sodium borohydride in methanol at 0 °C to give the corresponding 2-AG analogues *meso*-**7** (AM5504), (±)-**8** (AM5503), and *meso*-**9** (AM5505). These 2-arachidonoyl esters of 1,2,3-cyclohexanetriol were free of acyl migration byproducts usually observed for 2-acyl glycerols such as 2-AG[4,19–21] and have been characterized.[22]

Racemic compound (±)-**8** (AM5503) was not resolvable by chiral HPLC (CHIRALPAK AD). Therefore, enzymatic resolution of the chiral ketodiol (±)-**4** was carried out (Scheme 2) to give the required crucial intermediates (−)-**4** and (+)-**4** in optically active forms. The synthetic intermediates (−)-**11** and (+)-**12** were previously reported from an enzymatic method which utilized lipase from *Pseudomonas* sp. (SAM-II, Amano).[23] We utilized a different lipase from *Candida rugosa* (lipase L1754, Sigma) to selectively hydrolyze one enantiomer of ester (±)-**11** and give the corresponding alcohol (+)-**12** which was readily chromatographically separable from the unreactive enantiomeric ester (−)-**11**. Both ester (−)-**11** ($[\alpha]_D$ −48.1° (*c* 1.00, CH_3OH); Lit.[23] $[\alpha]_D$ −41.2° (*c* 0.7, $CHCl_3$)) and alcohol (+)-**12** ($[\alpha]_D$ +10.8° (*c* 1.00, CH_3OH); Lit.[23] $[\alpha]_D$ +12.3° (*c* 0.6, $CHCl_3$)) were obtained in good yields and high optical purities. Ketodiol (−)-(2*R*,3*R*)-**4** was then prepared from (−)-**11** and converted to (−)-**8** (AM4435, $[\alpha]_D$ −16.4° (*c* 1.2, $CHCl_3$)), while ketodiol (+)-(2*S*,3*S*)-**4** was prepared from (+)-**12** and converted to (+)-**8** (AM4434, $[\alpha]_D$ +17.0° (*c* 0.9, $CHCl_3$)).

Compounds were tested for their affinities for the CB1 and CB2 receptors using membrane preparations from rat brain or mouse spleen, respectively, as previously de-



Scheme 2. Chemoenzymatic syntheses of optically active starting 2,3-dihydroxycyclohexanones (−)-**4** and (+)-**4**.

Table 1. Substrate hydrolysis by FAAH and MGL enzymes with standard deviations

Compound	Name	Substrate assay (% hydrolysis)	
		FAAH	MGL
1	2-AG	100 ± 0	74 ± 4
(±)-**5**	AM5501	91 ± 1	48 ± 3
(±)-**6**	AM5502	100 ± 0	8 ± 3
meso-**7**	AM5504	2 ± 3	2 ± 2
(±)-**8**	AM5503	100 ± 0	1 ± 1
(+)-**8**	AM4434	88 ± 2	10 ± 3
(−)-**8**	AM4435	88 ± 3	13 ± 2
meso-**9**	AM5505	78 ± 6	19 ± 4

scribed[24–27] via competition-equilibrium binding with [^{3}H]CP55940 as the radioligand. The results were analyzed using nonlinear regression to determine the actual IC_{50} of the ligand (Prizm by GraphPad Software, Inc.) and the K_i values were calculated from the IC_{50}.[28] All data were in duplicate with IC_{50} and K_i values determined from single experiments. This series of rigid 2-AG analogues had affinities for the CB receptors that were comparable to the endogenous cannabinoid 2-AG (K_i CB1 472,[1] 2400,[2] 100,[20] 538[29]; K_i CB2 1400,[1] 100,[20] 1100[30]; review[8]) even in the presence of the bulky –$(CH_2)_3$– methylenes which were used to constrain the glycerol headgroup portion of the ligands. For example, the K_i's for (+)-**8** (AM4434, CB1 970 nM, CB2 370 nM) and (−)-**8** (AM4435, CB1 4200 nM, CB2 1200 nM) were determined, though some ester hydrolysis did occur during the course of the binding assays, as evidenced by the examples of the CB1 K_i's for (+)-**8** (AM4434) and (−)-**8** (AM4435), which were 360 and 770 nM, respectively, when the CB1-containing rat brain membrane preparations were pretreated[27] with phenylmethylsulfonyl fluoride (PMSF). All other compounds exhibited CB1 and CB2 K_i values larger than 1000 nM without substantial differences except for (±)-**5** (AM5501, CB2 K_i 410 nM).

FAAH and MGL enzymes were partially purified from adult Sprague–Dawley rat brains purchased from Pel-Freeze Biologicals according to a previously reported procedure.[31,32] The pellet from the last centrifugation step (microsomal fraction) was resuspended in 25 mM Tris–HCl, 5 mM $MgCl_2$, 1 mM EDTA, and pH 7.4 (TME) buffer for the FAAH assay (plus 0.1% BSA), and the supernatant from the last centrifugation step (cytosol fraction without BSA) was used for the MGL assay. All compound stock solutions used were 10 mM in DMSO, and the chemical stabilities of the compounds were first checked under the assay conditions (100 μM substrate) without any added FAAH or MGL enzymes. To screen compounds as substrates for FAAH, assays were carried out according to our previously reported procedures.[32,33] Samples (100 μL) were taken at the start of the assay to obtain the background concentration of arachidonic acid in the biological sample, and then after 15 min of FAAH hydrolysis. Samples were diluted 1:5 with acetonitrile and centrifuged (20,000*g*, 5 min, room temperature) to precipitate the proteins. The resulting supernatant was analyzed by HPLC to determine the percentage of compound hydrolyzed in the reaction time. The assays with cytosolic MGL were carried out in similar fashion to the FAAH assay described above, except that TME buffer was used without BSA, the MGL enzyme preparation used 30 μg of protein, and the reaction time was 20 min.

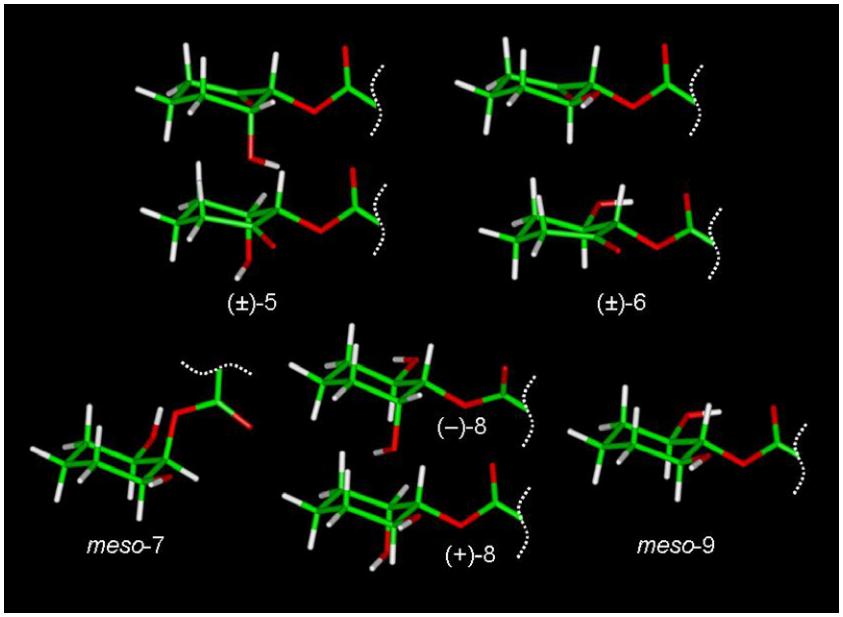


Figure 2. Molecular models for (±)-**5** (AM5501), (±)-**6** (AM5502), *meso*-**7** (AM5504), the enantiomers (−)-**8** (AM4435) and (+)-**8** (AM4434), and *meso*-**9** (AM5505) were obtained on a Silicon Graphics Fuel workstation using Insight II (2000). The *O*-arachidonoyl groups were modeled in extended conformations[34,35] but are not displayed. All structures were subject to molecular mechanics calculations using the steepest descent method for the first 1000 iterations, then the conjugate gradient method until the maximum derivative was less than 0.001 kcal/mol. Only for *meso*-7 (AM5504) was the 2-*O*-arachidonoyl group found to be axial in the lowest energy conformation (see Fig. 3).

These raw data in Table 1 from the enzyme susceptibility screenings have not been adjusted to reflect the stability of the substrates in the absence of enzyme, where (+)-**5** (AM5501) showed the most chemical instability (12% and 11% hydrolyses) and *meso*-**7** (AM5504) showed the least instability (2% and 1% hydrolyses) under control conditions for FAAH and MGL screenings, respectively. However, the data clearly indicated that the stereochemical features of the triol ester headgroup of some analogues could be used to distinguish the active sites for ester hydrolysis at the MGL and FAAH enzyme active sites. The preferred conformations of the individual analogues are represented in Figure 2. In general, FAAH was very effective in hydrolyzing all equatorial arachidonoyl esters. However, with the energy difference of 2.0 kcal/mol between the axial and equatorial conformations (see Fig. 3), *meso*-**7** (*cis*,*cis*-**7**, AM5504) exists primarily (97% at 25 °C) in a conformation where the arachidonoyl ester is axial, and it was not a good substrate for FAAH. The arachidonoyl ester of *meso*-**9** (*trans*,*trans*-**9**, AM5505) was only somewhat less susceptible to FAAH hydrolysis than (±)-**8** (AM5503), which represents a conformation of 2-AG that is readily hydrolyzable by FAAH. Interestingly, no difference was seen between the two enantiomers (+)-**8** (AM4434) and (−)-**8** (AM4435) for this observed excellent FAAH specificity, but it should be noted that both endocannabinoid substrates (AEA and 2-AG) are achiral. FAAH also readily hydrolyzes ketone (±)-**6**, which, like **8**, has one hydroxyl group adjacent and in the plane of the arachidonoyl ester.

The best access to the ester linkage by MGL was in the cases of ketone (±)-**5**, and to a much lesser extent, *meso*-**9** (*trans*,*trans*-**9**, AM5505). Thus, the combination of a carbonyl which is adjacent and in the plane of the arachidonoyl ester with a hydroxyl group axial as in (±)-**5** resulted in the highest MGL activity for 2-*O*-arachidonoylglycerol analogue hydrolysis.

This screening of conformationally restricted 2-AG analogues has identified key structural features which distinguish the endocannabinoid hydrolytic enzymes MGL and FAAH, and the K_m values for these compounds are being determined. We are also interested in evaluating this series of 2-AG analogues as competitive inhibitors of MGL and FAAH, and for their effects on signal transduction via the cannabinoid receptors in both the forskolin-stimulated cyclic AMP accumulation assay and the [^{35}S]GTPγS binding assay. Future work on constrained analogues of 2-AG will involve, not only modifying the triol ester headgroups through the use of

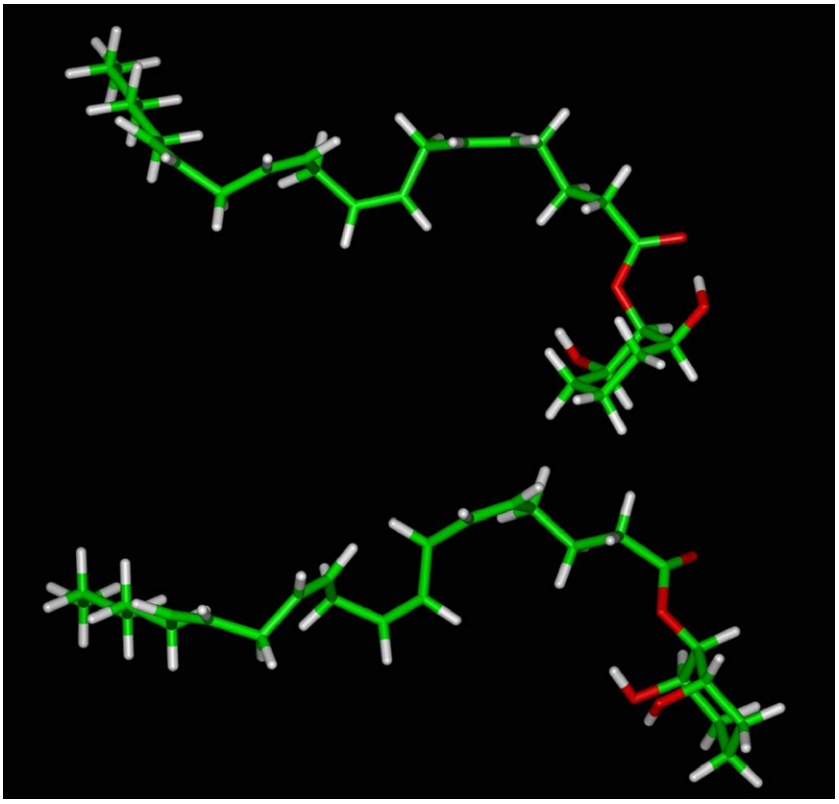


Figure 3. Molecular models of both possible chair conformations of *meso*-**7** (AM5504) were obtained on a Silicon Graphics Fuel workstation using Insight II (2000). The *O*-arachidonoyl groups were modeled in extended conformations as reported by Reggio et al.[34] for 2-AG and were constrained between C1 and C15. A restraint file for the cyclohexyl ring was also incorporated during the dynamics run in order to prevent possible isomerization and/or racemization at high temperature. The energy-minimized structures underwent constrained molecular dynamics performed by heating it to 1200 °K and recording 100 atomic coordinate trajectories every 10,000 iterations (1 fs per iteration). Next, each trajectory was subjected to simulated annealing followed by energy minimization using the steepest descent method for 100 iterations followed by conjugate gradient method until the maximum derivative was less than 0.001 kcal/mol. Two families of low-energy conformers were identified, and the conformer with the axial 2-*O*-arachidonoyl group (top) was found to be 2.0 kcal/mol lower in energy than the corresponding equatorial 2-*O*-arachidonoyl conformer (bottom).

different-sized rings, but also modifications of the 2-arachidonoyl group. These conformationally well-defined 2-AG analogues can now be used to develop lead compounds which are selective inhibitors of MGL or FAAH.

Acknowledgments

The authors are grateful to Ying Pei, Yan Peng, Alexander A. (Sasha) Zvonok, and Pusheng Fan for the biochemical assays of these compounds and to Lakshmipathi Pandarinathan for helpful discussions. This work was supported by grants from the National Institute on Drug Abuse DA03801, DA09158, and DA07215.

References and notes

1. Mechoulam, R.; Ben-Shabat, S.; Hanuš, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almog, S.; Martin, B. R.; Compton, D. R.; Pertwee, R. G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. *Biochem. Pharmacol.* **1995**, *50*, 83.
2. Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89.
3. Sugiura, T.; Kodaka, T.; Nakane, S.; Miyashita, T.; Kondo, S.; Suhara, Y.; Takayama, H.; Waku, K.; Seki, C.; Baba, N.; Ishima, Y. *J. Biol. Chem.* **1999**, *274*, 2794.
4. Savinainen, J. R.; Järvinen, T.; Laine, K.; Laitinen, J. T. *Br. J. Pharmacol.* **2001**, *134*, 664.
5. Sugiura, T.; Kondo, S.; Kishimoto, S.; Miyashita, T.; Nakane, S.; Kodaka, T.; Suhara, Y.; Takayama, H.; Waku, K. *J. Biol. Chem.* **2000**, *275*, 605.
6. Wilson, R. I.; Nicoll, R. A. *Science* **2002**, *296*, 678.
7. Diana, M. A.; Marty, A. *Br. J. Pharmacol.* **2004**, *142*, 9.
8. Lambert, D. M.; Fowler, C. J. *J. Med. Chem.* **2005**, *48*, 5059.
9. Gulyas, A. I.; Cravatt, B. F.; Bracey, M. H.; Dinh, T. P.; Piomelli, D.; Boscia, F.; Freund, T. F. *Eur. J. Neurosci.* **2004**, *20*, 441.
10. Ligresti, A.; Cascio, M. G.; Di Marzo, V. *Curr. Drug Targets—CNS Neurol. Disord.* **2005**, *4*, 615.
11. Vandevoorde, S.; Lambert, D. M. *Curr. Pharm. Des.* **2005**, *11*, 2647.
12. Bari, M.; Battista, N.; Fezza, F.; Gasperi, V.; Maccarrone, M. *Mini-Rev. Med. Chem.* **2006**, *6*, 257.
13. Sugiura, T.; Kobayashi, Y.; Oka, S.; Waku, K. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2002**, *66*, 173.
14. Kozak, K. R.; Gupta, R. A.; Moody, J. S.; Ji, C.; Boeglin, W. E.; DuBois, R. N.; Brash, A. R.; Marnett, L. J. *J. Biol. Chem.* **2002**, *277*, 23278.
15. Kozak, K. R.; Crews, B. C.; Morrow, J. D.; Wang, L.-H.; Ma, Y. H.; Weinander, R.; Jakobsson, P.-J.; Marnett, L. J. *J. Biol. Chem.* **2002**, *277*, 44877.
16. Di Marzo, V.; Bifulco, M.; De Petrocellis, L. *Nature Rev. Drug Disc.* **2004**, *3*, 771.
17. Makriyannis, A.; Mechoulam, R.; Piomelli, D. *Neuropharmacology* **2005**, *48*, 1068.
18. Pertwee, R. G. *AAPS J.* **2005**, *7*, E625.
19. Rouzer, C. A.; Ghebreselasie, K.; Marnett, L. J. *Chem. Phys. Lipids* **2002**, *119*, 69.
20. van der Stelt, M.; van Kuik, J. A.; Bari, M.; van Zadelhoff, G.; Leeflang, B. R.; Veldink, G. A.; Finazzi-Agrò, A.; Vliegenthart, J. F. G.; Maccarrone, M. *J. Med. Chem.* **2002**, *45*, 3709.
21. Saario, S. M.; Savinainen, J. R.; Laitinen, J. T.; Järvinen, T.; Niemi, R. *Biochem. Pharmacol.* **2004**, *67*, 1381.
22. (±)-**5** ($CDCl_3$, 500 MHz) δ 5.35–5.44 (m, 8H), 5.25 (d, J = 2.9 Hz, 1H), 4.48 (br s, 1H), 2.82 (m, 6H), 2.41–2.55 (m, 4H), 2.14–2.25 (m, 4H), 2.09–2.10 (m, 2H), 1.96–2.09 (m, 2H), 1.65–1.83 (m, 2H), 1.22–1.42 (m, 6H), 0.91 (t, J = 6.6 Hz, 3H); (±)-**6** ($CDCl_3$, 500 MHz) δ 5.28–5.33 (m, 8H), 4.98 (d, J = 9.9 Hz, 1H), 3.85–3.92 (dt, J = 10.4, 4.7 Hz, 1H), 2.82 (m, 6H), 2.47–2.55 (m, 4H), 2.14–2.25 (m, 4H), 2.09–2.10 (m, 2H), 1.96–2.09 (m, 2H), 1.78–1.83 (m, 2H), 1.22–1.42 (m, 6H), 0.91 (t, J = 6.6 Hz, 3H); *meso*-**7** ($CDCl_3$, 400 MHz) δ 5.30–5.43 (m, 8H), 4.95 (br s, 1H), 3.95 (br s, 2H), 2.79–2.85 (m, 6H), 2.43 (t, J = 7.6 Hz, 2H), 2.14–2.15 (m, 2H), 2.04–2.06 (m, 2H), 1.73–1.77 (m, 4H), 1.60–1.67 (m, 2H), 1.27–1.37 (m, 8H), 0.89 (t, J = 7.0 Hz, 3H); (±)-**8** ($CDCl_3$, 400 MHz) δ 5.30–5.43 (m, 8H), 4.72–4.82 (m, 1H), 4.04–4.05 (m, 1H), 3.68–3.71 (m, 1H), 2.84–2.88 (m, 6H), 2.38 (t, J = 7.6 Hz, 2H), 2.06–2.20 (m, 4H), 1.67–1.78 (m, 8H), 1.28–1.39 (m, 6H), 0.89 (t, J = 7.0 Hz, 3H); *meso*-**9** ($CDCl_3$, 400 MHz) δ 5.30–5.43 (m, 8H), 4.63–4.65 (m, 1H), 3.48–3.53 (m, 1H), 3.34–3.40 (dt, J = 3.4, 9.1 Hz, 1H), 2.79–2.85 (m, 6H), 2.35 (t, J = 7.4 Hz, 2H), 2.00–2.17 (m, 4H), 1.68–1.75 (m, 4H), 1.19–1.39 (m, 10H), 0.88 (t, J = 6.9 Hz, 3H).
23. Dumortier, L.; Van der Eycken, J.; Vandewalle, M. *Tetrahedron Lett.* **1989**, *30*, 3201.
24. Guo, Y.; Abadji, V.; Morse, K. L.; Fournier, D. J.; Li, X.; Makriyannis, A. *J. Med. Chem.* **1994**, *37*, 3867.
25. Morse, K. L.; Fournier, D. J.; Li, X.; Grzybowska, J.; Makriyannis, A. *Life Sci.* **1995**, *56*, 1957.
26. Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769.
27. Li, C.; Wei, X.; Vadivel, S. K.; Makriyannis, A. *J. Med. Chem.* **2005**, *48*, 6423.
28. Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
29. Ghafouri, N.; Tiger, G.; Razdan, R. K.; Mahadevan, A.; Pertwee, R. G.; Martin, B. R.; Fowler, C. J. *Br. J. Pharmacol.* **2004**, *143*, 774.
30. Mukherjee, S.; Adams, M.; Whiteaker, K.; Daza, A.; Kage, K.; Cassar, S.; Meyer, M.; Yao, B. B. *Eur. J. Pharmacol.* **2004**, *505*, 1.
31. Lang, W.; Qin, C.; Hill, W. A. G.; Lin, S.; Khanolkar, A. D.; Makriyannis, A. *Anal. Biochem.* **1996**, *238*, 40.
32. Lang, W.; Qin, C.; Lin, S.; Khanolkar, A. D.; Goutopoulos, A.; Fan, P.; Abouzid, K.; Meng, Z.; Biegel, D.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 896.
33. Qin, C.; Lin, S.; Lang, W.; Goutopoulos, A.; Pavlopoulos, S.; Mauri, F.; Makriyannis, A. *Anal. Biochem.* **1998**, *261*, 8.
34. Barnett-Norris, J.; Guarnieri, F.; Hurst, D. P.; Reggio, P. H. *J. Med. Chem.* **1998**, *41*, 4861.
35. Tian, X.; Guo, J.; Yao, F.; Yang, D.-P.; Makriyannis, A. *J. Biol. Chem.* **2005**, *280*, 29788.