Chapter 39
Further Advances in the Synthesis of Endocannabinoid-Related Ligands

Anu Mahadevan[1] and Raj K. Razdan[1]

Abstract Recent advances in the synthesis of endocannabinoid-related ligands for the period 2001–2004 are covered in this review. During this period the first solid phase synthesis of anandamide (AEA) analogs was developed, which allows modification at both the head group and the end pentyl chain. Synthesis of water-soluble prodrugs of noladin ether was reported, which are chemically stable, rapidly release noladin ether under enzymatic conditions and are shown to reduce intraocular pressure. The structure-activity relationships (SAR) of alkylcarbamic acid aryl esters and the discovery of potent archidonylsulfonyl derivatives as fatty acid amide hydrolase (FAAH) inhibitors are summarized. Recent synthetic developments in the controversial area of anandamide membrane transporter (AMT) inhibitors are also discussed.

Keywords endocannabinoids, synthesis, structure-activity relationship.

Introduction

The past decade has seen a sudden spurt of interest in the cannabinoid field.[1-5] Two types of cannabinoid receptors have been discovered: the CB1 receptor located both inside and outside the central nervous system (CNS), and the CB2 receptor located mainly in the periphery.[6,7] The CB1 receptor has been established to be a G-protein-coupled receptor. The first potent CB1 receptor antagonist SR141716A was reported by Rinaldi-Carmona et al,[8] who also reported the CB2 selective receptor antagonist SR144522.[8,9] Anandamide (AEA) was identified as the endogenous ligand for the CB1 receptor. Another important endogenous ligand is 2-arachidonyl-glycerol (2-Ara-Gl), which activates both CB1 and CB2 receptors. Both AEA and

[1] Organix, Inc, Woburn, MA 01801

Corresponding Author: Anu Mahadevan, Organix, Inc, 240 Salem Street, Woburn, MA 01801. Tel: (781) 932-4142; Fax: (781) 933-6695; E-mail: mahadevan@organixinc.com

2-Ara-Gl are found together with a family of related fatty acid glycerol esters and fatty acyl ethanolamides. It was found that the potency of 2-Ara-Gl was enhanced in the presence of the related 2-acyl-glycerols. This effect has been termed the "entourage effect," which probably represents the pathway for molecular regulation of endogenous cannabinoid activity.[10] Hanus et al[11] isolated yet another endogenous ligand "noladin ether" (2-arachidonylglyceryl ether) from porcine brain.[11] It binds to CB1 receptors and very weakly to CB2 receptors. During this review period 2 other endocannabinoids, virodhamine and N-arachidonyl dopamine, were discovered.[12,13] Virodhamine was found to be a CB1 receptor antagonist and partial agonist of CB2 receptor and N-arachidonyl dopamine was CB1 selective and also active at vanilloid VR1 receptors. The pharmacological activities of Δ^9-tetrahydrocannabinol (Δ^9-THC) and the endogenous ligand AEA are alike, however the onset of action of AEA is much faster and the duration of action is much shorter compared with Δ^9-THC. This has been attributed to the enzymatic hydrolysis of the amide bond in AEA; the enzyme involved in the metabolic degradation of AEA has been characterized as "fatty acid amide hydrolase" (FAAH). Several metabolically stable analogs of AEA have been synthesized that have greater binding affinities than AEA.[14,15] Makriyannis and coworkers[15] have improved the metabolic stability of the AEA part by incorporation of the methanandamide head group; our group has enhanced the metabolic stability by introduction of substituents on the carbon in the 2-position of the arachidonic acid part of AEA. AEA is produced from phospholipid precursors and it has been proposed that for its termination AEA is carried into the neurons by the anandamide membrane transporter (AMT) and then undergoes enzymatic hydrolysis into AEA and ethanolamine. The synthetic approaches to ligands that are associated with the endocannabinoid system, eg, AEA, 2-Ara-Gl, noladin ether, FAAH and AMT, have been discussed in detail in our previous review.[16] In this article we will be covering the chemistry and SAR, which have been developed subsequently in the above-mentioned areas.

AEA and 2-Ara-Gl Analogs

The structures of AEA and 2-Ara-Gl are shown in Fig. 39.1. The ethanol amine group is described as the head group moiety **a**; the arachidonyl backbone is moiety **b**; and the end pentyl chain is moiety **c**. During this period, no new chemistry was

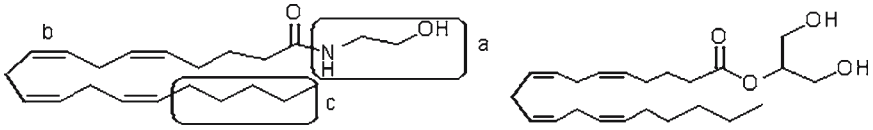


Fig. 39.1 Structures of AEA and 2-Ara-GI.

described related to either the head group (moiety **a**) or the arachidonyl backbone (moiety **b**). Only a new route was described for the introduction of different substituents in moiety **c**.

Altundas et al[17] described a flexible route to the synthesis of AEA analogs with modifications in the end pentyl chain, which is moiety **c**. The key step in the strategy involves copper-catalyzed coupling of the bromo intermediate **1** (Fig. 39.2) with the appropriate acetylenic derivatives. The target amides in the AEA series were synthesized by hydrolysis of the esters **2a-d** (Fig. 39.2) to the acids using LiOH, followed by their conversion to the corresponding acid chloride and coupling with (R)-2-aminopropanol. Alternatively, the target amides in this series were also synthesized by the mixed anhydride procedure. The SAR studies of these compounds have not yet been published.

An important development is the report by Qi et al[18] on the first solid phase synthesis of AEA analogs. The synthetic route (Fig. 39.3) is very versatile and it involves attachment of 5-hexynoic acid to Wang resin via an ester linkage followed by repetitive copper-catalyzed coupling with propargylic alcohols to provide the tetrayne skeleton. Cleavage of the tetrayne was accomplished by treatment with TFA or $AlMe_3$/amine to furnish the corresponding acids and amides, respectively, which was then reduced with P-2 Ni as the catalyst to furnish the desired products. Advantages of this procedure are that it allows modifications at both the head group moiety **a** and the end pentyl chain moiety **c** and thus allows for the effective synthesis and SAR of several modified AEA analogs.

To examine the SAR in the arachidonyl alcohol series, Parkkari et al[19] synthesized several ester, carbonate, and carbamate derivatives of arachidonyl alcohol. The major hurdle that they encountered in the synthesis of archidonyl alcohol esters was its self-degradation and polymerization. In general, the synthesis of these

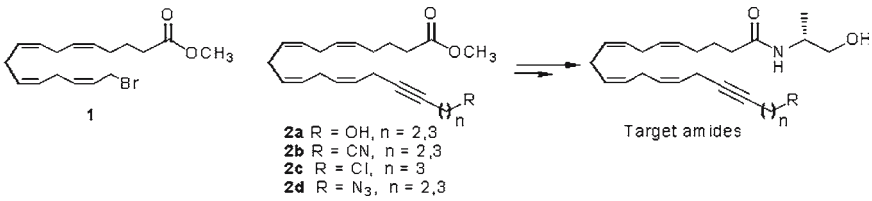


Fig. 39.2 AEA analogs with modifications in the end pentyl chain.

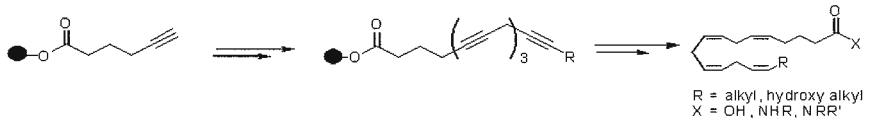


Fig. 39.3 Solid phase synthesis of AEA analogs.

analogs was accomplished by dicyclohexylcarbodiimide (DCC) coupling of the appropriate acid partners with arachidonyl alcohol. The silyl protecting groups, which were used to protect the alcohols, were deprotected using tetrabutyl ammonium fluoride (TBAF) in tetrahydrofuran (THF). The carbonates and the carbamates were synthesized via p-nitro-phenylchloroformate-activated intermediates as shown in Fig. 39.4. The results obtained from their SAR study indicate that among the different analogs that were tested only the ester analogs **3a** and **3b** (Fig. 39.4) showed some activity at the CB1 receptor, however even these compounds were found to be less potent and efficacious when compared with AEA.

Fangour et al[20] synthesized analogs based on 2-Ara-Gl and AEA template (Fig. 39.5) where one hydroxymethylene group of the glycerol moiety was replaced

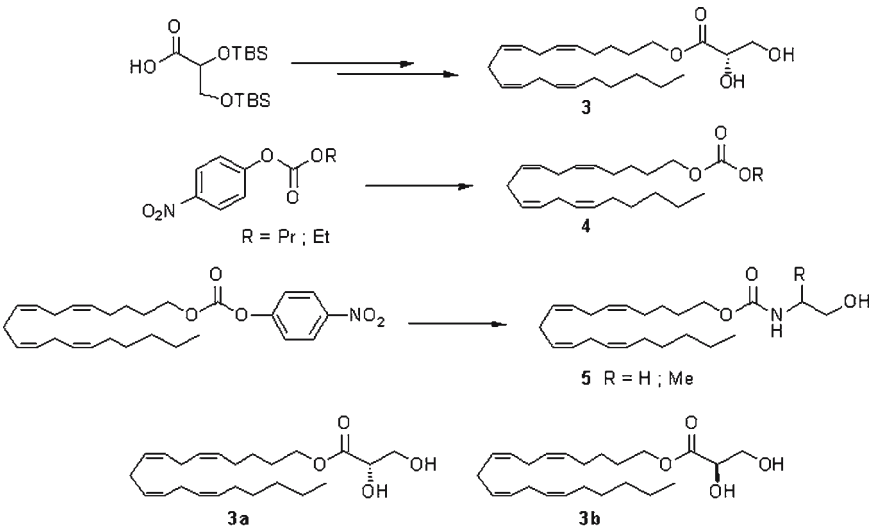


Fig. 39.4 Synthetic route towards arachidonyl alcohol derivatives.

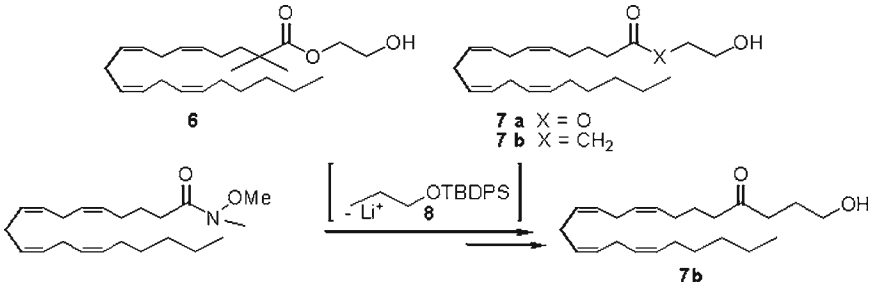


Fig. 39.5 Synthesis of arachidonyl ketone analog.

with hydrogen to give compound **7a**. The stability of the ester analog was improved by introduction of substituents on the carbon in the 2-position of the arachidonic acid part to give compound **6**. They also prepared the methylene-linked analog of AEA compound **7b** where the amide bond of AEA was replaced by a ketone functionality. The synthesis of the ketone analog **7b** was accomplished by treatment of Weinreb amide of arachidonic acid with the lithiated anion **8** to give the compound in an overall 27% yield. The analogs **6** and **7a** were prepared by standard procedures.[14] The affinity of these compounds for the CB1 receptor was investigated and it was found that the ketone analog **7b** showed moderate binding affinity (binding affinity [K_i] = 360 nM) to CB1 receptor, while the other two compounds **6** and **7a** were found to be inactive.

Noladin Ether

To increase the metabolic stability of 2-Ara-Gl, Mechoulam's group[14] synthesized noladin ether and it was later isolated from porcine brain as an endogenous cannabinoid ligand. To improve the aqueous solubility properties of these lipophilic compounds, Juntunen et al[21] synthesized water-soluble prodrugs of noladin ether. Monophosphate and diphosphate esters of noladin ether **9** and **10** (Fig. 39.6) were synthesized by treatment of noladin ether with dimethylchloro phosphate in the presence of pyridine as base. Juntunen et al found that both compounds **9** and **10** showed good chemical stability in buffer solutions at pH 7.4 and were also more soluble in aqueous solutions (>40 000-fold) when compared with noladin ether. These compounds rapidly converted into noladin ether under enzymatic hydrolysis conditions and the monophosphate ester **9** was able to reduce intraocular pressure in normotensive rabbits.

FAAH Inhibitors

A detailed discussion of the synthetic approaches and SAR of various classes of FAAH inhibitors can be found in our previous review.[16] Since then, the synthesis and SAR of alkylcarbamic acid aryl esters as FAAH inhibitors has been reported

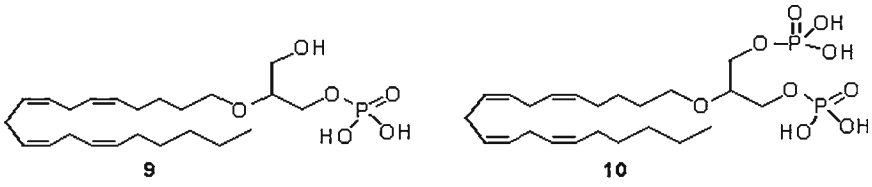


Fig. 39.6 Phosphate esters of noladin ether.

by Tarzia et al[22] and Mor et al.[23] One of the potent analogs, URB524, displayed profound anxiolytic-like properties in rats. The design of the alkylcarbamic acid aryl esters were based on the structural modifications of the known inhibitor of serine hydrolase acetylcholine esterase compound **11** (Fig. 39.7). In general these compounds were synthesized by addition of the appropriate alcohols or amines to isocyanatocyclohexane in the presence of triethyl amine as base. A brief summary of the SAR of this class of inhibitors is given below.

1. Replacement of the methyl group in **12** with the cyclohexyl group gave compound **13,** which showed a 60-fold improvement in inhibitor potency.
2. Modification of the carbamate function was found to be detrimental to its FAAH inhibitory activity. Ester and urea analogs were found to be inactive and replacement of the oxygen with sulfur to give the thiocarbamic acid derivatives was also found to be inactive.
3. Based on conformational analysis, Tarzia et al hypothesized that the shape of the aromatic substituent on the oxygen atom might be critical. Hence, they synthesized a series of analogs with different aromatic substituents in which they tried to mimic the "U-shaped" conformation of the fatty acid chain of AEA. The best inhibitor in this series was found to be URB524 (Inhibitor Potencies [IC_{50}] = 63 nM).
4. Modifications in which the aromatic substituents were replaced by alkyl chains were also found to be detrimental to the activity. Reversing the groups on the N- and O-substituents of URB524 gave compound **14,** which was found to be an inactive compound.
5. SAR was then conducted based on the structural template of URB524. The most potent compound in the series was URB597 (IC_{50} = 4.6 nM), which has a

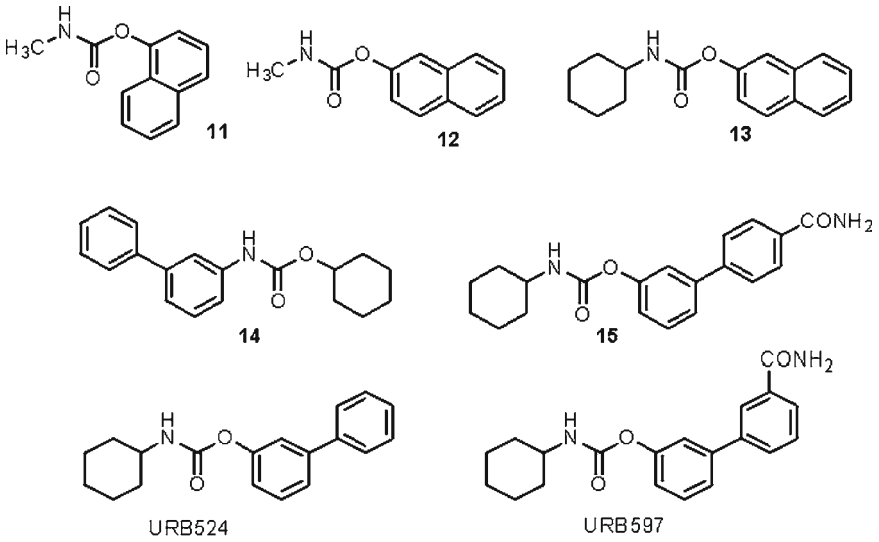


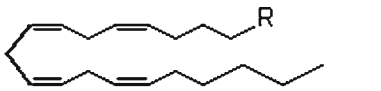
Fig. 39.7 Structures of Alkylcarbamic acid aryl esters.

substituent in the meta position of the distal phenyl ring. The meta-substituted compounds were in general found to be more active than the corresponding para isomers, for example compound **15** has an IC_{50} = 5909 nM.

Arachidonylsulfonyl derivatives **16** and **17** (Fig. 39.8) were reported by Segall et al[24] as novel inhibitors of FAAH with the potency of **16** being similar to methyl arachidonyl-fluorophosphonate. The synthesis involves conversion of arachidonyl bromide into arachidonylsulfonyl chloride via Grignard reaction. Subsequent treatment of arachidonylsulfonyl chloride with tetrabutylammonium fluoride and ethanolamine provided compounds **16** and **17** respectively.

AMT Transporter Inhibitors

A comprehensive review of AMT inhibitors and their potential as drugs for CNS-related disorders was recently reported by Lopez-Rodriguez and Ortega-Gutierrez.[25] However, the AMT protein has not been isolated or cloned and the whole issue of AMT has since become controversial. Nevertheless, Di Marzo et al[26] synthesized a series of AMT transporter inhibitors based on arachidonyl **18b** and oleoyl templates **18a** (Fig. 39.9) where the head group moiety **a** contains different aromatic moieties.



16 R = CH_2SO_2F
17 R = $CH_2SO_2NHCH_2CH_2OH$

Fig. 39.8 Structures of Arachidonylsulfonyl derivatives.

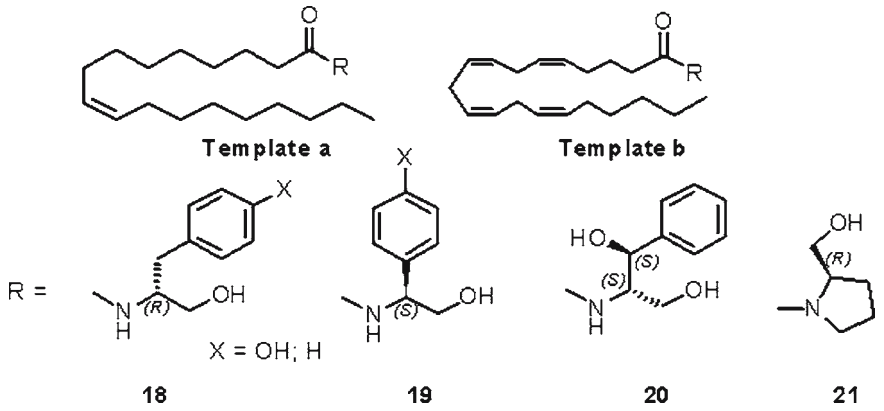


Fig. 39.9 AMT transporter inhibitors.

Their design is based on the observation that AMT transporter inhibitors, which have been designed to date, all contain aromatic moieties in the polar head group region. In general, the synthesis of these compounds has been accomplished by N-ethyl-N'-(3-dimethylamino propyl) carbodiimide (EDCI) coupling of the appropriate acids and amines to yield the desired products in good yields. The results obtained from their SAR study are summarized below.

1. Comparison of the arachidonyl template **b** and oleoyl template **a** show that the most potent and selective inhibitors in this series (**19a**, X=H K_i = 12.9 μM; **20a** K_i = 6.4 μM) have the oleoyl template.
2. Deletion of the phenolic hydroxyl from compounds **18a** and **18b** (for **18a** X = OH, K_i = 3.0 μM vs X = H, K_i = 11.1 μM) results in a decrease in the affinity for AMT. An opposite effect is seen in the compounds with general structure **19**. In the series of compounds with general structure **20** the (S,S)-diasteromer of oleic acid series shows an appreciable increase in activity with introduction of the hydroxyl group, however the trend is reversed in the arachidonic acid series where the (R,R)-diastereomer shows increase in activity.
3. Next, the restriction of the hydroxyalkyl group was investigated and it was observed that the conformationally restricted analogs **21** (both R and S) were found to be completely inactive.

Lopez-Rodriguez et al[27] synthesized a series of esters and amides with the general structural template **22** (Fig. 39.10) and explored the structure-affinity relationship between **22** and AMT. The compounds were synthesized from arachidonic acid by conversion to the acid chloride and coupling with the appropriate amine or alcohol or by direct DCC coupling of arachidonic acid with the appropriate amine or

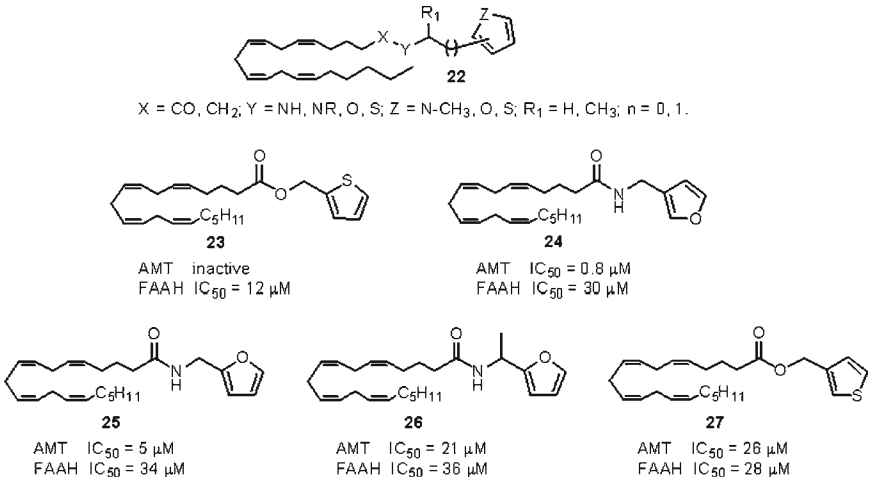


Fig. 39.10 Further examples of AMT inhibitors.

alcohol. From the results obtained, they concluded that there is no correlation between the effects on FAAH and on AMT. Compound **23** is a more potent FAAH inhibitor than compound **24**, however it is found to be inactive as an AMT inhibitor. Compounds **24 to 27** display similar FAAH inhibitory potencies, but differ in their ability to inhibit anandamide uptake (IC_{50} range from 0.8 to 26 μM). The most potent AMT inhibitor in this series is compound **24,** which is selective (CB1 K_i = 4700 nM; CB2 K_i = 67 nM; VR1 K_i > 5000 nM) except for moderate binding affinity to the CB2 receptor.

Conclusions

Since the discovery of the first endocannabinoids, anandamide and 2-arachidonyl glycerol, other endocannabinoids have been discovered, the pathway to their biosynthesis and degradation has been elucidated, and, as a result, the role of endocannabinoids in the brain has been firmly established. Progress is being made in understanding the full implications of their presence in the brain and the periphery, which may lead to the development of novel therapies in medicine.

References

1. Di Marzo V. *Cannabinoids*. New York: Plenum Publishers; 2004.
2. Di Marzo V, Bisogno T, De Petrocellis L, Melck D, Martin BR. Cannabimimetic fatty acid derivatives: the anandamide family and other "endocannabinoids." *Curr Med Chem*. 1999;6:721-744.
3. Pertwee RG, ed. *Cannabinoid Receptors*. London, UK: Academic Press Ltd; 1995.
4. Pertwee RG. Pharmacology of cannabinoid CB_1 and CB_2 receptors. *Pharmacol Ther*. 1997;74:129-180.
5. Pertwee RG. Pharmacology of cannabinoid receptor ligands. *Curr Med Chem*. 1999;6:635-664.
6. Devane WA, III, Dysarz FA, III, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol*. 1988;34:605-613.
7. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature*. 1993;365:61-65.
8. Rinaldi-Carmona M, Barth F, Heaulme M, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett*. 1994;350:240-244.
9. Rinaldi-Carmona M, Barth F, Millan J, et al. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther*. 1998;284:644-650.
10. Martin BR, Mechoulam R, Razdan RK. Discovery and characterization of endogenous cannabinoids. *Life Sci*. 1999;65:573-595.
11. Hanus L, Abu-Lafi S, Fride E, et al. 2-Arachidonylglyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA*. 2001;98:3662-3665.
12. Porter AC, Sauer JM, Knierman MD, et al. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther*. 2002;301:1020-1024.

13. Huang SM, Bisogno T, Trevisani M. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA*. 2002;99:8400-8405.
14. Sheskin T, Hanus L, Slager J, Vogel Z, Mechoulam R. Structural requirements for binding of anandamide type compounds to the brain cannabinoid receptor. *J Med Chem*. 1997;40:659-667.
15. Khanolkar AD, Makriyannis A. Structure-activity relationships of anandamide, an endogenous cannabinoid ligand. *Life Sci*. 1999;65:607-616.
16. Razdan RK, Mahadevan A. Recent advances in the synthesis of endocannabinoid related ligands. *Chem Phys Lipids*. 2002;121:21-33.
17. Altundas R, Mahadevan A, Razdan RK. A synthetic route to anandamide analogues carrying a substituent at the terminal carbon and an acetylene group in the end pentyl chain. *Tetrahedron Lett*. 2004;45:5449-5451.
18. Qi L, Meijler MM, Lee S-H, Sun C, Janda KD. Solid-phase synthesis of anandamide analogues. *Org Lett*. 2004;6:1673-1675.
19. Parkkari T, Savinainen JR, Rauhala AL, et al. Synthesis and CB1 receptor activities of novel arachidonyl alcohol derivatives. *Bioorg Med Chem Lett*. 2004;14:3231-3234.
20. Fangour SE, Balas L, Rossi J-C, et al. Hemisynthesis and preliminary evaluation of novel endocannabinoid analogues. *Bioorg Med Chem Lett*. 2003;13:1977-1980.
21. Juntunen J, Vepsalainen J, Niemi R, Laine K, Jarvinen T. Synthesis, in vitro evaluation, and intraocular pressure effects of water-soluble prodrugs of endocannabinoid noladin ether. *J Med Chem*. 2003;46:5083-5086.
22. Tarzia G, Duranti A, Tontini A, et al. Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. *J Med Chem*. 2003;46:2352-2360.
23. Mor M, Rivara S, Lodola A, et al. Cyclohexylcarbamic acid 3′- or 4′-substituted biphenyl-3-yl esters as fatty acid amide hydrolase inhibitors: synthesis, quantitative structure-activity relationships, and molecular modeling studies. *J Med Chem*. 2004;47:4998-5008.
24. Segall Y, Quistad GB, Nomura DK, Casida JE. Arachidonylsulfonyl derivatives as cannabinoid CB1 receptor and fatty acid amide hydrolase inhibitors. *Bioorg Med Chem Lett*. 2003;13:3301-3303.
25. Lopez-Rodriguez ML, Ortega-Gutierrez S. The anandamide degradation system as potential targets for the treatment of central nervous system related disorders. *Curr Med Chem Cent Nerv Syst Agents*. 2004;4:155-160.
26. Di Marzo V, Ligresti A, Morera E, Nalli M, Ortar G. The anandamide membrane transporter. Structure-activity relationships of anadamide and oleoylethanolamine analogs with phenyl rings in the polar head group region. *Bioorg Med Chem*. 2004;12:5161-5169.
27. Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, et al. Design, synthesis, and biological evaluation of new inhibitors of the endocannabinoid uptake: comparison with effects on fatty acid amidohydrolase. *J Med Chem*. 2003;46:1512-1522.