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Structural Requirements for Binding of Anandamide-Type Compounds to the Brain Cannabinoid Receptor

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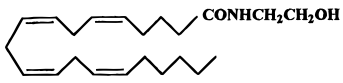
In order to establish the structural requirements for binding to the brain cannabinoid receptor (CB_1), we have synthesized numerous fatty acid amides, ethanolamides, and some related simple derivatives and have determined their K_i values. A few α-methyl- or α,α-dimethylarachidonoylalkylamides were also examined. In the 20:4, *n*-6 series, the unsubstituted amide is inactive; N-monoalkylation, at least up to a branched pentyl group, leads to significant binding. N,N-Dialkylation, with or without hydroxylation on one of the alkyl groups, leads to elimination of activity. Hydroxylation of the *N*-monoalkyl group at the ω carbon atom retains activity. In the 20:*x*, *n*-6 series, *x* has to be either 3 or 4; the presence of only two double bonds leads to inactivation. In the *n*-3 series, the limited data reported suggest that the derived ethanolamides are either inactive or less active than comparable compounds in the *n*-6 series. Alkylation or dialkylation of the α carbon adjacent to the carbonyl group retains the level of binding in the case of anandamide (compounds **48**, **49**); however, α-monomethylation or α,α-dimethylation of *N*-propyl derivatives (**50**−**53**) potentiates binding and leads to the most active compounds seen in the present work (K_i values of 6.9 ± 0.7 to 8.4 ± 1.1 nM). We have confirmed that the presence of a chiral center on the *N*-alkyl substituent may lead to enantiomers which differ in their levels of binding (compounds **54**, **57** and **55**, **56)**.

Several years ago we isolated from porcine brain arachidonoylethanolamide (anandamide) (**1**), the first known animal constituent that binds to the brain cannabinoid receptor (CB_1).[1] Later we found two additional active fatty acid ethanolamides: dihomo-γ-linolenoylethanolamide (**2**) and docosatetraenoylethanolamide (**3**).[2−4] The activity of anandamide parallels



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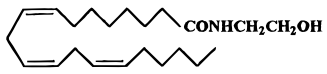

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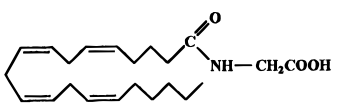
to a large extent that of Δ^9-tetrahydrocannabinol (Δ^9-THC), the active constituent of Cannabis,[5] including *in vitro* inhibition of adenylylcyclase[6] and *in vivo* effects on the hypothalamo−pituitary−adrenal axis,[7] sedation,[8] inhibition of working memory,[9] reduction of blood pressure,[10] etc.[11] The presence of anandamide in fresh pig brain has been confirmed.[12] Kempe et al. have reported that it could not be detected in fresh rat brain.[13] However Sugiura et al. have recently recorded its presence in this tissue,[14a] and a further group has confirmed this observation (D. Piomelli, private communication). The discrepancies reported may be due to the differences in the extraction and purification methods employed. Anandamide has also been detected in rat testis.[14b]

In order to establish the structural requirements for binding to CB_1, we have synthesized numerous fatty acid amides, ethanolamides, and some related simple derivatives and have determined their K_i values in a competition assay against the binding of [^{3}H]HU-243, a potent tricyclic cannabinoid.[1] A few α-methyl or α,α-dimethylarachidonoylalkylamides were also examined.

The monoalkyl, dialkyl and hydroxyalkyl amides were prepared by reaction of the appropriate acyl chloride with an alkyl-, dialkyl-, or hydroxyalkylamine, following the procedure previously described.[1] The phosphate (**4**) was prepared by condensation of the *N*-hydroxysuccinimide ester of arachidonic acid (**5**) with *O*-phosphorylethanolamine (Figure 1).[15]

The glycine derivative **6** was prepared by reaction of arachidonoyl chloride with glycine in potassium hydroxide solution. The carboxylic acid derivatives **7a** and **7b** were prepared by condensation of **5** with L-serine and D-serine, respectively.



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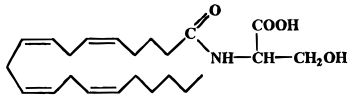

7a, D-
7b, L-

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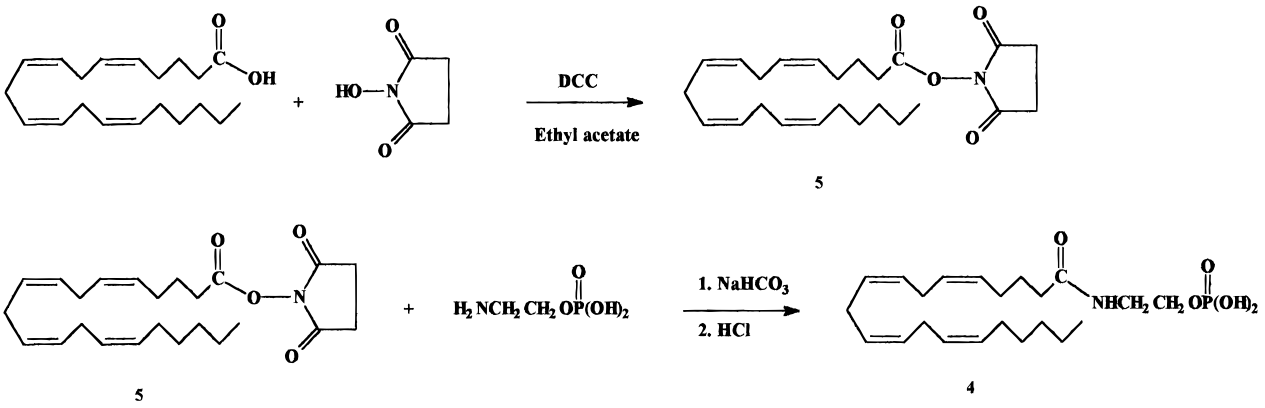


Figure 1. Synthesis of anandamide *O*-phosphate.

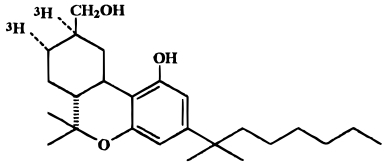
Several groups have published data on binding of various anandamide derivatives to CB_1.[16−23] Some of the published compounds were independently prepared and evaluated by us. The results obtained are presented now. Binding data are notoriously sensitive to experimental conditions, and it is difficult to compare results from different laboratories except with regard to trends in structure−activity changes. In the anandamide series such comparisons are further complicated due to the facile hydrolysis of the amide bond. In some of the early work very high K_i values (i.e., low binding) were reported for the compounds tested.[16] Later it was found that the addition of the amidase inhibitor phenylmethanesulfonyl fluoride (PMSF) prevents (or reduces) hydrolysis, and indeed, lower K_i values were noted.[24]

Two binding assay methods are generally used. In our original and later publications,[1,2a] we reported the use of a centrifugation-based ligand binding assay with rat synaptosomal membranes. However most other groups have prefered a "filtration" method, which in the absence of an amidase inhibitor apparently allows enzymatic hydrolysis to take place and produces high K_i values. In the centrifugation method, as used in our laboratory, the addition of an amidase inhibitor does not lower the K_i.

All compounds were tested for displacement of [^{3}H]-HU-243 (**8**), a highly potent probe prepared in our laboratory, bound to brain synaptosomal membranes (Tables 1−3).[25]

Structure−Activity Relationships (SAR)

Anandamide (20:4, *n*-6) (**1**) inhibited the specific binding of [^{3}H]HU-243 (**8**) to synaptosomal membranes in a manner typical of competitive ligands, under the conditions described in the Experimental Section, with an inhibition constant (K_i) of 39.2 ± 5.7 nM. The inhibition constants of the two other known additional endogenous anandamides, namely dihomo-γ-linolenoylethanolamide (anandamide 20:3, *n*-6) (**2**) and docosatetraenoylethanolamide (anandamide 22:4, *n*-6) (**3**) are very close, 53.4 ± 5.5 and 34.4 ± 3.2 nM, respectively.[2a,b] For comparison, the K_i of Δ^9-THC (**9**) is 46 ± 3 nM. These data indicate that the endogenous brain constituents and the main psychoactive Cannabis plant constituent bind to the CB_1 receptor at the same level of potency. However a comparison of the *in vivo* activities of anandamide (**1**) with those of Δ^9-THC (**9**) does not lead to analogous results. We have reported that in the open field ambulation test, which measures locomotor activity, and in the ring test, which measures catalepsy, anandamide, administered ip, was as active as Δ^8-THC (**10**).[3a] This isomer of Δ^9-THC is generally only slightly less active than Δ^9-THC in most *in vivo* tests. However, in the induction of hypothermia, and as an analgesic (measured on the hot plate), anandamide was considerably less active than Δ^8-THC.[3a] Smith et al. have noted that anandamide administered iv is 1.3−18 times less potent than Δ^9-THC in all behavioral assays.[26] This discrepancy may be due in part to the *in vivo* hydrolysis of anandamide, a well established metabolic route.



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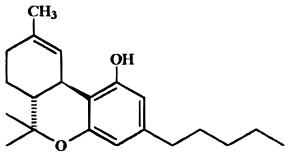


Δ^9-THC

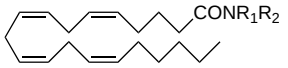
9

The above-mentioned results, as well as preliminary unpublished *in vivo* data on a few of the compounds discussed in this paper, indicate that *in vivo* and binding SAR's in the anandamide series do not necessarily parallel each other. In a separate publication, we expect to report data on *in vivo* SAR with anandamide-type compounds.

Table 1 lists, in an increasing order of potency, the synthetic arachidonoylamide derivatives prepared in our laboratory and their K_i values. An upper limit of 1 μM was arbitrarily set for binding potency. We do not report the exact inhibition constant of an assayed compound if it was above 1 μM, i.e., about 25 times less potent than anandamide. Such compounds we consider "inactive".

We were surprised to note that the hydroxyl group of the alkanolamine moiety is not a requirement for binding. This unexpected result has been independently observed by Pinto et al.[17] Arachidonoylethyl amide (**11**) is slightly more active than anandamide (**1**). A short-

Table 1. Binding of Arachidonoyl Amides to the Brain Cannabinoid Receptor (CB_1)

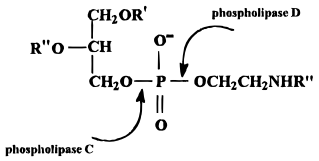


compd	R_1	R_2	K_i (nM)	lit. K_i (nM)
14	H	$CH_2CH_2CH_3$	11.7 ± 2.1	7.3 (5.7−9.5)17
15	H	$CH(CH_3)_2$	13.6 ± 1.1	
22	H	$CH_2CH_2CH_2OH$	29.9 ± 0.4	364 ± 95[16] 13 (11−17)[17] 189 ± 73 (with PMSF)[22]
11	H	CH_2CH_3	34.0 ± 2.7	
1	H	CH_2CH_2OH	39.2 ± 5.7	
12	H	CH_3	60.0 ± 7.4	
25	H	$CH_2CH_2OCH_3$	85.2 ± 3.8	650 (470−880)[17] 1820 ± 280 (with PMSF)[22]
17	H	$C(CH_3)_3$	138.6 ± 9.4	
23	H	$CCH_2(CH_3)_2OH$	161.8 ± 34.1	
4	H	$CH_2CH_2OP(=O)(OH)_2$	190.8 ± 11.1	
16	H	$CH_2CH_2CH_2CH_3$	235.7 ± 14.2	30 (22−43)[17]
21	H	$CH(CH_3)CH_2CH_3$	(*R*)-(−), 239.9 ± 63.8 (*S*)-(+), 377.2 ± 55.4	
20	H	$CCH_2(CH_3)_2CH_3$	446.7 ± 40.3	
24	H	$CH_2CH_2CH_2CH_2OH$	497.4 ± 27.1	220 (190−260)[17]
19	H	$CH_2CH_2CH(CH_3)_2$	575.1 ± 35.3	1860 ± 300 (with PMSF)[22]
13	H	H	>1000	9600 ± 100[16]
28	CH_3	CH_3	>1000	
29	CH_2CH_3	CH_2CH_3	>1000	
35	H	$CH(CH_3)CH(OH)C_6H_5$	>1000	
27	H	$CH_2CH_2N(CH_2CH_3)_2$	>1000	
18	H	$CH_2CH_2CH_2CH_2CH_3$	>1000	
34	H	OH	>1000	
30	CH_3	CH_2CH_2OH	>10000	5240 ± 2025[19] 4980 ± 376 (with PMSF)[19]
31	CH_2CH_3	CH_2CH_2OH	>10000	
32	$CH_2CH_2CH_3$	CH_2CH_2OH	>10000	
33	CH_2CH_2OH	CH_2CH_2OH	>10000	
6	H	CH_2COOH	>10000	
7	H	$CH(COOH)CH_2OH$	(a) D, >10000 (b) L, >10000	

ened side chain as in arachidonoylmethylamide (**12**) lowers activity, compared to that of **1**, while removal of the alkyl group, as in arachidonoyl amide (**13**),[16] eliminates binding. Activity increases with elongation of the *N*-alkyl side chain, the K_i of arachidonoyl-*n*-propyl amide (**14**) being 11.7 ± 2.1 nM and that of arachidonoylisopropyl amide (**15**), 13.6 ± 1.1 nM. However, further elongation, with or without branching, sharply reduces or eliminates binding (see compounds **16**−**21**). Another point of interest is that the straight chain pentyl amide (**18**) is inactive, i.e., has a K_i value above 1 μM, while the branched chain pentyl amides (**19**−**21**) were active with K_i values between ca. 235 and 575 nM. The same regularity is observed in the butyl series. The straight chain butyl derivative (**16**)[17] has a K_i of 235.7 ± 14.2 nM, while the branched butyl one (**17**) has a K_i of 138.6 ± 9.4 nM. Only a small difference was observed between the *R*-(−) enantiomer (**21a**) with a K_i of 239.9 ± 63.8 nM and the *S*-(+) enantiomer (**21b**) with a K_i of 377.2 ± 55.4 nM.

We noted the same regularity with regard to elongation of the side chain in the arachidonoylalkanolamide series. Elongation of the alkanol chain, from ethanol, as in **1**, to propanol, leading to **22**,[16,17,22,23] causes a slight increase in activity; however, the isobutyl alcohol **23** or 1-pentanol **24**[17,22,23] derivatives are considerably less active.

Blocking of the hydroxyl group in anandamide as a methyl ether (compound **25**)[17,22,23] decreased the activity about 2-fold. Anandamide phosphate (**4**) was about 5 times less active than anandamide. This material was prepared in view of the biogenesis of anandamide, which is considered to be formed by the action of phospholipase D on *N*-arachidonoylphosphatidylethanolamine (**26**).[14,27]



R' = acyl, R" = arachidonoyl

26

Assuming that the latter compound could also be cleaved by phospholipase C, compound **4** would be obtained. We have not observed **4** to be present in brain (unpublished observations), and in view of the low activity of **4** recorded now, we doubt whether phospholipase C is involved in the formation of anandamide-type compounds. However, we cannot rule out the possibility that **4**, once produced in the body, is quickly modified by phosphatases to anandamide.

Disubstitution of the amide nitrogen, forming *N*,*N*-dialkyl or *N*-alkyl, *N*-(2-hydroxyethyl) derivatives, led to compounds that did not bind up to 1 μM (see **28**−**33**). Apparently a secondary amide grouping is essential in the anandamide series to allow binding to CB_1.

Several serine amide derivatives of fatty acids have been isolated from body tissues alongside the *N*-ethanol derivatives.[28] Hence, we synthesized the D- and L-serine

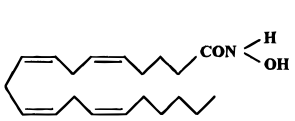
Table 2. Binding of *N*-Acyl Ethanolamines to the Brain Cannabinoid Receptor (CB_1)[a]

compd	acyl moiety	K_i (nM)	lit. K_i (nM)
3	22:4, *n*-6	34.4 ± 3.2	
2	20:3, *n*-6	53.4 ± 5.5	598 ± 254[22]
42	20:5, *n*-3	162.3 ± 13.6	1470 ± 500 (with PMSF)[22]
43	22:6, *n*-3	324.1 ± 9.2	12200 ± 500[16]
36	20:2, *n*-6	1500	
37	18:3, *n*-6	4600 ± 300	>41400 ± 6000[16]
44	20:1, *n*-9	>1000	>10000 (with PMSF)[22]
40	18:4, *n*-3	>1000	
39	20:3, *n*-3	>10000	
38	18:2, *n*-6	>25000	
45	16:0	no activity	no activity[16]
41	18:3, *n*-3	no activity	
47	eicosatetraynoic acid	no activity	

[a] The acyl group is designated following the accepted shorthand nomenclature for long-chain unsaturated fatty acids. Thus the acyl moiety 20:4, *n*-6, in anandamide indicates the presence of a 20-carbon atom chain; four homoallylic double bonds, the first one of which is on the sixth carbon atom, counting from the non-carboxyl end of the acid.

amides of arachidonic acid (**7a,b**). These compounds did not bind to CB_1. Likewise no binding was observed for the related glycine amide of arachidonic acid (**6**).

Corey et al.[29] have reported that the *N*-hydroxyl derivative of arachidonoyl amide (**34**) is an inhibitor of leukotriene synthesis. We found that this compound does not displace the binding of [^{3}H]HU-243 in concentrations up to 1 μM. The arachidonoyl amide of (±)-norephedrine (**35**) was also inactive.



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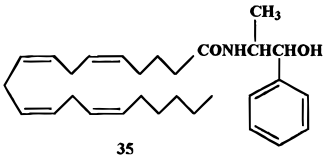


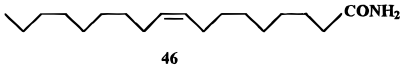
35

Table 2 presents the results obtained with a variety of amides of fatty acids other than arachidonic acid. As mentioned above, the endogenous anandamides (20:3, *n*-6) (**2**) and (22:4, *n*-6) (**3**) bind to CB_1 at the level of anandamide (20:4, *n*-6) (**1**). However anandamide (20:2, *n*-6) (**36**) is inactive. Apparently in the 20, *n*-6 series, three or four double bonds are required for activity. Anandamide (18:3, *n*-6) (**37**)[16,20] and anandamide (18:2, *n*-6) (**38**) are also inactive. Further work is required to establish the lower limit of the length of the fatty acid and the number of double bonds in them needed for binding to CB_1. We also looked into the *n*-3 series: anandamides (20:3, *n*-3) (**39**), (18:4, *n*-3) (**40**), and (18:3 *n*-3) (**41**) are inactive. However anandamides (20:5, *n*-3) (**42**)[22,23] and (22:6, *n*-3) (**43**)[16] retain activity (K_i, 162.3 ± 13.6 nM and 324.1 ± 9.2 nM respectively). A definite conclusion with regard to differences in activity in the *n*-3 and *n*-6 series cannot be drawn as few compounds that differ only in the positions of the double bonds (n-6 versus *n*-3) have been tested in both series (**37** versus **41** and **2** versus **39**). However the high activity of **2** as compared to the inactivity of **39**, as well as the general low activity of those compounds in the *n*-3 series that exhibit binding (**42**, **43**), compared to related active compounds in the *n*-6 series (for example **1** and **3)** points out that activity in the *n*-6 series is apparently higher than in the *n*-3 series. An *n*-9 anandamide (20:1, *n*-9) (**44**)[20,22,23] was inactive.

Palmitoylethanolamide (anandamide, 16:0) (**45**)[16,20] under our conditions did not bind to either CB_1 (membrane receptor or transfected cells) or CB_2 (transfected cells). This is in contrast to the data published by Facci et al. who found that **45** binds and activates CB_2 in mast cells.[30] It is possible that in mast cells **45** binds to a yet unidentified subtype of CB_2.

Oleoyl amide (**46**) has been reported to be a "sleep factor".[31] As cannabinoids are known to cause sedation and promote sleep we assumed that **46** may cause its activity *via* CB_1 or CB_2. We observed no binding. Apparently any sleep promotion caused by **46** does not take place *via* CB_1 or CB_2.

Eicosatetraynoylethanolamide (**47**) was prepared as the parent acid is known to block prostaglandin synthesis.[32] However, **47** did not bind to CB_1.



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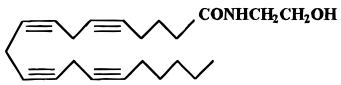


47

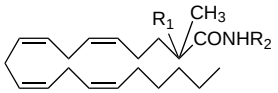
It has been established that the major metabolic degradation of anandamide is through hydrolysis, yielding arachidonic acid and ethanolamine.[33] We assumed that the introduction of one or two alkyl groups α to the carbonyl moiety may prevent, or at least reduce, the rate of hydrolysis, which may be expressed as a lower K_i value (assuming that during the binding assay anandamide is partially hydrolyzed). In Table 3 we present the K_i values of several α-mono- and α,α-dimethylarachidonoyl amides. We first determined the K_i values of α-monomethylanandamide (**48**) and α,α-dimethylanandamide (**49**). Neither **48** nor **49** was significantly more potent than anandamide (**1**). After the completion of these determinations, a paper describing compounds **48** and **49** and their K_i values was published.[19] The α-monomethyl and α,α-dimethyl derivatives of the *n*-propyl and isopropyl amides (**50**−**53**) were the most potent compounds obtained by us thus far, with K_i values in the range 6.9−8.4 nM.

Makriyannis et al. have reported the introduction of an asymmetry center on the *N*-alkyl side of anandamide, through placement of the hydroxyl group on the secondary rather than primary carbon atom of the ethanol side chain or by alkylation of the secondary carbon atom.[18] These changes led to a significant variation in activity between the *S* and *R* compounds. We have now produced the same type of derivatives of α,α-dimethylarachidonic acid. We also observe significant differences in binding: (**55**, *R*) and (**54**, *S*) are about as active as anandamide, while their enantiomers (**56**, *S*) and (**57**, *R*) are significantly less potent.

In summary, we have found so far several structure−activity regularities concerning binding of fatty acid amides to CB_1.

(1) In the 20:4, *n*-6 series, the unsubstituted amide (**13**) is inactive; N-monoalkylation, at least up to a branched pentyl group, leads to significant binding. The following regularities in binding were noted for anandamide-type compounds with the indicated N-alkyl moieties: *n*-C_5H_{11} (**18**) < branched C_5H_{11} (**19**, **20**) < CH(CH_3)CH_2CH_2 (either *R* or *S*) (**21**) < *n*-C_4H_9 (**16**) < $C(CH_3)_3$ (**17**) < CH_3 (**12**) < C_2H_5 (**11**) < C(CH_3)$_2$ (**15**) < *n*-C_3H_7 (**14**). The last two compounds were the most

Table 3. Binding of α-Methyl and α,α-Dimethyl Arachidonoyl Amides to the Brain Cannabinoid Receptor (CB_1)



compd	R_1	R_2	K_i (nM)	lit K_i (nM)
51	CH_3	$CH_2CH_2CH_3$	6.9 ± 0.7	
53	CH_3	$CH(CH_3)_2$	7.2 ± 0.1	
50	H	$CH_2CH_2CH_3$	7.4 ± 0.2	
52	H	$CH(CH_3)_2$	8.4 ± 1.1	
49	CH_3	CH_2CH_2OH	25.5 ± 2.8	47 ± 3 (with PMSF)[19] 41 ± 3[19] 47 ± 2 (with PMSF)[22] 41 ± 3[22]
55	CH_3	$CH(CH_3)CH_2OH$	(*R*)-(−), 31.1 ± 1.0	
48	H	CH_2CH_2OH	32.5 ± 5.1	53 ± 15 (with PMSF)[19] 137 ± 20[19] 53 ± 11 (with PMSF)[22] 137 ± 20[22]
54	CH_3	$CH_2CH(OH)CH_3$	(*S*)-(+), 46.6 ± 2.2	
57	CH_3	$CH_2CH(OH)CH_3$	(*R*)-(−), 153.9 ± 30.0	
56	CH_3	$CH(CH_3)CH_2OH$	(*S*)-(+), 191.4 ± 24.5	

active in these homologous series, with K_i values about three times lower than that of anandamide (20:4, *n*-6) (**1**).

2. N,N-Dialkylation, with or without hydroxylation on one of the alkyl groups, leads to elimination of activity (compounds **28**−**33**).

3. Hydroxylation of the *N*-monoalkyl group at the ω carbon atom retains activity, as compared to the parent *N*-alkyl group. However in most cases this activity is slightly lower, at least in the relatively potent compounds (cf. compound **22** versus **14**; compound **1** versus **11**; compound **23** versus **17**). However this relationship is not retained with the rather weak **24** (K_i 497.4 ± 27.1 μM) versus the inactive **18**.

4. The methyl ether (**25**) and the phosphate (**4**) are less active than the parent alcohol (**1**). The carboxyl acid derivatives (**6**) and (**7**) are inactive, but these are singular examples of these structural types.

5. In the 20:*x*, *n*-6 series, *x* has to be 3 or 4; two double bonds only leads to inactivation.

6. In the *n*-3 series, the limited data suggest that the derived ethanolamides are either inactive or less active than related compounds in the *n*-6 series.

7. Alkylation or dialkylation of the α carbon adjacent to the carbonyl group retains the level of binding in the case of anandamide (compounds **48**, **49**); however α-monomethylation or α,α-dimethylation of N-propyl derivatives (compounds **50**−**53**) potentiated binding and led to the most active compounds seen in the present work (K_i values of 6.9 ± 0.7 to 8.4 ± 1.1 nM).

8. We have confirmed previous work that the presence of a chiral center on the *N*-alkyl substituent leads to enantiomers with significantly different levels of binding.

Experimental Section

Chemistry. ^{1}H NMR spectra were measured on a Varian VXR-300S spectrophotometer using TMS as the internal standard. All chemical shifts are reported in ppm. Specific rotations were detected with a Perkin-Elmer 141 polarimeter. Melting points (uncorrected) were determined on a Buchi 530 apparatus. Column chromatography was performed with ICN silica 60A. Elemental analyses were obtained for all the newly synthesized compounds and are ±0.4% of the theoretical values.

All amines and amino alcohols were obtained from Aldrich Chemical Co. Amino acids and fatty acids were obtained from Sigma Chemical Co.

Anandamides **1**, **2**, and **3** were prepared as described previously.[1]

***N*-Hydroxysuccinimide Ester of Arachidonic Acid (5).** Arachidonic acid (440 mg, 1.44 mmol) was added to a solution of *N*-hydroxysuccinimide (184 mg, 1.6 mmol) in dry ethyl acetate (10 mL). After 5 min of stirring, a solution of dicyclohexylcarbodiimide (330 mg, 1.6 mmol) in dry ethyl acetate (2 mL) was added, and the reaction mixture was left overnight at room temperature under a nitrogen atmosphere. Dicyclohexylurea was filtered, and the crude material was chromatographed on silica gel (eluting with chloroform) to give 440 mg (76%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.32−5.43 (m, 8H), 2.82−2.84 (m, 10H), 2.62 (t, J = 7.5 Hz, 2H), 2.19−2.22 (m, 2H), 2.04−2.07 (m, 2H), 1.80−1.86 (m, 2H), 1.28−1.38 (m, 6H), 0.89 (t, J = 6.9 Hz, 3H). Anal. ($C_{24}H_{35}NO_4$) C, H, N.

Anandamide *O*-Phosphate (4). A solution of *N*-hydroxysuccinimide ester of arachidonic acid (**5**) (410 mg, 1.02 mmol) in tetrahydrofuran (5 mL) was added to a solution of *O*-phosphoethanolamine (360 mg, 2.55 mmol) and sodium bicarbonate (536 mg, 6.38 mmol) in water (3 mL). The reaction mixture was left overnight at room temprature under a nitrogen atmosphere, then it was acidified to pH 2 with 0.1 N HCl, and the organic solvent was evaporated under reduced pressure. After addition of water (50 mL), the product was extracted with methylene chloride (4 × 50 mL) and dried ($MgSO_4$), and solvent was evaporated to give 265 mg (60%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.34−5.42 (m, 8H), 3.98 (br s, 2H), 3.46 (br s, 2H), 2.77−2.81 (m, 6H), 2.26 (t, J = 6.8 Hz, 2H), 2.00−2.06 (m, 4H), 1.60−1.69 (m, 2H), 1.29−1.42 (m, 6H), 0.88 (t, J = 6.9 Hz, 3H). Anal. ($C_{22}H_{38}NO_5P$) C, H, N.

***N*-Arachidonoylglycine (6).** To a solution of arachidonic acid (150 mg, 0.49 mmol) and *N*,*N*-dimethylformamide (38 μL, 0.49 mmol) in dry methylene chloride (5 mL) was added dropwise oxalyl chloride (2.0 M solution in methylene chloride, 0.49 mL, 0.98 mmol) under nitrogen atmosphere. The reaction mixture was stirred for 1 h and then the solvent was evaporated under a nitrogen flow. The crude material in methylene chloride (5 mL) was added to a solution of glycine (110 mg, 1.46 mmol) and 2 N potassium hydroxide in an ice bath. Then, the reaction mixture was stirred for 1 h, water (10 mL) was added, and the mixture was acidified to pH 3 with 1 N HCl. The product was extracted with ether (3 × 50 mL) and dried ($MgSO_4$), and solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel (eluting with ether:petroleum ether, 40:6) to give 60 mg (34%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.25 (br s, 1H), 5.30−5.37 (m, 8H), 4.05 (d, J = 5.1 Hz, 2H), 2.76−2.82 (m,

6H), 2.22 (t, J = 7.8 Hz, 2H), 2.04−2.18 (m, 2H), 1.70−1.82 (m, 4H), 1.25−1.35 (m, 6H), 0.89 (t, J = 7.1 Hz, 3H). Anal. ($C_{22}H_{35}NO_3$) C, H, N.

***N*-Arachidonoyl-D-serine (7a)** was prepared as described by Shinitzky et al. for *N*-stearoylserine.[34] A solution of D-serine (190 mg, 1.8 mmol) in 20 mL of 0.1 M sodium carbonate and 0.1 M sodium bicarbonate was added to a solution of *N*-hydroxysuccinimide ester of arachidonic acid (200 mg, 0.5 mmol) in tetrahydrofuran (20 mL). The reaction mixture was stirred overnight at 35 °C, evaporated down to 20 mL, and acidified to pH 1 with 1 N HCl. The product was extracted with methylene chloride (2 × 30 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give 118 mg (60%) as a colorless oil. ^{1}H NMR (CD_3OD) δ 5.30−5.43 (m, 8H), 4.49 (t, J = 4.8 Hz, 1H), 3.80−3.88 (m, 2H), 2.80−2.86 (m, 6H), 2.30 (t, J = 6.6 Hz, 2H), 2.04−2.18 (m, 4H), 1.66−1.72 (m, 2H), 1.29−1.39 (m, 6H), 0.90 (t, J = 6.9 Hz, 3H); $[\alpha]^{25}_D$ −8.9° (c = 1, $CHCl_3$). Anal. ($C_{23}H_{37}NO_4$) C, H, N.

***N*-Arachidonoyl-L-serine (7b)** was prepared by the same method as for arachidonoyl-D-serine **(7a)** to give 107 mg (55%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 5.33−5.40 (m, 8H), 4.50 (t, J = 4.8 Hz, 1H), 3.78−3.90 (m, 2H), 2.80−2.86 (m, 6H), 2.29 (t, J = 6.6 Hz, 2H), 2.04−2.18 (m, 4H), 1.64−1.72 (m, 2H), 1.29−1.39 (m, 6H), 0.90 (t, J = 7.2 Hz, 3H); $[\alpha]^{25}_D$ +8.9° (c = 1, $CHCl_3$). Anal. ($C_{23}H_{37}NO_4$) C, H, N.

General Procedure for Preparing Compounds 11−25 and 27−35. The following procedure was used to prepare the amide derivatives of arachidonic acid and the other fatty acid amides. The preparation of *N*-ethyl arachidonoylamide (**11**) is given as a representative example.

***N*-Ethyl Arachidonoyl Amide (11).** To a solution of arachidonic acid (100 mg, 0.33 mmol) and *N*,*N*-dimethylformamide (25 μL, 0.33 mmol) in dry methylene chloride (5 mL) was added dropwise oxalyl chloride (2.0 M solution im methylene chloride, 0.33 mL, 0.66 mmol) under nitrogen atmosphere. The reaction mixture was stirred for 1 h and then the solvent was evaporated under nitrogen flow. The crude material in methylene chloride (5 mL) was added to an ice-cold solution of ethylamine (70 wt % solution in water, 0.2 mL, 3.55 mmol) in methylene chloride (5 mL). The reaction mixture was stirred for 15 min, then it was washed with water (3 × 20 mL) and dried ($MgSO_4$), and solvent was evaporated under reduced pressure. The residue was chromatographed on silica gel (eluting with chloroform:petroleum ether, 40:6) to give 80 mg (73%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.31−5.41 (m, 8H), 3.22−3.34 (m, 2H), 2.78−2.84 (m, 6H), 2.01−2.18 (m, 6H), 1.68−1.78 (m, 4H), 1.22−1.40 (m, 6H), 1.13 (t, J = 7.3 Hz, 3H), 0.88 (t, J = 7.1 Hz, 3H). Anal. ($C_{22}H_{37}NO$) C, H, N.

***N*-Methyl arachidonoyl amide (12)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and methylamine (40 wt % solution in water, 0.12 mL, 3.55 mmol) as described for compound **11**: yield, 70 mg (67%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.80 (br s, 1H) 5.30−5.40 (m, 8H), 2.78−2.85 (m, 9H), 2.03−2.19 (m, 6H), 1.66−1.76 (m, 2H), 1.25−1.34 (m, 6H), 0.88 (t, J = 9 Hz, 3H). Anal. ($C_{22}H_{37}NO$) C, H, N.

Arachidonoyl amide (13)[16,29] was prepared from arachidonic acid (100 mg, 0.33 mmol) and ammonium hydroxide (0.2 mL, 5.2 mmol) as described for compound **11**: yield, 80 mg (80%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.82 (br s, 1H) 5.31−5.42 (m, 8H), 2.79−2.85 (m, 6H), 2.23 (t, J = 8.1 Hz, 2H), 2.04−2.15 (m, 4H), 1.70−1.77 (m, 2H), 1.25−1.38 (m, 6H), 0.89 (t, J = 6.8 Hz, 3H). Anal. ($C_{22}H_{33}NO$): C, H, N.

***N*-Propyl arachidonoyl amide (14)**[17] was prepared from arachidonic acid (100 mg, 0.33 mmol) and n-propylamine (0.27 mL, 3.3 mmol) as described for compound **11**: yield, 81 mg (71%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.62 (br s, 1H) 5.26−5.38 (m, 8H), 3.13 (q, J = 6 Hz, 2H), 2.62−2.80 (m, 6H), 2.10 (t, J = 7.3 Hz, 2H), 1.96−2.06 (m, 4H), 1.56−1.66 (m, 2H), 1.38−1.48 (m, 2H), 1.20−1.32 (m, 6H), 0.79−0.86 (m, 6H).

***N*-Isopropyl arachidonoyl amide (15)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and isopropylamine (0.28mL, 3.3 mmol) as described for compound **11**: yield, 52 mg (46%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.44 (m, 8H), 4.02−4.12 (m, 1H), 2.76−2.86 (m, 6H), 2.02−2.16 (m, 6H), 1.66−1.76 (m, 2H), 1.26−1.38 (m, 6H), 1.14 (d, J = 6.6 Hz, 6H), 0.89 (t, J = 6.9 Hz, 3H).

***N*-Butyl arachidonoyl amide (16)**[17] was prepared from arachidonic acid (100 mg, 0.33 mmol) and *n*-butylamine (0.33 mL, 3.3 mmol) as described for compound **11**: yield, 70 mg (56%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.26−5.44 (m, 8H), 3.24 (q, J = 6 Hz, 2H), 2.76−2.86 (m, 6H), 2.02−2.22 (m, 6H), 1.64−1.78 (m, 2H), 1.22−1.56 (m, 10H), 0.86−0.98 (m, 6H).

***N*-*tert*-Butyl arachidonoyl amide (17)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and *tert*-butylamine (0.35 mL, 3.3 mmol) as described for compound **11**: yield, 85 mg (72%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.40 (m, 8H), 5.22 (br s, 1H), 2.76−2.84 (m, 6H), 2.02−2.16 (m, 6H), 1.62−1.70 (m, 2H), 1.33 (s, 9H), 1.22−1.30 (m, 6H), 0.89 (t, J = 6.9 Hz, 3H).

***N*-Amyl arachidonoyl amide (18)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and amylamine (0.38 mL, 3.3 mmol) as described for compound **11**: yield, 90 mg (73%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.70 (br s, 1H), 5.23−5.33 (m, 8H), 3.15 (q, J = 3 Hz, 2H), 2.71−2.77 (m, 6H), 1.97−2.16 (m, 6H), 1.58−1.68 (m, 2H), 1.18−1.42 (m, 12H), 0.79−0.85 (m, 6H). Anal. ($C_{25}H_{43}NO$) C, H, N.

***N*-(3-Methylbutyl) arachidonoyl amide (19)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and isoamylamine (0.38 mL, 3.3 mmol) as described for compound **11**: yield, 87 mg (71%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 3.26 (q, J = 3 Hz, 2H), 2.76−2.86 (m, 6H), 2.02−2.20 (m, 6H), 1.68−1.78 (m, 2H), 1.54−1.64 (m, 1H), 1.26−1.40 (m, 8H), 0.82−0.96 (m, 9H). Anal. ($C_{25}H_{43}NO$) C, H, N.

***N*-(1,1-Dimethylpropyl) arachidonoyl amide (20)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and 1,1-dimethylpropylamine (0.38mL, 3.3 mmol) as described for compound **11**: yield, 71 mg (58%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.28−5.38 (m, 8H), 5.10 (br s, 1H), 2.67−2.82 (m, 6H), 2.04−2.12 (m, 6H), 1.66−1.76 (m, 4H), 1.24−1.32 (m, 12H), 0.81−0.90 (m, 6H). Anal. ($C_{25}H_{43}NO$) C, H, N.

***N*-((*R*-(−)-1-Methylpropyl) arachidonoyl amide (21a)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and (*R*)-(−)-*sec*-butylamine (0.33 mL, 3.3 mmol) as described for compound **11**: yield, 75 mg (63%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 5.20 (br s, 1H), 3.86−4.00 (m, 1H), 2.75−2.86 (m, 6H), 2.00−2.20 (m, 6H), 1.65−1.80 (m, 2H), 1.22−1.50 (m, 8H), 1.10 (d, J = 7.5 Hz, 3H), 0.82−0.92 (m, 6H); $[\alpha]^{25}_D$= −7.08° (c = 1, EtOH). Anal. ($C_{24}H_{41}NO$) C, H, N.

***N*-((*S*)-(+)-1-Methylpropyl) arachidonoyl amide (21b)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and (*S*)-(+)-*sec*-butylamine (0.33 mL, 3.3 mmol) as described for compound **11**: yield, 65 mg (55%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 5.22 (br s, 1H), 3.88−3.98 (m, 1H), 2.75−2.90 (m, 6H), 2.00−2.20 (m, 6H), 1.65−1.76 (m, 2H), 1.22−1.50 (m, 8H), 1.12 (d, J = 7.5 Hz, 3H), 0.82−0.96 (m, 6H); $[\alpha]^{25}_D$ +7.08° (c = 1, EtOH). Anal. ($C_{24}H_{41}NO$) C, H, N.

***N*-(3-Hydroxypropyl) arachidonoyl amide (22)**[16,17,22,23] was prepared from arachidonic acid (100 mg, 0.33 mmol) and 3-amino-1-propanol (0.25 mL, 3.3 mmol) as described for compound **11**: yield, 81 mg (68%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 6.42 (br s, 1H), 5.22−5.38 (m, 8H), 3.90 (br s, 1H), 3.54 (t, J = 5.5 Hz, 2H), 3.32 (q, J = 6 Hz, 2H), 2.62−2.80 (m, 6H), 1.92−2.18 (m, 6H), 1.50−1.70 (m, 4H), 1.20−1.34 (m, 6H), 0.90 (t, J = 7 Hz, 3H).

***N*-(1,1-Dimethyl-2-hydroxyethyl) arachidonoyl amide (23)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and 2-amino-2-methyl-1-propanol (0.32 mL, 3.3 mmol) as described for compound **11**: yield, 60 mg (48%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.50 (br s, 1H), 5.28−5.40 (m, 8H), 3.57 (s, 2H), 2.78−2.90 (m, 6H), 2.02−2.20 (m, 6H), 1.62−1.74 (m, 2H), 1.20−1.40 (m, 12H), 0.89 (t, J = 7.1 Hz, 3H). Anal ($C_{24}H_{41}NO_2$): C, H, N.

***N*-(5-Hydroxypentyl) arachidonoyl amide (24)**[17,22,23] was prepared from arachidonic acid (100 mg, 0.33 mmol) and 5-amino-1-pentanol (340 mg, 3.3 mmol) as described for compound **11**: yield, 78 mg (61%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.60 (br s, 1H), 5.22−5.42 (m, 8H), 3.64 (t, J = 5.5 Hz, 2H), 3.20−3.32 (m, 2H), 2.76−2.89 (m, 6H), 2.00−2.22 (m,

6H), 1.90 (br s, 1H), 1.20−1.80 (series of m, 14H), 0.89 (t, J = 7.1 Hz, 3H). Anal. ($C_{25}H_{43}NO_2$): C, H, N.

***N*-(2-Methoxyethyl) arachidonoyl amide (25)**[17,22,23] was prepared from arachidonic acid (100 mg, 0.33 mmol) and methoxyethylamine (0.43 mL, 3.3 mmol) as described for compound **11**: yield, 75 mg (41%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.80 (br s, 1H), 5.30−5.42 (m, 8H), 3.45 (d, J = 2.1 Hz, 4H), 3.35 (s, 3H), 2.75-2.85 (m, 6H), 2.00−2.20 (m, 6H), 1.62−1.80 (m, 2H), 1.20−1.40 (m, 6H), 0.89 (t, J = 6.8 Hz, 3H).

***N*′-Arachidonoyl-*N*″-diethylethylenediamine (27)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and *N*,*N*-diethylenediamine (0.46 mL, 3.3 mmol) as described for compound **11**: yield, 93 mg (70%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 6.10 (br s, 1H), 5.26−5.42 (m, 8H), 3.22−3.30 (m, 2H), 2.76−2.90 (m, 6H), 2.50−2.62 (m, 6H), 2.00−2.20 (m, 6H), 1.66−1.80 (m, 2H), 1.20−1.40 (m, 6H), 1.02 (t, J = 7.1 Hz, 6H), 0.89 (t, J = 6.5 Hz, 3H). Anal. ($C_{26}H_{46}N_2O$) C, H, N.

***N*,*N*-Dimethyl arachidonoyl amide (28)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and dimethylamine (40% aqueous solution, 0.17 mL, 1.4 mmol) as described for compound **11**: yield, 65 mg (59%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.24−5.44 (m, 8H), 2.99 (s, 3H), 2.94 (s, 3H), 2.76−2.86 (m, 6H), 2.31 (t, J = 7 Hz, 2H), 2.00−2.18 (m, 4H), 1.66−1.78 (m, 2H), 1.20−1.38 (m, 6H), 0.89 (t, J = 7.1 Hz, 3H). Anal. ($C_{22}H_{37}NO$) C, H, N.

***N*,*N*-Diethyl arachidonoyl amide (29)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and diethylamine (0.34 mL, 3.3 mmol) as described for compound **11**: yield, 78 mg (66%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 3.20−3.42 (m, 4H), 2.76−2.86 (m, 6H), 2.29 (t, J = 7.1 Hz, 2H), 2.00−2.20 (m, 4H), 1.60−1.80 (m, 2H), 1.22−1.40 (m, 6H), 1.04−1.20 (m, 6H), 0.90 (t, J = 6.1 Hz, 3H). Anal. ($C_{24}H_{41}NO$) C, H, N.

***N*-Methyl *N*-(2-hydroxyethyl) arachidonoyl amide (30)**[19] was prepared from arachidonic acid (152 mg, 0.5 mmol) and 2-(methylamino)ethanol (0.40 mL, 5 mmol) as described for compound **11**: yield, 136 mg (75%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 3.77 (t, J = 5 Hz, 2H), 3.55 (t, J = 4.6 Hz, 2H) 3.06 (s, 3H), 2.78−2.85 (m, 6H), 2.35 (t, J = 7.8 Hz, 2H), 2.04−2.15 (m, 4H), 1.64−1.75 (m, 2H), 1.25−1.38 (m, 6H), 0.89 (t, J = 6.8 Hz, 3H). Anal. ($C_{23}H_{39}NO_2$) C, H, N.

***N*-Ethyl *N*-(2-hydroxyethyl) arachidonoyl amide (31)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and 2-(ethylamino)ethanol (0.49 mL, 5 mmol) as described for compound **11**: yield, 156 mg (83%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.31−5.42 (m, 8H), 3.75 (t, J = 4.6 Hz, 2H), 3.51 (t, J = 4.6 Hz, 2H), 3.34 (q, J = 7.1 Hz, 2H), 2.79−2.86 (m, 6H), 2.35 (t, J = 7.8 Hz, 2H), 2.04−2.15 (m, 4H), 1.64−1.77 (m, 2H), 1.25−1.40 (m, 6H), 1.19 (t, J = 7.1 Hz, 3H), 0.89 (t, J = 7.1 Hz, 3H). Anal. ($C_{24}H_{41}NO_2$) C, H, N.

***N*-Propyl *N*-(2-hydroxyethyl) arachidonoyl amide (32)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and 2-(propylamino)ethanol (0.57 mL, 5 mmol) as described for compound **11**: yield, 119 mg (61%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 3.76 (t, J = 4.9 Hz, 2H), 3.52 (t, J = 4.4 Hz, 2H), 3.24 (t, J = 8.1 Hz, 2H), 2.79−2.86 (m, 6H), 2.35 (t, J = 8.1 Hz, 2H), 2.02−2.20 (m, 4H), 1.54−1.76 (m, 4H), 1.25−1.38 (m, 6H), 0.86−0.89 (m, 6H). Anal. ($C_{25}H_{43}NO_2$) C, H, N.

***N*,*N*-(Di-2-hydroxyethyl) arachidonoyl amide (33)** was prepared from arachidonic acid (100 mg, 0.33 mmol) and diethanolamine (0.32 mL, 3.3 mmol) as described for compound **11**: yield, 58 mg (45%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 3.83 (t, J = 4.9 Hz, 2H), 3.77 (t, J = 5.1 Hz, 2H), 3.54 (t, J = 5.1 Hz, 2H), 3.49 (t, J = 4.9 Hz, 2H), 2.77−2.86 (m, 6H), 2.40 (t, J = 7.3 Hz, 2H), 2.02−2.16 (m, 4H), 1.69−1.76 (m, 2H), 1.25−1.38 (m, 6H), 0.88 (t, J = 6.6 Hz, 3H). Anal. ($C_{24}H_{41}NO_3$) C, H, N.

***N*-Hydroxy *N*-arachidonoyl amide (34)**[29] was prepared from arachidonic acid (200 mg, 0.66 mmol), hydroxylamine hydrochloride (220 mg, 3.17 mmol) and triethylamine (0.44 mL, 3.17 mmol) as described for compound **11**: yield, 103 mg (49%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 5.30−5.43 (m, 8H), 2.78−2.86 (m, 6H), 2.02−2.17 (m, 6H), 1.70−1.78 (m, 2H), 1.22−1.38 (m, 6H), 0.89 (t, J = 6.9 Hz, 3H).

(±)-*N*-(1-Methyl-2-hydroxy-2-phenylethyl) arachidonoyl amide (35) was prepared from arachidonic acid (152 mg, 0.5 mmol), (±)-phenylpropanolamine hydrochloride (938 mg, 5 mmol) and triethylamine (0.7 mL, 5 mmol) as described for compound **11**: yield, 146 mg (67%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.27−7.35 (m, 5H), 5.54 (br s, 1H), 5.30−5.41 (m, 8H), 4.84 (br s, 1H), 4.31−4.36 (m, 1H), 3.60 (br s, 1H), 2.78−2.85 (m, 6H), 2.01−2.22 (m, 6H), 1.67−1.77 (m, 2H), 1.25−1.37 (m, 6H), 1.01 (d, J = 7.1 Hz, 3H), 0.89 (t, J = 7.1 Hz, 3H). Anal. ($C_{29}H_{43}NO_2$) C, H, N.

Compounds 36−47 were synthesized as described for anandamide (**1**).[1]

Anandamide (20:2, *n*-6) (36) was prepared from *cis*-11,-14-eicosadienoic acid (100 mg, 0.32 mmol) and ethanolamine (0.19 mL, 3.2 mmol) in 81% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.90 (br s, 1H), 5.30−5.38 (m, 4H), 3.70 (t, J = 4.4 Hz, 2H), 3.40−3.45 (m, 2H), 2.78 (t, J = 5.4 Hz, 2H), 2.18 (t, J = 7.1 Hz, 2H), 2.00−2.12 (m, 2H), 1.50−1.70 (m, 8H), 1.30 (br s, 8H), 0.89 (t, J = 7.5 Hz, 3H). Anal. ($C_{22}H_{37}NO_2$) C, H, N.

Anandamide (18:3, *n*-6) (37)[16,20] was prepared from *cis*-6,9,12-octadecatrienoic acid (100 mg, 0.36 mmol) and ethanolamine (0.22 mL, 3.6 mmol) in 79% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.13 (br s, 1H), 5.29−5.41 (m, 6H), 3.71 (t, J = 5.1 Hz, 2H), 3.41 (q, J = 5.1 Hz, 2H), 2.80 (t, J = 5.7 Hz, 4H), 2.20 (t, J = 8.1 Hz, 2H), 2.00−2.12 (m, 4H), 1.60−1.70 (m, 4H), 1.31 (br s, 6H), 0.88 (t, J = 7.5 Hz, 3H).

Anandamide (18:2, *n*-6) (38) was prepared from *cis*-9,*cis*-12-octadecadienoic acid (200 mg, 0.71 mmol) and ethanolamine (0.43 mL, 7.1 mmol) in 84% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.90 (br s, 1H), 5.30−5.39 (m, 4H), 3.72 (t, J = 5.1 Hz, 2H), 3.42 (q, J = 5.4 Hz, 2H), 2.76 (t, J = 5.7 Hz, 2H), 2.20 (t, J = 8.1 Hz, 2H), 2.01−2.06 (m, 4H), 1.60−1.70 (m, 2H), 1.30 (br s, 14H), 0.88 (t, J = 7.2 Hz, 3H). Anal. ($C_{20}H_{37}NO_2$) C, H, N.

Anandamide (20:3, *n*-3) (39) was prepared from *cis*-11,-14,17-eicosatrienoic acid (100 mg, 0.33 mmol) and ethanolamine (0.20 mL, 3.3 mmol) in 50% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.90 (br s, 1H), 5.30−5.39 (m, 4H), 3.72 (t, J = 5.1 Hz, 2H), 3.42 (q, J = 5.4 Hz, 2H), 2.80 (t, J = 5.7 Hz, 4H), 2.20 (t, J = 8.1 Hz, 2H), 2.01−2.12 (m, 4H), 1.56−1.67 (m, 4H), 1.26 (br s, 10H), 0.97 (t, J = 7.5 Hz, 3H). Anal. ($C_{22}H_{39}NO_2$) C, H, N.

Anandamide (18:4, *n*-3) (40) was prepared from *cis*-6,9,-12,15-octadecatetraenoic acid (50 mg, 0.18 mmol) and ethanolamine (0.11 mL, 1.8 mmol) in 76% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.92 (br s, 1H), 5.33−5.40 (m, 8H), 3.72 (t, J = 5.1 Hz, 2H), 3.42 (q, J = 5.2 Hz, 2H), 2.79−2.84 (m, 6H), 2.22 (t, J = 7.8 Hz, 2H), 2.02−2.12 (m, 4H), 1.60−1.72 (m, 2H), 1.38−1.48 (m, 2H), 0.98 (t, J = 7.5 Hz, 3H). Anal. ($C_{20}H_{33}NO_2$) C, H, N.

Anandamide (18:3, *n*-3) (41) was prepared from *cis*-9,12,-15-octadecatrienoic acid (100 mg, 0.36 mmol) and ethanolamine (0.22 mL, 3.6 mmol) in 74% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.13 (br s, 1H), 5.29−5.42 (m, 6H), 3.71 (t, J = 5.1 Hz, 2H), 3.41 (q, J = 5.1 Hz, 2H), 2.80 (t, J = 5.7 Hz, 4H), 2.20 (t, J = 8.1 Hz, 2H), 2.04−2.12 (m, 4H), 1.63 (t, J = 6.9 Hz, 2H), 1.31 (br s, 10H), 0.97 (t, J = 7.5 Hz, 3H). Anal. ($C_{20}H_{35}NO_2$) C, H, N.

Anandamide (20:5, *n*-3) (42)[22,23] was prepared from *cis*-5,8,11,14,17-eicosapentaenoic acid (50 mg, 0.16 mmol) and ethanolamine (0.10 mL, 1.6 mmol) in 72% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.90 (br s, 1H), 5.22−5.42 (m, 10H), 3.71 (t, J = 5.1 Hz, 2H), 3.41 (q, J = 4.9 Hz, 2H), 2.79−2.86 (m, 8H), 2.05−2.22 (m, 6H), 1.60−1.72 (m, 2H), 0.97 (t, J = 7.9 Hz, 3H).

Anandamide (22:6, *n*-3) (43)[16] was prepared from *cis*-4,7,-10,13,16,19-docosahexaenoic acid (100 mg, 0.3 mmol) and ethanolamine (0.18 mL, 3 mmol) in 65% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.90 (br s, 1H), 5.28−5.42 (m, 12H), 3.72 (t, J = 5.1 Hz, 2H), 3.41 (q, J = 4.9 Hz, 2H), 2.77−2.88 (m, 10H), 2.42 (t, J = 8.1 Hz, 2H), 2.22−2.30 (m, 2H), 2.02−2.14 (m, 2H), 0.97 (t, J = 7.8 Hz, 3H).

Anandamide (20:1, *n*-9) (44)[20,22,23] was prepared from *cis*-11-eicosenoic acid (100 mg, 0.32 mmol) and ethanolamine (0.19 mL, 3.2 mmol) in 70% yield: mp 67−68 °C; ^{1}H NMR ($CDCl_3$)

δ 5.90 (br s, 1H), 5.32−5.36 (m, 2H), 3.70 (t, J = 5.1 Hz, 2H), 3.40−3.42 (m, 2H), 2.18 (t, J = 7.8 Hz, 2H), 1.96−2.02 (m, 4H), 1.54−1.63 (m, 6H), 0.86 (t, J = 6.1 Hz, 3H). Anal. ($C_{22}H_{43}NO_2$) C, H, N.

Anandamide (16:0) (45)[16,20] was prepared from palmitic acid (100 mg, 0.39 mmol) and ethanolamine (0.23 mL, 3.9 mmol) in 79% yield: mp 100−101 °C; ^{1}H NMR ($CDCl_3$) δ 6.00 (br s, 1H), 3.72 (t, J = 5.1 Hz, 2H), 3.41 (q, J = 4.9 Hz, 2H), 2.22 (t, J = 7.8 Hz, 2H), 1.56−1.80 (m, 4H), 1.00−1.40 (m, 22H), 0.86 (t, J = 6.1 Hz, 3H).

Oleoyl amide (46)[31] was prepared from oleic acid (882 mg, 3.12 mmol) and ammonium hydroxide (2.34 mL, 0.12 mol) in 60% yield: mp 74−75 °C; ^{1}H NMR ($CDCl_3$) δ 5.42 (br s, 2H), 5.32−5.36 (m, 2H), 2.22 (t, J = 7.8 Hz, 2H), 1.98−2.02 (m, 6H), 1.62−1.66 (m, 4H), 1.27−1.31 (m, 16H), 0.88 (t, J = 6.9 Hz, 3H).

***cis*-5,8,11,14-Eicosatetrayoyl ethanolamide (47)** was prepared from *cis*-5,8,11,14-eicosatetrayoic acid (25 mg, 0.084 mmol) and ethanolamine (0.05 mL, 0.84 mmol) in 64% yield as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.15 (br s, 1H), 3.73 (t, J = 5.1 Hz, 2H), 3.42 (q, J = 4.9 Hz, 2H), 3.06−3.22 (m, 6H), 2.36 (t, J = 7.8 Hz, 2H), 2.20−2.24 (m, 2H), 2.10−2.18 (m, 2H), 1.78−1.88 (m, 2H), 1.40−1.56 (m, 2H), 1.20−1.38 (m, 4H), 0.90 (t, J = 6.1 Hz, 3H). Anal. ($C_{22}H_{29}NO_2$) C, H, N.

Compounds 48−57. α-Methylarachidonic acid was synthesized from methyl arachidonate with 2 equivalents of LDA (lithium diisopropylamine, prepared *in situ*) and methyl iodide at −50 °C, as described by Adams et al.[19] The α,α-dimethyl arachidonic acid was synthesized by recycling the α-methylarachidonic acid through the same reaction a second time.

After hydrolysis of the ester with lithium hydroxide in 3:1 methanol:water, the acid derivatives were converted to the corresponding amides by reaction with oxalyl chloride and the appropriate amine.

α-Methyl anandamide (48)[19,22] was prepared from arachidonic acid (152 mg, 0.5 mmol) and ethanolamine (0.15 mL, 2.5 mmol) in 82% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.10 (br s, 1H), 5.22−5.40 (m, 8H), 3.75 (t, J = 5.1 Hz, 2H), 3.42 (q, J = 4.9 Hz, 2H), 2.74−2.86 (m, 6H), 2.20−2.30 (m, 1H), 2.00−2.12 (m, 4H), 1.68−1.80 (m, 1H), 1.20−1.50 (m, 6H), 1.16 (d, J = 6.6 Hz, 3H), 0.89 (t, J = 6.9 Hz, 3H).

α,α-Dimethyl anandamide (49)[19,22,23] was prepared from arachidonic acid (152 mg, 0.5 mmol) and ethanolamine (0.15 mL, 2.5 mmol) in 47% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.10 (br s, 1H), 5.30−5.42 (m, 8H), 3.75 (t, J = 5.1 Hz, 2H), 3.42 (q, J = 4.9 Hz, 2H), 2.78−2.86 (m, 6H), 2.00−2.10 (m, 4H), 1.50−1.62 (m, 4H), 1.24−1.40 (m, 4H), 1.22 (s, 6H), 0.90 (t, J = 6.9 Hz, 3H).

***N*-Propyl α-methylarachidonoyl amide (50)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and propylamine (0.21 mL, 2.5 mmol) in 84% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.20−5.45 (m, 9H), 3.18−3.25 (m, 2H), 2.70−2.86 (m, 6H), 2.00−2.20 (m, 5H), 1.70−1.80 (m, 1H), 1.40−1.60 (m, 4H), 1.10 (d, J = 6.6 Hz, 3H), 0.86−0.95 (m, 6H). Anal ($C_{24}H_{41}NO$): C, H, N.

***N*-Propyl α,α-dimethylarachidonoyl amide (51)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and propylamine (0.21 mL, 2.5 mmol) in 70% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.60 (br s, 1H), 5.30−5.40 (m, 8H), 3.20−3.23 (m, 2H), 2.79−2.84 (m, 6H), 2.00-2.10 (m, 4H), 1.20−1.60 (series of m, 10H), 1.15 (s, 6H), 0.86−0.94 (m, 6H). Anal. ($C_{25}H_{43}NO$) C, H, N.

***N*-Isopropyl α-methylarachidonoyl amide (52)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and isopropylamine (0.21 mL, 2.5 mmol) in 85% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.30−5.42 (m, 8H), 4.02−4.14 (m, 1H), 2.76−2.90 (m, 6H), 1.92−2.12 (m, 5H), 1.10−1.60 (series of m, 17H), 0.89 (t, J = 6.9 Hz, 3H). Anal. ($C_{24}H_{41}NO$) C, H, N.

***N*-Isopropyl α,α-dimethylarachidonoyl amide (53)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and isopropylamine (0.21 mL, 2.5 mmol) in 78% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.20−5.50 (m, 9H), 4.04−4.14 (m, 1H), 2.76−2.90 (m, 6H), 1.90−2.15 (m, 4H), 1.10−1.60 (series of m, 20H), 0.90 (t, J = 6.9 Hz, 3H). Anal. ($C_{25}H_{43}NO$) C, H, N.

***N*-((*S*)-(+)-2-hydroxypropyl) α,α-dimethylarachidonoyl amide (54)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and (*S*)-(+)-1-amino-2-propanol (0.16 mL, 2 mmol) in 57% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.95 (br s, 1H), 5.30−5.42 (m, 8H), 3.92−3.96 (m, 1H), 3.40−3.50 (m, 1H), 3.02-3.23 (m, 1H), 2.70−2.95 (m, 7H), 2.20−2.28 (m, 1H), 2.00−2.15 (m, 4H), 1.70−1.75 (m, 2H), 1.20−1.52 (series of m, 14H), 0.89 (t, J = 6.7 Hz, 3H). Anal. ($C_{25}H_{43}NO_2$) C, H, N.

***N*-((*R*)-(−)-1-Methyl-2-hydroxyethyl) α,α-dimethylarachidonoyl amide (55)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and (*R*)-(−)-2-amino-1-propanol (0.16 mL, 2 mmol) in 45% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.80 (br s, 1H), 5.30−5.42 (m, 8H), 4.01−4.12 (m, 1H), 3.60−3.68 (m, 1H), 3.50-3.55 (m, 1H), 2.98−3.01 (m, 1H), 2.76−2.84 (m, 6H), 1.90−2.10 (m, 4H), 1.15−1.62 (series of m, 17H), 0.90 (t, J = 7.1 Hz, 3H). Anal. ($C_{25}H_{43}NO_2$) C, H, N.

***N*-((*S*)-(+)-1-Methyl-2-hydroxyethyl) α,α-dimethylarachidonoyl amide (56)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and (*S*)-(+)-2-amino-1-propanol (0.16 mL, 2 mmol) in 44% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.80 (br s, 1H), 5.30−5.42 (m, 8H), 4.01−4.12 (m, 1H), 3.60−3.68 (m, 1H), 3.50-3.55 (m, 1H), 2.98−3.01 (m, 1H), 2.76−2.84 (m, 6H), 1.90−2.10 (m, 4H), 1.15−1.62 (series of m, 17H), 0.90 (t, J = 7.1 Hz, 3H). Anal. ($C_{25}H_{43}NO_2$) C, H, N.

***N*-((*R*)-(−)-2-hydroxypropyl) α,α-dimethylarachidonoyl amide (57)** was prepared from arachidonic acid (152 mg, 0.5 mmol) and (*R*)-(−)-1-amino-2-propanol (0.16 mL, 2 mmol) in 63% yield, as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.95 (br s, 1H), 5.30−5.42 (m, 8H), 3.92−3.96 (m, 1H), 3.40−3.50 (m, 1H), 3.02-3.23 (m, 1H), 2.70−2.95 (m, 7H), 2.20−2.28 (m, 1H), 2.00−2.15 (m, 4H), 1.70−1.75 (m, 2H), 1.20−1.52 (series of m, 14H), 0.89 (t, J = 6.7 Hz, 3H). Anal. ($C_{25}H_{43}NO_2$) C, H, N.

Ligand Binding Assay. The synaptosomal brain preparation was made from whole rat brain as described previously.[1,25] Radiolabeled HU-243[25] was stored at a concentration of 1 μM (40.5 μCi/mmol) in the absolute ethanol at −20 °C. Unlabeled drugs were stored as 10 mM and 100 μM ethanol solutions at −20 °C. After evaporation of the ethanol, drug dilutions were performed serially from 1 or 100 μM solutions in a vehicle of 5 mg/mL of fatty acid-free BSA (Sigma Chemical Co., St. Louis, MO) in water. The radioligand was placed in a beaker, the ethanol was evaporated, and then 0.2 mL of 50 mg/mL of BSA was added, followed by 100 mL of TME buffer. This preparation was aliquoted in 0.8 mL volumes to siliconized microfuge tubes (Sigma), followed by 0.1 mL of drug or vehicle and 0.1 mL of synaptosomal membrane preparation in TME buffer. Thus, the final assay volume of 1 ml contained 45 mM Tris-HCl, 2.7 mM $MgCl_2$, 0.9 mM EDTA, 0.58 mg of BSA, 2.4−3.8 μg of protein of synaptosomal membrane preparation, 38−48 fmol of [^{3}H]HU-243, and vehicle or drug.

Tubes were incubated at 30 °C for 90 min and then centrifuged at 13 000 rpm for 6 min. After centrifugation, samples of the supernatant were saved and counted. The remaining supernatant was aspired off, and the tubes were placed inverted on drying pins. After 20 min, the tips of the tubes containing the pelleted membranes were removed with a heated scalpel blade in a rig designed to ensure consistency. The tips were then placed in Opti-Fluor, LSC-coctail (Packard, A Canberra Co.) and vortexed, and after several hours, radioactivity was determined as disintegrations per minute. The radioligand adhering to the remaining part of the vial after tip removal was also determined. Nonspecific binding to the microfuge tip was assessed in tubes containing radioligand and protein, which were not centrifuged. This value was subtracted from the total binding of the cut tips to give the total bound to the pelleted membranes. Specific binding was defined as the difference between the total bound to the pelleted membranes in the absence and presence of 50 nM unlabeled HU-243 and was typically 70−80% of the total bound. Assays were performed in triplicate, and experiments were repeated three times.

Receptor binding assays with transfected COS-7 cells for either CB_1 or CB_2 were performed as described in ref 35.

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(4) In view of the existence of numerous related unsaturated fatty acid ethanolamides which bind to the cannabinoid receptor, we suggest to name this group of compounds "anandamides" with each individual member identified with the parent fatty acid indicated (in parentheses) using the generally accepted fatty acid shorthand designation. Thus the anandamide derived from arachidonic acid will be anandamide (20:4, *n*-6). According to this proposed nomenclature dihomo-γ-linolenylethanolamide (**2**) will be anandamide (20:3, *n*-6) and docosatetraenylethanolamide (**3**) will be anandamide (22:4, *n*-6). When only ther term "anandamide" is used, it refers to arachidonoylethanolamide (**1**).

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