Unique Analogues of Anandamide: Arachidonyl Ethers and Carbamates and Norarachidonyl Carbamates and Ureas

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To examine the effect of changing the amide bond of anandamide (**5**, AN) to a less hydrolyzable moiety, analogues **1a**−**1l**, **2a**−**2c**, **3a**−**3c**, and **4a**−**4h** were synthesized from commercially available arachidonyl alcohol or arachidonic acid and tested for their pharmacological activity. Arachidonyl ethers **1a**−**1k** were obtained through the coupling of the arachidonyl mesylate (**6**) (generated from the mesylation of arachidonyl alcohol) with the appropriate alcohol in potassium hydroxide. Arachidonyl ether **1l** was obtained through the phase-transfer coupling of arachidonyl alcohol with 2-(2-iodoethoxy)tetrahydropyran (which was generated from its bromide) followed by cleavage of the tetrahydropyran group with Dowex resin. Arachidonyl carbamates **2a**−**2c** were obtained through the coupling of arachidonyl alcohol with the appropriate isocyanates. Norarachidonyl carbamates **3a**−**3c** and ureas **4a**−**4h** were obtained through the coupling of the norarachidonyl isocyanate (generated from arachidonic acid using diphenyl phosphorazidate and triethylamine upon heating) with the appropriate alcohols and amines, respectively. AN analogues **1**−**3** have shown poor binding affinities to the CB1 receptor and fail to produce significant pharmacological effect at doses up to 30 mg/kg. Several ether analogues **1** were also evaluated in the CB2 binding assay and were found to be of low affinity. However, norarachidonyl urea analogues **4** have shown generally good binding affinities to the CB1 receptor (K_i = 55−746 nM) and pharmacological activity with AN-like profiles. The most potent analogue of this series is the 2-fluoroethyl analogue **4f** which binds 2 times better than AN and was more active in several mouse behavioral assays. It was also observed that urea analogues **4a** and **4g**, which have weak binding affinities to the CB1 receptor (K_i = 436 and 347 nM, respectively), produced surprisingly potent pharmacological activity. These urea analogues have also shown hydrolytic stability toward the amidase enzymes, responsible for the primary degradation pathway of anandamide, in binding affinity assays in the absence of the enzyme inhibitor PMSF.

Introduction

Since the discovery of anandamide (arachidonylethanolamide, **5**, AN) as a putative endogenous ligand which competitively binds to the central cannabinoid receptor (CB1),[1] it has been established that AN exhibits cannabimimetic effects similar to those of Δ^9-tetrahydrocannabinol (Δ^9-THC).[2−4] AN has a faster onset and shorter duration and is less potent than Δ^9-THC in the pharmacological tests of hypoactivity, analgesia, hypothermia, and catalepsy. In addition, like the THCs, it inhibits forskolin-stimulated adenylate cyclase activity[5,6] as well as calcium currents as a partial agonist in N18 neuroblastoma cells.[7,8]

Despite its faster onset than Δ^9-THC, its short duration is attributed to its susceptibility to rapid enzymatic hydrolysis of the amide bond by amidases.[9−11] This anandamide amidase has been shown to be sensitive to serine protease inhibitors such as phenylmethanesulfonyl fluoride, PMSF.[10] Binding studies using a filtration assay demonstrated that AN displaces [^{3}H]-CP-55940 from rat whole brain P_2 membrane preparations with a K_D value of 89 ± 10 nM in the presence of the enzyme inhibitor PMSF.[12] However, in the absence of PMSF, a K_D value of 5400 ± 1600 nM was observed as a result of metabolic degradation. The hydrolyzable amide moiety was the focus of study for increasing stability and thus longer activity. Altering the headgroup of AN[13−17] met with some success in generating less hydrolyzable amide analogues. Introducing a substituent at the 2-position of the arachidonyl moiety to sterically hinder access to the amide bond also produced analogues which were more stable than AN.[12,14,18−20]

The availability of more stable AN analogues to amidase hydrolysis will prove useful for future in vitro and in vivo studies aimed at delineating their mode of action in the brain. To design novel AN analogues less susceptible to enzymatic hydrolysis, we have replaced the amide bond in AN by a nonhydrolyzable moiety. We employed groups which are chemically somewhat unreactive, are isosteric, and yet can participate in hydrogen bonding, a phenomenon which is very important in ligand−receptor interaction for the CB1 receptor. These analogues were designed to incorporate headgroups of amide analogues which have previously shown good binding affinities.[12−18] With this background, we synthesized the various arachidonyl ethers **1** and carbamates **2** and norarachidonyl carbamates **3** and ureas **4** that are presented in this paper.

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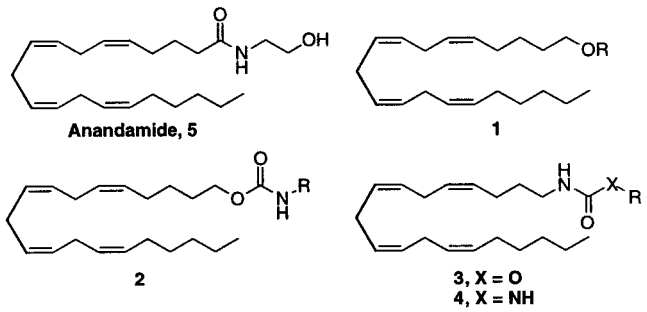


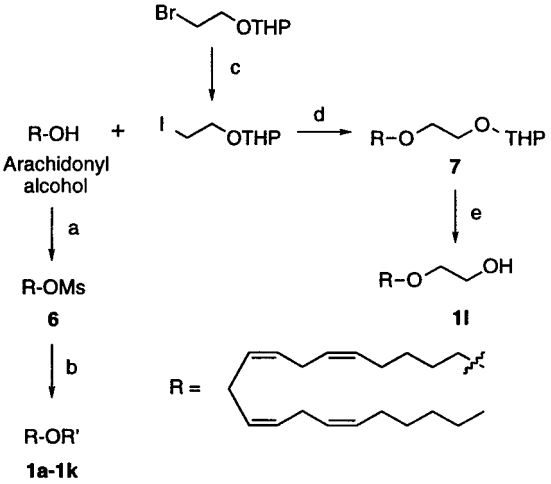
Chemistry

Arachidonyl ethers **1a**−**1k** were prepared by the alcoholic KOH reaction of arachidonyl mesylate (**6**) in the appropriate alcohol at 50 °C (Scheme 1). The arachidonyl mesylate was prepared by treatment of the commercially available arachidonyl alcohol with methanesulfonyl chloride in the presence of triethylamine in methylene chloride. These ether analogues were synthesized in 73−96% yields. Arachidonyl ether **1l** was prepared by the phase-transfer[21] coupling of arachidonyl alcohol with THP-protected 2-iodoethanol (prepared from the corresponding 2-bromo analogue) using tetrabutylammonium bisulfate (TBAB) in 50% aqueous NaOH heated at 55 °C (Scheme 1). The THP group of ether **7** is then cleaved off in the presence of Dowex resin in methanol to give the hydroxyethyl analogue.

The arachidonyl carbamates **2a**−**2c** were prepared by coupling arachidonyl alcohol with the appropriate alkyl isocyanate in benzene at 65 °C (Scheme 2). These carbamate analogues were synthesized in 89−98% yields.

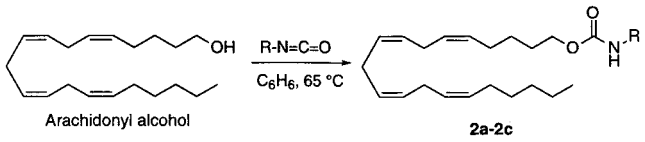
The norarachidonyl carbamates **3a**−**3c** and ureas **4a**−**4h** were prepared by coupling norarachidonyl isocyanate with the appropriate alcohol or amine, respectively, in benzene at 65 °C. The norarachidonyl isocyanate was prepared by treatment of the commercially available arachidonic acid with diphenyl phosphorazidate in the presence of triethylamine in benzene to generate the acyl azide which undergoes a Curtius rearrangement[22,23] upon heating (Scheme 3). These syntheses were carried out in one-pot reactions and afforded the carbamates in 36−64% yields and the ureas in 55−80% yields.

Scheme 1[a]

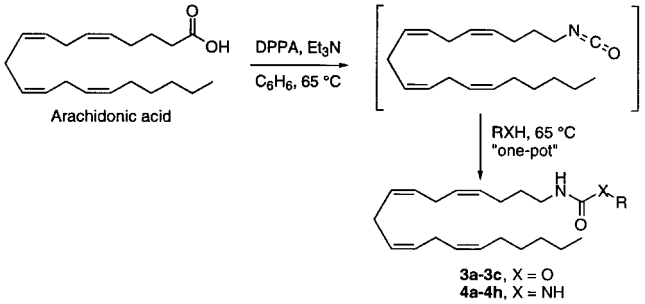


[a] (a) MsCl, Et_3N, CH_2Cl_2, 0 °C−rt; (b) KOH, R′OH, 50 °C; (c) NaI, acetone, reflux; (d) TBAB, 50% NaOH/H_2O, CH_2Cl_2, 50 °C; (e) Dowex 50W-XB, MeOH, rt.

Scheme 2



Scheme 3



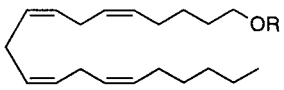
Results and Discussion

The arachidonyl ether analogues **1a**−**1l** (Table 1) were devised such that the carbonyl of AN was reduced to a methylene and the amide nitrogen was replaced by an oxygen; i.e., the *trans*-amide bond has been replaced by an oxymethylene ether linkage. The geometry of the amide bond remains unaffected; this approach has been successfully applied in renin inhibitors.[24] Whereas the oxymethylene moiety is not susceptible to enzymatic hydrolysis, its ability to hydrogen bond is reduced. However, all the arachidonyl ether analogues **1a**−**1l** possessed relatively low affinity to the CB1 receptor, displacing [^{3}H]CP-55,940 with K_i values ranging from 578 to 4260 nM, which show poor competitive binding at the cannabinoid receptor CB1. In vivo testing of some of these analogues supported the correlation of poor in vitro CB1 binding to poor in vivo activity (data not shown). The low pharmacological activities indicated that further testing of these analogues could not be justified and was not carried out. In addition, analogues **1b**−**1f** and **1i**−**1l** were evaluated in the CB2 receptor binding assay, and all failed to produce significant displacement at 10 μM.

The arachidonyl carbamate analogues **2a**−**2c** (Table 1) were devised to study the presence of an oxygen heteroatom at the 2-position of arachidonic acid. Although these carbamates are two atoms longer than AN, we regard this of limited significance. The reason for this is that previously reported results on docosatetraenylethanolamide, with 2-fold lower affinity as well as being two carbon atoms longer than AN, has shown similar pharmacological activity as AN.[25,26] In addition, work from our laboratories[27] and Seltzman et al.[28] had shown that increasing the terminal chain length of the arachidonyl moiety led to very potent and pharmacologically active AN analogues. With this rationale we synthesized the carbamate analogues **2a**−**2c**, but they all showed poor binding affinity for the receptor (K_i = 2200, 995, and 471 nM, respectively).

The norarachidonyl carbamate and urea analogues **3a**−**3c** and **4a**−**4h**, respectively (Table 2), were designed such that the carbon at the 2-position of AN is replaced

Table 1. Arachidonyl Ether (**1a**−**1l**) and Carbamate (**2a**−**2c**) Analogues[a]



analogue	R	K_i (nM)
5	AN	89 ± 10
1a	CH_3	4260 ± 628
1b	CH_2CH_3	2200 ± 561
1c	$CH(CH_3)_2$	871 ± 33
1d	$CH_2CH_2CH_3$	1080 ± 193
1e	$CH(CH_3)CH_2CH_3$	1310 ± 154
1f	$CH_2CH(CH_3)_2$	2480 ± 656
1g	$CH_2CH(CH_2)_2$	578 ± 38
1h	$CH_2CH_2CH_2CH_3$	1710 ± 451
1i	$CH_2CH{=}CH_2$	1170 ± 287
1j	$CH_2C{\equiv}CH$	1580 ± 263
1k	CH_2CH_2F	885 ± 79
1l	CH_2CH_2OH	797 ± 25
2a	$CONHCH_2CH_3$	2200 ± 257
2b	$CONHCH(CH_3)_2$	995 ± 208
2c	$CONHCH_2CH_2CH_3$	471 ± 44

[a] K_i's (nM) were determined in the presence of PMSF and are expressed as means of ±SE for at least three experiments.

by a nitrogen. Isosterically, the chain length on the arachidonyl part is increased by only one atom. This alteration also introduced a hydrogen-bonding site at the nitrogen contained within the arachidonyl part of AN. The norarachidonyl carbamate analogues **3a**−**3c** also showed weak binding affinities (K_i = 218, 1230, and 331 nM, respectively). In in vivo tests, they showed only marginal activity in the spontaneous activity test and were inactive in the tail flick test at a dose of 30 mg/kg.

The norarachidonyl urea analogues **4a**−**4h** showed greater activity than the carbamate analogues. These ureas were more potent in binding affinities (K_i values of 55−746 nM) and showed more robust pharmacological effects and AN-like profiles. The most active analogue of this series was the norarachidonyl 2-fluoroethyl urea **4f**. In binding studies, it was twice as active (K_i = 55 ± 8 nM) as AN, and in in vivo study its activity was equal to or greater than that of AN (5 times more active in spontaneous activity, ED_{50} 3.5 mg/kg; equiactive in antinociception, ED_{50} 6.4 mg/kg; 31 times more active in hypothermia, ED_{50} 0.85 mg/kg; and 4 times more active in catalepsy, ED_{50} 4.48 mg/kg). The other analogues, in general, showed pharmacological activities which correlated well with their binding affinities. However, the methyl analogue **4a** and the hydroxyethyl analogue **4g** were the two exceptions. Both exhibited weak binding affinities (K_i = 436 and 347 nM, respectively) but showed potent pharmacological activity. Analogue **4a** was more potent than AN in both the spontaneous activity (1.8-fold) and ring immobility (4.2-fold) tests and was 12-fold more active in the hypothermia test. Similarly, analogue **4g** showed robust pharmacological activity and was more potent that AN in all tests.

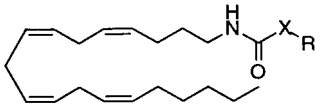
It is noteworthy that like the SAR of AN,[13,14] the propyl and isopropyl urea analogues **4d** and **4c** were both more active in binding affinity than the corresponding hydroxyethyl analogue **4g**. However, in pharmacological activity, the hydroxyethyl analogue **4g** was more potent in spontaneous activity, tail flick, and immobility than both **4c** and **4d**. In hypothermia, **4g** was more potent than **4d** but was one-half as potent as **4c**. The potent activities of **4a** and **4g** cannot be explained at this time but may be due to different pharmacokinetics of these analogues.

The susceptibility of these urea analogues toward enzyme hydrolysis was also tested. The in vitro binding assays for selected active analogues were studied in the absence of the enzyme inhibitor PMSF. The binding affinities were either unchanged (K_i = 52 ± 9 nM for the 2-fluoroethyl analogue) or halved (K_i = 170 ± 21 and 204 ± 13 nM for the propyl and isopropyl analogues, respectively) in these studies. These results show that these urea analogues are indeed stable toward enzyme hydrolysis.

Conclusions

A variety of novel ether, carbamate, and urea analogues of anandamide was synthesized, and their binding affinity and pharmacological activity in the mouse tetrad tests were determined. Only the urea analogues exhibited good binding affinity (**4f** was twice as potent

Table 2. Norarachidonyl Carbamate (**3a**−**3c**) and Urea (**4a**−**4h**) Analogues[a]



				ED_{50} (mg/kg)			
analogue	X-R	K_i (nM) (PMSF)	K_i (nM)	SA	TF	RT	RI
5	AN	89 ± 10	5400 ± 1600	17.9	6.2	26.5	19.1
3a	$O\text{-}CH_2CH_3$	218 ± 10	ND	36%[b]	5%[b]	ND	ND
3b	$O\text{-}CH(CH_3)_2$	1230 ± 149	ND	35%[b]	3%[b]	ND	ND
3c	$O\text{-}CH_2CH_2F$	331 ± 47	ND	18%[b]	20%[b]	ND	ND
4a	$NH\text{-}CH_3$	436 ± 91	ND	9.9	12.3	2.2	4.51
4b	$NH\text{-}CH_2CH_3$	156 ± 40	ND	2.8	3.3	16.2	19.9
4c	$NH\text{-}CH(CH_3)_2$	131 ± 5	204 ± 13	5.8	14.0	1.28	38.0
4d	$NH\text{-}CH_2CH_2CH_3$	91 ± 7	170 ± 21	7.2	8.9	3.4	5.4
4e	$NH\text{-}CH_2CH(CH_3)_2$	335 ± 46	ND	59%[b]	26%[b]	ND	ND
4f	$NH\text{-}CH_2CH_2F$	55 ± 8	52 ± 9	3.5	6.4	0.85	4.48
4g	$NH\text{-}CH_2CH_2OH$	347 ± 33	ND	3.1	4.0	2.6	3.9
4h	$NH\text{-}CH_2CH_2OCH_3$	746 ± 22	ND	69%[b]	32%[b]	−2.8 °C[b]	3%[b]

[a] K_i's (nM) were determined in the presence and absence of PMSF and are expressed as means of ±SE for at least three experiments. The pharmacological measures included inhibition of spontaneous activity (SA), antinociception as measured by tail flick response (TF), hypothermia as changes in rectal temperature (RT), and ring immobility (RI). ND, not determined. [b] Activity at 30 mg/kg dosage.

as AN) and showed robust pharmacological activity. It was also found that the urea analogues were relatively more stable to amidase hydrolysis than AN since their binding affinities were marginally affected when determined in the presence or absence of the enzyme inhibitor PMSF.

The present studies also contribute to the known SAR of the ethanolamido headgroup in AN. The known SAR is quite strict and indicates that (i) monosubstitution of the amide is a requirement for activity; (ii) substitution by an alkyl, fluoroalkyl, or hydroxyalkyl moiety increases activity with a two or three carbon chain being optimal; (iii) branching of the chain (methyl is optimal) enhances stability and in some cases binding affinity; and (iv) substitution of the hydroxyl in AN by a methyl ether, phenyl ether, or phosphate derivative of AN decreases activity, while introduction of an amino or a carboxyl group eliminates activity.[12−15,18]

A comparison of the biological activities of carbamate and urea analogues in the present study provides further insights on the interactions between the CB1 receptor and AN. It shows that the presence of an additional hydrogen-donating nitrogen atom, as in ureas, contributes to better binding affinity and pharmacological activity. This enhancement suggests that hydrogen bonding with the receptor is involved at this site, which is in complete agreement with the known SAR and emphasizes its role in the interaction with the CB1 receptor. All this information could prove valuable in the design of novel and metabolically stable analogues of AN.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on either a Bruker 100, a JEOL Eclipse 300, or a Varian XL400 spectrophotometer using $CDCl_3$ as the solvent with trimethylsilane as an internal standard. Thin-layer chromatography (TLC) was carried out on Baker Si 250F plates and was developed upon treatment with phosphomolybdic acid (PMA). Flash column chromatography was carried out on EM Science silica gel 60. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and were found to be within ±0.4% of calculated values for the elements shown, unless otherwise noted. Mass spectrometry was performed by the Chemistry Department at Boston University, Boston, MA.

All final compounds were oils. The purity of the products on which the high-resolution mass spectral data are reported was determined by ^{1}H and ^{13}C NMR spectroscopy as well as TLC. The presence of chloroform or water in the target compounds was confirmed by ^{1}H and ^{13}C NMR. For TLC solvent system, 10% EtOAc/hexanes was used for ethers **1a**−**1k**; 20% EtOAc/hexanes was used for ether **1l** and carbamates **2a**−**2c** and **3a**−**3c**; 50% EtOAc/hexanes was used for ureas **4a**−**4f** and **4h**; and EtOAc was used for urea **4g**.

Arachidonyl Mesylate (6). To a solution of arachidonyl alcohol (400 mg, 1.38 mmol) and triethylamine (0.58 mL, 4.14 mmol) in dichloromethane (20 mL) at 0 °C was added methanesulfonyl chloride (0.16 mL, 2.07 mmol). The mixture was warmed to room temperature and stirred for an additional 2 h. The mixture was quenched with water and extracted with ethyl acetate. The organic extracts were washed with water and brine and dried over magnesium sulfate. The filtrate was concentrated in vacuo, and the crude product was purified by silica gel column chromatography eluting with 15% EtOAc/hexanes to give a colorless oil (512 mg, 97%): ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.88 (bt, 3 H), 1.18−1.76 (m, 10 H), 2.03−2.14 (m, 4 H), 2.80 (m, 6 H), 2.99 (s, 3 H), 4.22 (t, J = 6.27 Hz, 2 H), 5.36 (m, 8 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.16, 22.65, 25.48, 25.71, 26.59, 27.29, 28.75, 29.39, 31.58, 37.42, 70.03, 127.59, 127.90, 128.17, 128.31, 128.66, 128.74, 129.20, 130.55.

General Procedure for the Syntheses of Arachidonyl Ethers. To a solution of arachidonyl mesylate (160 mg, 0.44 mmol) in an appropriate alcohol (8 mL) was added freshly ground potassium hydroxide (73 mg, 1.30 mmol), and the mixture was heated to 50 °C overnight. The mixture was cooled to room temperature, quenched with water, and extracted with ethyl acetate. The organic extracts were washed with water and brine and dried over magnesium sulfate. The filtrate was concentrated in vacuo, and the crude product was purified by silica gel column chromatography eluting with 1% EtOAc/hexanes.

Arachidonyl methyl ether (1a): pale-yellow oil, 93%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (bt, 3 H), 1.28−1.58 (m, 10 H), 2.04 (m, 4 H), 2.78 (m, 6 H), 3.32 (s, 3 H), 3.37 (t, J = 5.69 Hz, 2 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.99, 22.53, 25.60 (3), 26.14, 26.99, 27.18, 29.23, 29.29, 31.48, 58.44, 72.68, 127.53, 127.92 (2), 127.98, 128.32, 128.47, 129.91, 130.37; HRMS calcd for $C_{21}H_{37}O$ (MH^+) 305.2844, found 305.2826. Anal. ($C_{21}H_{36}O\cdot 0.1CHCl_3\cdot 0.2H_2O$) C, H.

Arachidonyl ethyl ether (1b): yellow oil, 96%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (bt, 3 H), 1.20 (t, J = 7.00 Hz, 3 H), 1.26−1.64 (m, 10 H), 2.06 (m, 4 H), 2.84 (m, 6 H), 3.41 (t, J = 6.39 Hz, 4 H), 3.47 (q, J = 14.0, 7.02 Hz, 2 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.95, 15.14, 22.50, 25.57 (3), 26.20, 26.98, 27.14, 29.26, 29.38, 31.45, 65.97, 70.44, 127.50, 127.84 (2), 127.92, 128.30, 128.41, 129.92, 130.31; HRMS calcd for $C_{22}H_{38}O$ (M) 318.2922, found 318.2952. Anal. ($C_{22}H_{38}O\cdot 0.8CHCl_3\cdot 0.2H_2O$) C, H.

Arachidonyl 1-methylethyl ether (1c): yellow oil, 88%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (bt, 3 H), 1.15 (d, J = 6.13 Hz, 3 H), 1.28−1.55 (m, 10 H), 2.05 (m, 4 H), 2.82 (m, 6 H), 3.40 (t, J = 6.36 Hz, 2 H), 3.46 (sept., J = 6.12 Hz, 1 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.02, 22.14 (2), 22.56, 25.63 (3), 26.32, 27.05, 27.21, 29.32, 29.81, 31.51, 67.99, 71.25, 127.56, 127.86, 127.92, 128.01, 128.41, 128.50, 130.10, 130.42; HRMS calcd for $C_{23}H_{41}O$ (MH^+) 333.3157, found 333.3131. Anal. ($C_{23}H_{40}O\cdot 0.1\ CHCl_3\cdot 0.2\ H_2O$) C, H.

Arachidonyl propyl ether (1d): colorless oil, 92%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.88 (m, 6 H), 1.28−1.69 (m, 12 H), 2.07 (m, 4 H), 2.79 (m, 4 H), 2.79 (m, 6 H), 3.36 (t, J = 6.63 Hz, 2 H), 3.41 (t, J = 6.09 Hz, 2 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.54, 13.97, 22.52, 22.91, 25.59 (2), 26.22, 27.00, 27.17, 29.28, 29.37, 31.48, 70.63, 72.51, 127.52, 127.86 (2), 127.96, 128.34, 128.44, 129.99, 130.34; HRMS calcd for $C_{23}H_{41}O$ (MH^+) 333.3157, found 333.3175. Anal. ($C_{23}H_{40}O\cdot 0.1CHCl_3$) C, H.

Arachidonyl (1-methylpropyl) ether (1e): yellow oil, 77%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (t, J = 7.00 Hz, 6 H), 1.11 (d, J = 6.15 Hz, 3 H), 1.26−1.66 (m, 12 H), 2.07 (m, 4 H), 2.78 (m, 6 H), 3.38 (m, 1 H), 3.38 (t, J = 6.15 Hz, 2 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 9.80, 14.00, 19.23, 22.54, 25.60 (2), 26.32, 27.04, 27.19, 29.24, 29.29, 29.81, 31.49, 68.22, 76.56, 127.54, 127.81, 127.89, 127.97, 128.46, 130.08, 130.38; HRMS calcd for $C_{24}H_{43}O$ (MH^+) 347.3314, found, 347.3252. Anal. ($C_{24}H_{42}O\cdot 0.1CHCl_3$) C, H.

Arachidonyl (2-methylpropyl) ether (1f): pale-yellow oil, 94%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (m, 3 H), 0.89 (d, J = 6.64 Hz, 6 H), 1.28−1.57 (m, 11 H), 2.05 (m, 4 H), 2.79 (m, 6 H), 3.16 (d, J = 6.64 Hz, 2 H), 3.40 (t, J = 6.02 Hz, 2 H), 5.57 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.01, 19.37 (2), 22.54, 25.62 (3), 26.26, 27.02, 27.20, 28.44, 29.33, 29.37, 31.50, 70.81, 77.83, 127.55, 127.88 (2), 127.98, 128.37, 128.46, 130.04, 130.37; HRMS calcd for $C_{24}H_{43}O$ (MH^+) 347.3314, found 347.3291. Anal. ($C_{24}H_{42}O\cdot 0.3H_2O$) C, H.

Arachidonyl cyclopropylmethyl ether (1g): yellow oil, 84%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.20 (m, 2 H), 0.53 (m, 2 H), 0.89 (bt, 3 H), 1.28−1.68 (m, 11 H), 2.04 (m, 4 H), 2.76 (m, 6 H), 3.24 (d, J = 6.81 Hz, 2 H), 3.43 (t, J = 6.36 Hz, 2 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 2.87 (2), 10.61, 13.97, 22.50, 25.57 (3), 26.19, 26.97, 27.14, 29.26, 29.35, 31.45, 70.48, 75.45, 127.50, 127.84 (2), 127.93, 128.32, 128.41, 129.95, 130.32; HRMS calcd for $C_{24}H_{41}O$ (MH^+) 345.3157, found 345.3173. Anal. ($C_{24}H_{40}O\cdot 0.3\ H_2O$) C, H.

Arachidonyl butyl ether (1h): yellow oil, 94%; ^{1}H NMR

(100 MHz, $CDCl_3$) δ 0.90 (m, 6 H), 1.25−1.61 (m, 14 H), 2.07 (m, 4 H), 2.79 (m, 6 H), 3.40 (t, J = 6.33 Hz, 4 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.86, 13.98, 19.35, 22.53, 25.61 (3), 26.23, 27.01, 27.19, 29.29, 29.39, 31.49, 31.86, 70.62, 70.68, 127.53, 127.87 (2), 127.97, 128.35, 128.46, 130.00, 130.36; HRMS calcd for $C_{24}H_{43}O$ (MH^+) 347.3314, found 347.3313. Anal. ($C_{24}H_{42}O\cdot 0.3CHCl_3$) C, H.

Arachidonyl allyl ether (1i): pale-yellow oil, 73%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (bt, 3 H), 1.26−1.59 (m, 10 H), 2.06 (m, 4 H), 2.79 (m, 6 H), 3.43 (t, J = 6.32 Hz, 2 H), 3.96 (td, J = 5.49, 1.36 Hz, 2 H), 5.10−5.23 (m, 2 H), 5.37 (m, 8 H), 5.74−6.12 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.96, 22.49, 25.56 (3), 26.16, 26.95, 27.14, 29.28 (2), 31.44, 70.14, 116.43, 127.49, 127.85 (2), 127.92, 128.28, 128.40, 129.87, 130.29, 135.01; HRMS calcd for $C_{23}H_{39}O$ (MH^+) 331.3000, found 331.29780. Anal. ($C_{23}H_{38}O\cdot 0.1CHCl_3$) C, H.

Arachidonyl propargyl ether (1j): colorless oil, 66%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (bt, 3 H), 1.26−1.63 (m, 8 H), 2.07 (m, 6 H), 2.41 (t, J = 2.38 Hz, 1 H), 2.60 (m, 6 H), 3.52 (t, J = 6.26 Hz, 2 H), 4.13 (d, J = 2.39 Hz, 2 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.99, 22.51, 25.59 (3), 26.05, 26.89, 27.16, 29.04, 29.26, 31.47, 57.93, 69.93, 73.99, 79.96, 127.51, 127.86, 127.98 (2), 128.29, 128.46, 129.83, 130.38; HRMS calcd for $C_{23}H_{37}O$ (MH^+) 329.2844, found 329.2818. Anal. ($C_{23}H_{36}O$) C, H.

Arachidonyl 2-fluoroethyl ether (1k): colorless oil, 94%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (bt, 3 H), 1.28−1.63 (m, 8 H), 2.11 (m, 6 H), 2.78 (m, 6 H), 3.48 (t, J = 3.85 Hz, 1 H), 3.50 (t, J = 6.15 Hz, 2 H), 3.81 (t, J = 4.21 Hz, 1 H), 4.31 (t, J = 4.20 Hz, 1 H), 4.79 (t, J = 4.21 Hz, 1 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.98, 22.50, 25.57 (3), 26.04, 26.92, 27.15, 29.20, 29.26, 31.45, 69.41, 70.19, 71.39, 79.65, 86.37, 127.50, 127.84, 127.96 (2), 128.29, 128.44, 129.85, 130.35; HRMS calcd for $C_{22}H_{37}OF$ (M) 336.2828, found 336.2831. Anal. ($C_{22}H_{37}OF\cdot 0.2CHCl_3\cdot 0.3H_2O$) C, H.

Arachidonyl 2-Hydroxyethyl Ether (1l). To a solution of 2-(2-bromoethoxy)tetrahydro-2*H*-pyran (1.00 g, 4.73 mmol) in acetone (20 mL) was added sodium iodide (1.44 g, 9.57 mmol). The mixture was refluxed for 3 h and then filtered to remove the solid. The filtrate was then concentrated in vacuo to isolate the 2-(2-iodoethoxy)tetrahydro-2*H*-pyran (69% yield) which was used for the next step.

To a mixture of arachidonyl alcohol (73 mg, 0.25 mmol), 2-(2-iodoethoxy)tetrahydro-2*H*-pyran (0.64 g, 2.5 mmol), and tetrabutylammonium bisulfate (TBAB) (85 mg, 0.25 mmol) was added a solution of sodium hydroxide (70 mg, 2.41 mmol) in water (0.14 mL). The mixture was heated to 55 °C for 3 days; however, the reaction remained incomplete. The mixture was cooled to room temperature, quenched with water, and extracted with ethyl acetate. The organic extracts were washed with water and brine and dried over magnesium sulfate. The filtrate was removed in vacuo, and the crude product was purified by silica gel column chromatography. The arachidonyl 2-ethoxytetrahydro-2*H*-pyranyl ether (**7**) was isolated as a beige oil (44 mg, 41% yield, 41% conversion). The arachidonyl 2-ethoxytetrahydro-2*H*-pyranyl ether was then treated with Dowex 50W-X8 (4 mg, 10 wt %) in methanol (1 mL) at room temperature overnight to cleave the THP protecting group. The mixture was filtered to remove the resin and concentrated in vacuo to give the crude product. The crude product was purified by silica gel column chromatography eluting with 10% EtOAc/hexanes to give a yellow oil (20 mg, 73%): ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.88 (bt, 3 H), 1.25−1.68 (m, 8 H), 2.07 (m, 6 H), 2.79 (m, 6 H), 3.49 (m, 4 H), 3.71 (m, 2 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.02, 22.53, 25.61 (3), 26.10, 26.96, 27.18, 29.24, 29.28, 31.48, 61.79, 71.18, 71.77, 127.52, 127.87, 128.04 (2), 128.29, 128.50, 129.84, 130.42; HRMS calcd for $C_{22}H_{39}O_2$ (MH^+), 335.2950; found, 335.2940. Anal. ($C_{22}H_{38}O_2\cdot 0.8CHCl_3$) C, H.

General Procedure for the Syntheses of Arachidonyl Carbamates. To a solution of arachidonyl alcohol (100 mg, 0.35 mmol) in benzene (5 mL) heated at 65 °C for 2 h was added an excess of the alkyl isocyanate (1.03 mmol) via syringe, and the mixture was stirred at 65 °C overnight. The mixture was cooled to room temperature, quenched with water, and extracted with ethyl acetate. The organic extracts were washed with water and brine and dried over magnesium sulfate. The filtrate was concentrated in vacuo, and the crude product was purified by silica gel column chromatography eluting with 10% EtOAc/hexanes.

Arachidonyl *N*-ethylcarbamate (2a): pale-yellow oil, 89%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.88 Hz, 3 H), 1.12 (t, J = 7.30 Hz, 3 H), 1.25−1.37 (m, 6 H), 1.43 (m, 2 H), 1.62 (m, 2 H), 2.08 (m, 4 H), 2.82 (m, 6 H), 3.20 (quint., 2 H), 4.04 (t, J = 6.47 Hz, 2 H), 4.60 (bs, 1 H), 5.35 (m, 8 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.14, 15.38, 22.65, 25.71 (3), 26.00, 26.91, 27.29, 28.77, 29.39, 31.59, 35.86, 64.72, 127.61, 127.97, 128.19, 128.25, 128.36, 128.62, 129.78, 130.55, 156.71; HRMS calcd for $C_{23}H_{40}NO_2$ (MH^+) 362.3059, found 362.3083. Anal. ($C_{23}H_{39}NO_2\cdot 4.0H_2O$) C, N; H: calcd, 10.92; found, 9.04.

Arachidonyl *N*-(1-methylethyl)carbamate (2b): pale-yellow oil, 94%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.88 Hz, 3 H), 1.14 (d, J = 6.33 Hz, 6 H), 1.26−1.30 (m, 8 H), 1.38 (m, 2 H), 1.63 (m, 2 H), 2.08 (m, 4 H), 2.82 (m, 6 H), 3.79 (bm, 1 H), 4.03 (t, J = 6.33 Hz, 2 H), 4.46 (bs, 1 H), 5.36 (m, 8 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 14.14, 22.65, 23.16 (2), 25.71 (3), 26.02, 26.92, 27.29, 28.77, 29.40, 31.59, 43.03, 64.62, 127.62, 127.97, 128.25 (2), 128.37, 128.63, 129.80, 130.55, 156.00; HRMS calcd for $C_{24}H_{42}NO_2$ (MH^+) 376.3215, found 376.3229. Anal. ($C_{24}H_{41}NO_2\cdot 3.8H_2O$) C, N; H: calcd, 11.03; found, 9.44.

Arachidonyl *N*-propylcarbamate (2c): pale-yellow oil, 98%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (m, 6 H), 1.28−1.34 (m, 8 H), 1.47 (m, 2 H), 1.61 (m, 2 H), 2.06 (m, 4 H), 2.81 (m, 6 H), 3.12 (m, 2 H), 4.04 (t, J = 6.47 Hz, 2 H), 4.65 (bs, 1 H), 5.36 (m, 8 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 11.27, 14.13, 22.64, 23.32, 25.71 (3), 26.00, 26.91, 27.29, 28.77, 29.39, 31.58, 42.76, 64.74, 127.61, 127.97, 128.19, 128.25, 128.36, 128.61, 129.78, 130.55, 156.85; HRMS calcd for $C_{24}H_{42}NO_2$ (MH^+) 376.3215, found 376.3197. Anal. ($C_{24}H_{41}NO_2\cdot 4.0H_2O$) C, N; H: calcd, 11.03; found, 9.35.

General Procedure for the Syntheses of Norarachidonyl Carbamates and Ureas. To a solution of arachidonic acid (150 mg, 0.49 mmol) in benzene (5 mL) were added triethylamine (0.07 mL, 0.49 mmol) and diphenyl phosphorazidate (DPPA) (0.11 mL, 0.49 mmol), and the mixture was heated to 65 °C for 2 h. An excess of the alcohol or amine (for the corresponding carbamate or urea, respectively) was added via syringe, and the mixture was stirred at 65 °C overnight. The mixture was cooled to room temperature, quenched with water, and extracted with ethyl acetate. The organic extracts were washed with water and brine and dried over magnesium sulfate. The filtrate was concentrated in vacuo, and the crude product was purified by silica gel column chromatography eluting with 10% EtOAc/hexanes for compounds **3a**−**3c**, 50% EtOAc/hexanes for compounds **4a**−**4f** and **4h**, and 75% EtOAc/hexanes for compound **4g**.

Ethyl *N*-norarachidonylcarbamate (3a): yellow oil, 64%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.88 (bt, 3 H), 1.23 (t, J = 7.11 Hz, 3 H), 1.28−1.37 (m, 6 H), 1.56 (quint., J = 6.90 Hz, 2 H), 2.08 (m, 4 H), 2.81 (m, 6 H), 3.17 (q, J = 13.4, 6.63 Hz, 2 H), 4.10 (q, J = 14.2, 7.09 Hz, 2 H), 4.60 (bs, 1 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.93, 14.54, 22.45, 24.36, 25.52 (3), 27.11, 29.20, 29.78, 31.41, 40.51, 60.53, 127.44, 127.72, 128.02, 128.13, 128.48, 128.52, 128.93, 130.35, 156.57; HRMS calcd for $C_{22}H_{38}NO_2$ (MH^+) 348.2902, found 348.2920. Anal. ($C_{22}H_{37}NO_2\cdot 0.3H_2O$) C, H, N.

1-Methylethyl *N*-norarachidonylcarbamate (3b): pale-yellow oil, 36%; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.89 (bt, 3 H), 1.22 (d, J = 6.25 Hz, 6 H), 1.28−1.38 (m, 6 H), 1.56 (quint., J = 6.90 Hz, 2 H), 2.08 (m, 4 H), 2.81 (m, 6 H), 3.16 (q, J = 13.6, 6.41 Hz, 2 H), 4.55 (bs, 1 H), 4.90 (sept., J = 6.23 Hz, 1 H), 5.37 (m, 8 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.01, 22.14 (2), 22.53, 24.44, 25.59 (3), 27.17, 29.28, 29.87, 31.47, 40.52, 67.83, 127.50, 127.80, 128.10, 128.19, 128.57 (2), 129.02, 130.44, 156.27; HRMS calcd for $C_{23}H_{40}NO_2$ (MH^+) 362.3059, found 362.3047. Anal. ($C_{23}H_{39}NO_2\cdot 0.3H_2O$) C, H, N.

2-Fluoroethyl *N*-norarachidonylcarbamate (3c): yel-

low oil, 60%;[1]H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.74 Hz, 3 H), 1.25−1.36 (m, 6 H), 1.57 (m, 2 H), 2.04 (q, J = 14.1, 7.03 Hz, 2 H), 2.09 (q, J = 14.1, 7.03 Hz, 2 H), 2.81 (m, 6 H), 3.18 (q, J = 13.5, 7.03 Hz, 2 H), 4.25 (t, J = 3.81 Hz, 1 H), 4.32 (t, J = 3.81 Hz, 1 H), 4.50 (t, J = 4.10 Hz, 1 H), 4.62 (t, J = 4.10 Hz, 1 H), 4.76 (bs, 1 H), 5.38 (m, 8 H); HRMS calcd for $C_{22}H_{37}NO_2F$ (MH^+) 366.2808, found 366.2798. Anal. ($C_{22}H_{36}NO_2F \cdot 0.1CHCl_3$) C, H, N.

1-Norarachidonyl-3-methylurea (4a): yellow oil, 77%; [1]H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.88 Hz, 3 H), 1.24−1.37 (m, 6 H), 1.56 (quint., 2 H), 2.07 (m, 4 H), 2.75 (d, J = 4.95 Hz, 3 H), 2.81 (m, 6 H), 3.16 (q, J = 6.60 Hz, 2 H), 4.65 (bm, 2 H), 5.36 (m, 8 H); [13]C NMR (300 MHz, $CDCl_3$) δ 14.15, 22.64, 24.68, 25.71 (3), 27.29 (2), 29.39, 30.22, 31.59, 40.29, 127.58, 127.90, 128.23, 128.32, 128.70 (2), 129.28, 130.61, 159.09; HRMS calcd for $C_{21}H_{37}N_2O$ (MH^+) 333.2906, found 333.2912. Anal. ($C_{21}H_{36}N_2O \cdot 1.3H_2O$) C, N; H: calcd, 11.18; found, 10.26.

1-Norarachidonyl-3-ethylurea (4b): yellow oil, 76%; [1]H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.88 Hz, 3 H), 1.12 (t, J = 7.30 Hz, 3 H), 1.24−1.34 (m, 7 H), 1.56 (quint., 1 H), 2.07 (m, 4 H), 2.80 (m, 6 H), 3.17 (m, 4 H), 4.37 (bt, 1 H), 4.43 (bt, 1 H), 5.37 (m, 8 H); [13]C NMR (300 MHz, $CDCl_3$) δ 14.15, 15.58, 22.65, 24.70, 25.71 (3), 27.30, 29.39, 30.20, 31.59, 35.41, 40.26, 127.58, 127.90, 128.23, 128.32, 128.70 (2), 129.28, 130.61, 158.50. Anal. ($C_{22}H_{38}N_2O \cdot 0.9H_2O$) C, N; H: calcd, 11.05; found, 10.60.

1-Norarachidonyl-3-(1′-methylethyl)urea (4c): yellow oil, 80%; [1]H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.78 Hz, 3 H), 1.12 (d, J = 6.59 Hz, 6 H), 1.26−1.37 (m, 6 H), 1.56 (quint., J = 7.32 Hz, 2 H), 2.03 (q, J = 13.9, 6.96 Hz, 2 H), 2.09 (q, J = 12.6, 6.78 Hz, 2 H), 2.80 (m, 6 H), 3.14 (q, 2 H), 3.82 (m, 1 H), 4.01 (bs, 1 H), 4.18 (bs, 1 H), 5.38 (m, 8 H); [13]C NMR (300 MHz, $CDCl_3$) δ 14.03, 22.51, 23.43 (2), 24.58, 25.54, 25.57 (2), 27.16, 29.27, 30.11, 31.45, 40.02, 42.07, 127.44, 127.76, 128.10, 128.19, 128.49, 128.57, 129.15, 130.46, 157.82; HRMS calcd for $C_{23}H_{41}N_2O$ (MH^+) 361.3219, found 361.3188. Anal. ($C_{23}H_{40}N_2O \cdot 0.1CHCl_3 \cdot 0.2H_2O$) C, H, N.

1-Norarachidonyl-3-propylurea (4d): orange oil, 80%; [1]H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.78 Hz, 3 H), 0.90 (t, J = 7.32 Hz, 3 H), 1.26−1.36 (m, 6 H), 1.49 (quint., J = 7.51 Hz, 2 H), 1.56 (quint., J = 7.32 Hz, 2 H), 2.03 (q, J = 14.1, 6.78 Hz, 2 H), 2.10 (q, J = 12.6, 7.14 Hz, 2 H), 2.80 (m, 6 H), 3.10 (t, J = 7.14 Hz, 2 H), 3.16 (t, J = 6.96 Hz, 2 H), 4.28 (bs, 2 H), 5.36 (m, 8 H); [13]C NMR (300 MHz, $CDCl_3$) δ 11.34, 14.05, 22.54, 23.44, 24.59, 25.58 (3), 27.17, 29.28, 30.15, 31.47, 40.08, 42.21, 127.45, 127.79, 128.13, 128.20, 128.49, 128.59, 129.18, 130.48, 158.53; HRMS calcd for $C_{23}H_{41}N_2O$ (MH^+) 361.3219, found 361.3186. Anal. ($C_{23}H_{40}N_2O \cdot 0.2CHCl_3 \cdot 0.2H_2O$) C, H, N.

1-Norarachidonyl-3-(2′-methylpropyl)urea (4e): orange oil, 58%; [1]H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 7.03 Hz, 3 H), 0.89 (d, J = 7.03 Hz, 6 H), 1.25−1.35 (m, 6 H), 1.56 (quint., J = 7.33 Hz, 2 H), 1.72 (sept., J = 6.74 Hz, 1 H), 2.03 (q, J = 13.8, 6.74 Hz, 2 H), 2.10 (q, J = 12.9, 7.03 Hz, 2 H), 2.80 (m, 6 H), 2.96 (bt, 2 H), 3.16 (bt, 2 H), 4.25 (bs, 2 H), 5.36 (m, 8 H); HRMS calcd for $C_{24}H_{43}N_2O$ (MH^+) 375.3375, found 375.3364. Anal. ($C_{24}H_{42}N_2O \cdot 0.3H_2O$) C, H, N.

1-Norarachidonyl-3-(2′-fluoroethyl)urea (4f): yellow oil, 55%;[1]H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 6.74 Hz, 3 H), 1.23−1.37 (m, 6 H), 1.56 (quint., J = 7.33 Hz, 2 H), 2.03 (q, J = 13.8, 6.74 Hz, 2 H), 2.10 (q, J = 13.8, 6.74 Hz, 2 H), 2.81 (m, 6 H), 3.16 (bm, 2 H), 3.46 (q, 1 H), 3.53 (q, 1 H), 4.34 (bs, 1 H), 4.41 (t, J = 4.69 Hz, 1 H), 4.53 (t, J = 4.69 Hz, 1 H), 4.59 (bs, 1 H), 5.38 (m, 8 H); [13]C NMR (300 MHz, $CDCl_3$) δ 14.08, 22.59, 24.57, 25.65 (3), 27.24, 29.34, 29.99, 31.53, 40.31, [40.78, 41.03], [82.60, 84.79], 127.51, 127.83, 128.14, 128.30, 128.72 (2), 129.07, 130.56, 157.74; HRMS calcd for $C_{22}H_{38}N_2OF$ (MH^+) 365.2968, found 365.2970. Anal. ($C_{22}H_{37}N_2OF \cdot 0.4CHCl_3$) C, H, N.

1-Norarachidonyl-3-(2′-hydroxyethyl)urea (4g): yellow oil, 70%; [1]H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, J = 6.74 Hz, 3 H), 1.29 (m, 6 H), 1.53 (quint., 2 H), 2.05 (m, 4 H), 2.80 (m, 6 H), 3.12 (m, 2 H), 3.26 (q, J = 5.23, 10.4 Hz, 2 H), 3.62 (t, J = 4.82 Hz, 2 H), 4.37 (bs, 1 H), 5.35 (m, 9 H), 5.59 (bt, 1 H); [13]C NMR (300 MHz, $CDCl_3$) δ 14.14, 22.64, 24.66, 25.69 (3), 27.29, 29.39, 30.20, 31.58, 40.19, 43.20, 62.90, 127.57, 127.88, 128.19, 128.37, 128.71 (2), 129.15, 130.60, 159.93; LRMS (CI) found for $C_{22}H_{39}N_2O_2$ (MH^+) 363.2. Anal. ($C_{22}H_{38}N_2O_2 \cdot 1.2H_2O$) C, N; H: calcd, 10.60; found, 10.04.

1-Norarachidonyl-3-(2′-methoxyethyl)urea (4h): yellow oil, 67%; [1]H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.88 Hz, 3 H), 1.24−1.37 (m, 6 H), 1.55 (quint., 2 H), 2.07 (m, 4 H), 2.81 (m, 6 H), 3.15 (q, J = 6.67, 13.3 Hz, 2 H), 3.35 (m, 5 H), 3.44 (t, J = 4.95 Hz, 2 H), 4.77 (bt, 1 H), 4.87 (bt, 1 H), 5.36 (m, 8 H); [13]C NMR (300 MHz, $CDCl_3$) δ 14.15, 22.65, 24.68, 25.71 (3), 27.29, 29.39, 30.18, 31.59, 40.23, 40.49, 58.81, 72.49, 127.59, 127.91, 128.31 (2), 128.61, 128.68, 129.27, 130.59, 158.58. Anal. ($C_{23}H_{40}N_2O_2 \cdot 0.5H_2O$) C, H, N.

Pharmacology. 1. Drug Preparation and Administration. For binding assays, compounds were prepared as 1 mg/mL stock solutions in absolute ethanol and were stored at −20 °C. For behavioral assays, drugs were dissolved in a 1:1:18 mixture of ethanol, emulphor (GAF Corp., Linden, NJ), and saline (0.9% NaCl) and were administered intravenously (iv) in the mouse tail vein in volumes of 0.1 mL/10 g of body weight.

2. Binding Assays. Radioligand binding to P_2 membrane preparations were performed as described elsewhere.[12,29] Ethanolic 1 mg/mL stock solutions of anandamide analogues were diluted in buffer (50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, and 5 mg/mL bovine serum albumin) without evaporation of the ethanol (final concentration not exceeding 0.4%). Analogue concentrations ranging from 1 nM to 10 μM and a 1 nM concentration of [3H]CP-55,940 were used. Binding was initiated with the addition of 100−150 μg of membrane preparation protein. Nonspecific binding was determined in the presence of 1 μM CP-55,940. The binding experiments were performed either with or without the amidase inhibitor phenylmethanesulfonyl fluoride (PMSF; 50 μM). After 1 h incubation at 30 °C, the reaction was terminated with the addition of 2 mL of ice-cold buffer (50 mM Tris-HCl, 1 mg/mL bovine serum albumin) followed by rapid filtration through PEI-treated filters. The assays were performed in triplicate, and the results represent the combined data from three to six independent experiments.

3. Behavioral Evaluations. Mice were acclimated to the laboratory overnight. Depression of locomotor activity and antinociception, as determined by the tail flick (TF) response to heat stimulus,[30] were measured in the same animal. Control tail flick latencies of 2−4 s were measured for each animal with a standard tail flick apparatus prior to drug or vehicle administration. Four minutes following an iv injection of either vehicle or drug, mice were tested for tail flick response. Immediately thereafter, the mice were placed into individual photocell activity cages (11 × 6.5 in.) for assessment of spontaneous activity. For the next 10 min the total number of beam interruptions in the 16 photocell beams/cage was recorded using a Digiscan Animal Activity Monitor (Omnitech Electronics Inc., Columbus, OH). Spontaneous activity was expressed as percent of control activity. Antinociception was expressed as the percent maximum possible effect (MPE) using a 10-s maximum test latency as described earlier.[30] Each dose tested in the antinociception and hypomotility assays represents one group of animals (6 mice/group). Cannabinoid-induced hypothermia and immobility were determined in a separate group of animals. Prior to vehicle or drug administration, rectal temperature was determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Four minutes after the iv injection of the drug, mice were tested for body temperature. The difference between pre- and postinjection rectal temperatures was calculated. Immediately after measure of body temperature, the mice were placed on a 5.5-cm ring attached at a height of 16 cm to a ring stand, and the amount of time the animals remained motionless during a 5-min period was recorded.[31] The time that each animal remained motionless on the ring was divided by 300 s and multiplied by 100 to obtain a percent immobility rating.

4. Data Analysis. IC_{50} values were converted to K_i values.[32] Statistical evaluation of parallelism between displacement curves generated in the presence and absence of PMSF was performed using ALLFIT.[33] Dose-dependent relationships were determined for each analogue in the pharmacological assays. Percent effect was determined based upon the maximal effects that are produced by Δ^9-THC and anandamide which are 90, 100, and 60% for spontaneous activity, antinociception, and ring immobility, respectively. The percent effect for hypothermia was based upon the maximal effect produced by anandamide (−3.0 °C) rather than that produced by Δ^9-THC (−6.0 °C). Antinociception, hypomotility, and immobility data were converted to probit values, and ED_{50}'s were calculated by unweighted least-squares linear regression analysis of the log dose versus the probit values. Several analogues were classified as having partial agonist effects because they produced dose−response effects that failed to exceed 60% of maximal effect. Analogues producing effects less than 30% or hypothermia less than 1 °C at the highest doses tested were considered to be inactive.

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