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Inhibition of Fatty Acid Amidohydrolase, the Enzyme Responsible for the Metabolism of the Endocannabinoid Anandamide, by Analogues of Arachidonoyl-serotonin

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Arachidonoyl-serotonin inhibits in a mixed-type manner the metabolism of the endocannabinoid anandamide by the enzyme fatty acid amidohydrolase. In the present study, compounds related to arachidonoyl-serotonin have been synthesised and investigated for their ability to inhibit anandamide hydrolysis by this enzyme in rat brain homogenates. Removal of the 5-hydroxy from the serotonin head group of arachidonoyl-serotonin produced a compound (*N*-arachidonoyltryptamine) that was a 2.3-fold weaker inhibitor of anandamide hydrolysis, but which also produced its inhibition by a mixed-type manner ($K_{i(slope)}$ 1.3 μM; $K_{i(intercept)}$ 44 μM). Replacement of the amide linkage in this compound by an ester group further reduced the potency. In contrast, replacement of the arachidonoyl side chain by a linolenoyl side chain did not affect the observed potency. *N*-(Fur-3-ylmethyl) arachidonamide (UCM707), *N*-(fur-3-ylmethyl)linolenamide and *N*-(fur-3-ylmethyl)oleamide inhibited anandamide hydrolysis with pI_{50} values of 4.53, 5.36 and 5.25, respectively. The linolenamide derivative was also found to be a mixed-type inhibitor. It is concluded that the 5-hydroxy group of arachidonoyl-serotonin contributes to, but is not essential for, inhibitory potency at fatty acid amidohydrolase.

Keywords: Fatty acid amidohydrolase; Arachidonoyl-serotonin; Cannabinoid receptors; Anandamide

INTRODUCTION

The *N*-acyl ethanolamines are a class of endogenous compounds with a variety of important physiological effects. The most well studied of these compounds, anandamide (AEA, *N*-arachidonoyl ethanolamine, 20:4), is a ligand for both cannabinoid and vanilloid receptors, and as such is involved in a wide variety of physiological processes, including nociception, retrograde neurotransmission in the brain, seizure threshold, control of motor function and memory, cardiovascular and immune regulation and reproduction.[1–7] Among other *N*-acyl ethanolamines, the three most important are *N*-palmitoylethanolamine (16:0), *N*-oleoylethanolamine (18:1) and *N*-stearoylethanolamine (18:0). *N*-Palmitoylethanolamine (16:0) is inactive at CB receptors *in vitro*, but reduces inflammatory pain *in vivo* by a cannabinoid CB_2 (or CB_2-like) receptor-mechanism.[1,8] *N*-Oleoylethanolamine (18:1) acts in the body as an endogenous regulator of appetite,[9] whilst *N*-stearoylethanolamine (18:0) produces AEA-like behavioural effects in a CB receptor-independent manner.[10] Given that the synthesis of *N*-acyl ethanolamines is drastically increased under conditions of severe inflammation and neurodegeneration,[11–13] compounds preventing their metabolism may be useful therapeutic agents for the treatment of inflammatory and neurodegenerative disorders (for a detailed discussion with respect to stroke, see Reference 14). Indeed, the spasticity found in a mouse multiple sclerosis model (chronic relapsing experimental allergic encephalomyelitis), which is accompanied by an increased spinal cord level of AEA, can be alleviated by such a treatment.[15]

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The enzyme responsible for the metabolism of *N*-acyl ethanolamines to their corresponding acids is fatty acid amidohydrolase (FAAH).[10,16–18] Mice lacking this enzyme have been shown to have a higher level of brain AEA and to display a raised pain threshold.[19] The enzyme can be inhibited by a variety of agents, varying from substrate analogues to phenylmethanesulphonyl fluoride and nonsteroidal anti-inflammatory agents such as ibuprofen and flurbiprofen.[20] Among substrate analogues, initial studies demonstrated that trifluoromethyl ketone derivatives were effective inhibitors,[21] although the most potent compounds so far discovered have α–keto heterocycle head groups.[22] It is not known, however, whether these compounds interact with cannabinoid receptors, a problem associated with the arachidonoyl trifluoromethyl ketone compound.[21]

In 1998, Bisogno *et al.*[23] reported that arachidonoyl-serotonin (AA-*N*-5HT, *N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]arachidonamide) was a mixed-type inhibitor of [^{3}H]AEA hydrolysis by N18TG2 neuroblastoma cell membranes with an IC_{50} value of 12 μM, but which did not produce effects upon cannabinoid receptors *in vivo*. In contrast, arachidonoyl–dopamine and –histamine were 5 to 6-fold weaker FAAH inhibitors. Replacement of the arachidonic (20:4) side chain with an icosapentaenoic (20:5) side chain also reduced the potency of the serotonin derivative by 2.5-fold.[23] The authors concluded that "It is possible that the presence of the 5-hydroxy-indole moiety rather than the imidazole of histamine or the di-hydroxy-phenyl of dopamine allows the molecule to interact more strongly with the enzyme active site. The rather acidic 5-hydroxy-group in the serotonin moiety may allow the formation of a hydrogen bond with one of the active site amino acid residues, thus further strengthening the stability of the enzyme-inhibitor complex".[23] If this hypothesis is correct, then removal of the hydroxy group should greatly reduce the potency of the compound as an FAAH inhibitor. This hypothesis has been investigated in the present study. In addition, the effect of the replacement of the arachidonic side chain with a linolenic side chain has been investigated.

MATERIALS AND METHODS

Compounds

Arachidonylethanolamide-[1-^{3}H] ([^{3}H]AEA, specific activity 20 Ci mmol^{-1}) was obtained from American Radiolabelled Chemicals Inc., St. Louis, MO, USA. Arachidonoyl-serotonin (*N*-[2-(5-hydroxy-1*H*-indol-3-yl)ethyl]arachidonamide, AA-*N*-5HT) and non-radioactive anandamide were obtained from the Cayman Chemical Company, Ann Arbor, MI, USA. *N*-(Fur-3-ylmethyl)arachidonamide (AA-*N*-Fur, UCM707) was synthesised as previously described.[24,25] Synthesis of *N*-(Fur-3-ylmethyl)linolenamide will be published elsewhere. Fatty acid-free bovine serum albumin was obtained from the Sigma Chemical Co. (St Louis, MO, USA). [^{3}H]WIN 55212,2 (specific activity 180 Ci mmol^{-1}), [^{3}H]CP 55,940 (specific activity 180 Ci mmol^{-1}) and [^{3}H]resiniferatoxin (specific activity 48 Ci mmol^{-1}) were purchased from New England Nuclear, Boston, MA, USA.

Chemistry

Infrared (IR) spectra were determined on a Perkin-Elmer 781 or Shimadzu-8300 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR-300S, Bruker Avance 3000-AM or Bruker 200-AC instrument at room temperature. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (J) are in Hertz. Satisfactory elemental analyses were obtained for all the newly synthesized analogs and are within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. For normal pressure chromatography, Merck silica gel type 60 (size 70–230) was used. Unless stated otherwise, starting materials used were high-grade commercial products from Aldrich, Acros, Fluka, Merck or Panreac except arachidonic acid (90% of purity) which was purchased from Sigma. Starting materials were used as supplied except methylene chloride which was distillated over CaH_2 prior to use.

General Procedure for the Synthesis of Fatty Acid Derivatives

To a stirred solution of one equivalent (0.33 mmol) of fatty acid in dry methylene chloride (1 mL/mmol) and the appropriate alcohol or amine (1.5 equivalents) in dry methylene chloride (1 mL/mmol) at −20°C in a salt-ice-bath and under argon was added dropwise a solution of dicyclohexylcarbodiimide (DCC; 1 equivalent) and 4-(dimethylamino)pyridine (DMAP; 0.068 equivalents) in dry methylene chloride (3 mL/mmol DCC). The mixture was stirred for 5 min at this temperature and then removed from the cooling bath and stirred at room temperature (3–6 h) until no further evolution was observed by thin layer chromatography (TLC) in chloroform: methanol (95:5). The dicyclohexylurea was filtered off, the filtrate evaporated under reduced pressure and the obtained residue taken up in methylene chloride (20 mL/mmol of fatty acid). This resulting organic phase was washed with a cooled 0.5 M hydrochloric acid solution and brine and the organic

extracts dried over Na_2SO_4 or $MgSO_4$. Then, the solvent was evaporated under reduced pressure and the product purified by column chromatography on silica gel using the appropriate eluent.

(5*Z*,8*Z*,11*Z*,14*Z*)-*N*-[2-(1*H*-Indol-3-yl)ethyl]icosa-5,8,11,14-tetraenamide (*N*-[2-(1*H*-indol-3-yl)ethyl]-arachidonamide, *N*-arachidonoyltryptamine, AA-*N*-T)

$R_f = 0.24$ (chloroform:diethyl ether, 9:1). Yield = 69%. IR (CH_2Cl_2, cm^{-1}): 3408, 3292, 3011, 2926, 2854, 1651, 1526, 1456, 1434, 1229. ^{1}H-NMR (200 MHz, $CDCl_3$-δ): 0.90 (t, 3H, $J = 6.8$ Hz, 3H-20), 1.27–1.37 (m, 6H, 2H-17, 2H-18, 2H-19), 1.68 (quint, 2H, $J = 7.3$ Hz, 2H-3), 2.01–2.18 (m, 6H, 2H-4, 2H-16, 2H-2), 2.78–2.84 (m, 6H, 2H-7, 2H-10, 2H-13), 2.97 (t, 2H, $J = 6.8$ Hz, NH-CH_2–CH_2), 3.60 (q, 2H, $J = 6.8$ Hz, NH-CH_2), 5.27–5.42 (m, 8H, vinylic-H), 5.67 (br s, 1H, NH-CO), 7.00 (br s, 1H, H-2′), 7.08–7.25 (m, 2H, H-5′, H-6′), 7.38 (dm, 1H, $J = 7.8$ Hz, H-7′), 7.60 (d, 1H, $J = 7.1$ Hz, H-4′), 8.48 (br s, 1H, NH). ^{13}C-NMR (50 MHz, $CDCl_3$-δ): 13.9, 22.4, 25.4, 25.6 (4C), 26.7, 27.2, 29.1, 31.3, 36.0, 39.6, 111.2, 112.6, 118.5, 119.2, 121.9, 127.4, 127.5, 127.9 (2C), 128.2 (2C), 128.6, 128.7, 129.1, 130.4, 136.3, 172.8.

2-(1*H*-Indol-3-yl)ethyl (5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoate (AA-*O*-T)

$R_f = 0.38$ (methylene chloride:hexane, 8:2). Yield = 65%. IR (CH_2Cl_2, cm^{-1}): 3412, 3013, 2957, 2928, 2856, 1728, 1458, 1225, 704. ^{1}H-NMR (200 MHz, $CDCl_3$-δ): 0.89 (t, 3H, $J = 6.8$ Hz, 3H-20), 1.27–1.37 (m, 6H, 2H-17, 2H-18, 2H-19), 1.72 (quint, 2H, $J = 7.3$ Hz, 2H-3), 1.93–2.07 (m, 4H, 2H-4, 2H-16), 2.35 (t, 2H, $J = 7.3$ Hz, 2H-2), 2.79–2.86 (m, 6H, 2H-7, 2H-10, 2H-13), 3.02 (td, 2H, $J = 7.2$, 0.6 Hz, O-CH_2-CH_2), 4.28 (t, 2H, $J = 7.2$ Hz, O-CH_2), 5.20–5.40 (m, 8H, vinylic-H), 6.94 (d, 1H, $J = 2.3$ Hz, H-2′), 7.10–7.26 (m, 2H, H-5′, H-6′), 7.37 (dm, 1H, $J = 7.6$ Hz, H-7′), 7.66 (dm, 1H, $J = 7.6$ Hz, H-4′), 8.07 (br s, 1H, NH). ^{13}C-NMR (50 MHz, $CDCl_3$-δ): 14.1, 22.6, 24.8 (2C), 25.6 (3C), 26.6, 27.2, 29.3, 31.5, 33.8, 64.3, 111.1, 112.1, 118.8, 119.4, 122.0, 122.1, 127.4, 127.5, 127.9, 128.2 (2C), 128.6, 128.8, 129.0, 130.5, 136.0, 173.6.

(9*Z*,12*Z*,15*Z*)-*N*-[2-(1*H*-Indol-3-yl)ethyl]octadeca-9,12,15-trienamide (*N*-[2-(1*H*-indol-3-yl)-ethyl]linolenamide, *N*-linolenoyltryptamine, Lin-*N*-T)

$R_f = 0.26$ (chloroform:diethyl ether, 9:1). Yield = 47%. IR (CH_2Cl_2, cm^{-1}): 3402, 3288, 3011, 2928, 2854, 1651, 1554, 1529, 1456, 1095. ^{1}H-NMR (200 MHz, $CDCl_3$-δ): 0.89 (t, 3H, $J = 7.5$ Hz, 3H-18), 1.15–1.29 (m, 8H, 2H-4, 2H-5, 2H-6, 2H-7), 1.58–1.62 (m, 2H, 2H-3), 1.93–2.10 (m, 6H, 2H-2, 2H-8, 2H-17), 2.70–2.85 (m, 4H, 2H-11, 2H-14), 2.89 (t, 2H, $J = 6.6$ Hz, NH-CH_2-CH_2), 3.52 (q, 2H, $J = 6.6$ Hz, NH-CH_2), 5.17–5.38 (m, 6H, vinylic-H), 5.54 (br s, 1H, NH-CO), 7.01 (d, 1H, $J = 2.0$ Hz, H-2′), 7.08–7.26 (m, 2H, H-5′, H-6′), 7.30 (d, 1H, $J = 7.8$ Hz, H-7′), 7.53 (d, 1H, $J = 7.6$ Hz, H-4′), 8.37 (br s, 1H, NH). ^{13}C-NMR (50 MHz, $CDCl_3$-δ): 14.2, 20.5, 25.4, 25.5, 25.6, 25.8, 27.2, 29.1, 29.3, 29.6, 33.7, 36.8, 39.7, 111.3, 112.8, 118.6, 119.4, 122.1 (2C), 127.1, 127.4, 127.7, 128.2, 128.3, 130.3, 132.0, 136.4, 173.2.

Assay of FAAH Activity

The assay of Omeir *et al.*[26] adapted to the tritiated substrate,[27] was used. Briefly, frozen (−70°C) brains (minus cerebellum) from adult female Sprague–Dawley rats were thawed and homogenized at 4°C in 20 mM HEPES buffer, pH 7.0, with 1 mM $MgCl_2$. After the homogenates had been centrifuged twice (36,000 g, 20 min at 4°C), the tissue pellets were washed by resuspension in homogenisation buffer, incubated at 37°C for 15 min, and recentrifuged as before. Membranes were then resuspended in 50 mM Tris–HCl buffer, pH 7.4, containing 1 mM EDTA and 3 mM $MgCl_2$, and stored at −70°C until used for assay. Upon assay, membranes, test compounds (10 μl, dissolved in ethanol), [^{3}H]AEA and assay buffer (10 mM Tris–HCl, 1 mM EDTA, 1% (w/v) fatty acid-free bovine serum albumin, pH 7.6) (final assay volume of 200 μl) were incubated for 10 min at 37°C. The ethanol concentration was kept constant throughout (5% v/v). Controls contained the same concentration of ethanol in place of the test compounds. Reactions were stopped by placing the tubes in ice. Following addition of chloroform: methanol (1:1 v/v, 400 μl) and thorough mixing, phases were separated by centrifugation. Aliquots (200 μl) of the methanol / buffer phase were analyzed for radioactivity by liquid scintillation spectroscopy with quench correction. Blanks contained distilled water instead of the membranes.

Assay of Affinity at Cannabinoid and Vanilloid Receptors

Assay of affinity of the test compounds at cannabinoid and vanilloid receptors was assessed as described previously.[25] Tissue and ligands, respectively, were for CB_1 receptor, rat cerebellar membranes and 0.5 nM [^{3}H]WIN 55212,2; CB_2 receptor, HEK293EBNA membranes and 0.3 nM [^{3}H]CP 55,940; VR1 receptor, rat spinal cord membranes and 25 pM [^{3}H]resiniferatoxin (for details, see Reference 25).

Calculation of pI_{50} Values

Maximal attainable inhibition and pI_{50} values were determined as described previously[28] using the GraphPad Prism computer programme (GraphPad Software Inc., San Diego, CA, USA). Briefly, data as % of control were analysed using the built-in

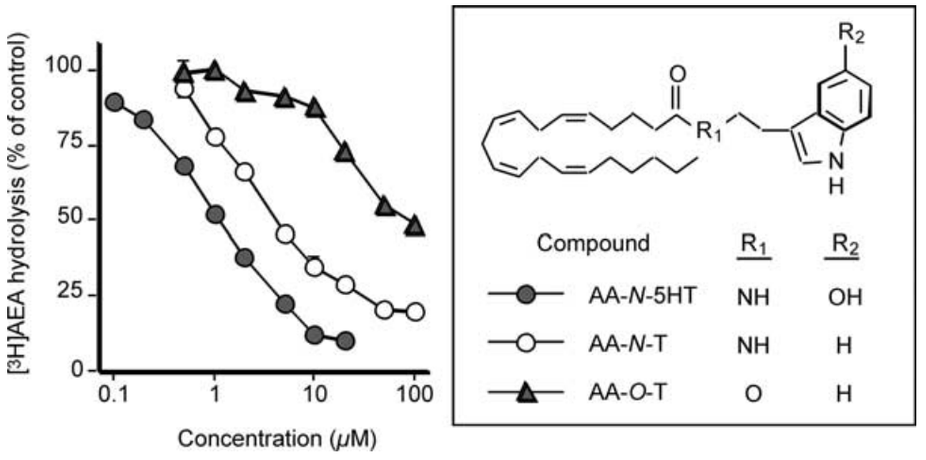

FIGURE 1 Inhibition of 2 μM [^{3}H]AEA hydrolysis by rat brain homogenates by AA-*N*-5HT and structurally related compounds. Shown are means ± s.e.m. (when not enclosed by the symbols), N = 3. The data for AA-*N*-5HT is taken from Figure 2 of Jonsson *et al.*[28] using an identical assay and is shown for comparative purposes.

equation "sigmoid dose-response (variable slope)" with the "top" (i.e. uninhibited) value set to 100% and the "bottom" (i.e. minimum activity remaining) allowed to vary. This allows determination of both the maximum attainable inhibition and the pI_{50} value, i.e. $-\log_{10}$ of the concentration producing half the maximum attainable inhibition. When the maximum attainable inhibition was not significantly different from 0%, a bottom value of 0% was used.[28]

Calculation of $K_{i(slope)}$ and $K_{i(intercept)}$ Values

In the experiments determining the mode of inhibition by selected compounds, $K_{M(app)}$ and $V_{max(app)}$ values for [^{3}H] AEA metabolism for each concentration of the test compounds were calculated from the mean data using the Direct Linear Plot analysis[29] and the Enzyme Kinetics v 1.4 software package, Trinity Software, Campton, NH, USA. The values were then used in secondary replots of [test compound] vs $K_{Mapp}/V_{max\ app}$ and $1/V_{max\ app}$ to determine $K_{i(slope)}$ and $K_{i(intercept)}$ values, respectively.

RESULTS AND DISCUSSION

The ability of the compounds to interact with FAAH has been investigated by measurement of the rate of metabolism of [^{3}H]AEA by rat brain homogenates. Dose response curves for the test compounds are shown in Figures 1 and 2. Inhibition curves were analysed to determine the maximum inhibition attainable and the pI_{50} values for the inhibitable component. In studies with analogues of

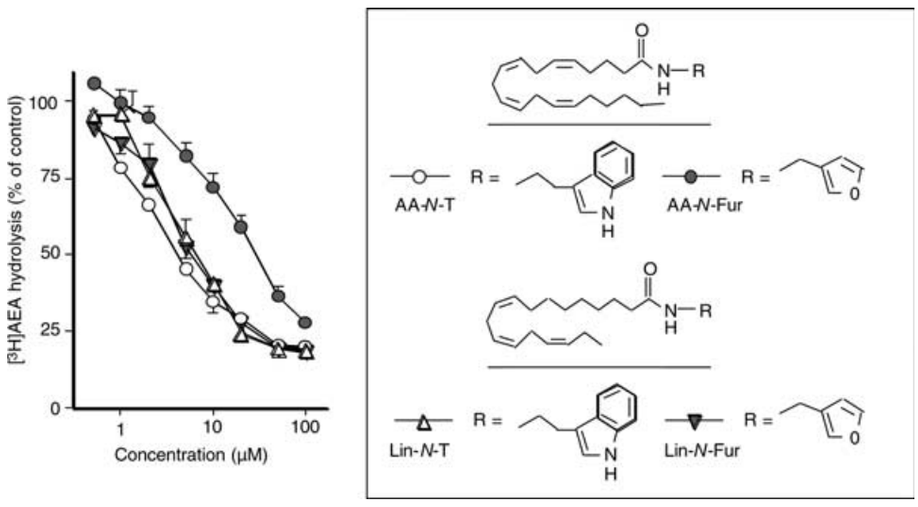

FIGURE 2 Inhibition of 2 μM [^{3}H]AEA hydrolysis by rat brain homogenates by four fatty acid derivatives. Shown are means ± s.e.m. (when not enclosed by the symbols), N = 3. The data for AA-*N*-T is the same as in Figure 1.

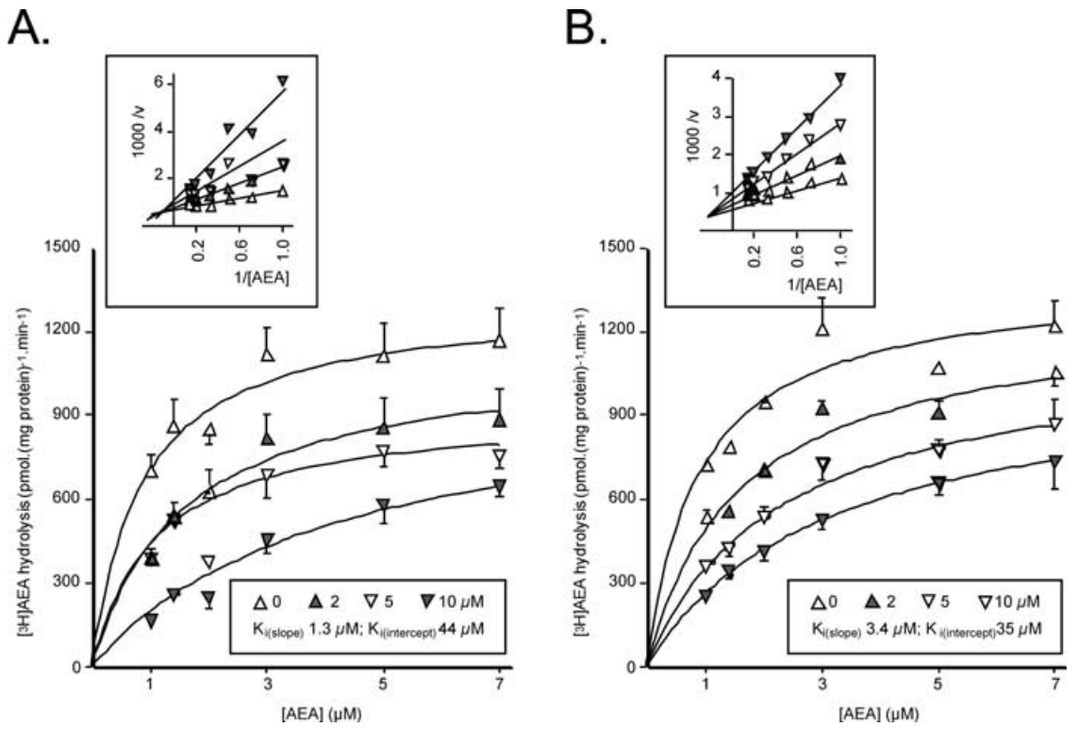

FIGURE 3 Mode of inhibition of rat brain [^{3}H]AEA metabolism by **A**, AA-*N*-T and **B**, Lin-*N*-Fur. Shown are means ± s.e. mean of three experiments. Secondary replots of the mean data to illustrate the mixed-type nature of the inhibition are shown as inserts. The $K_{i(slope)}$ and $K_{i(intercept)}$ values were calculated from the mean data as described in *Materials and Methods*.

***N*-palmitoylethanolamine and *N*-oleoylethanolamine, the maximum inhibition attainable is pH-dependent and reflects the limited solubility of these lipophilic compounds, whereas the pI_{50} values are the true measure of potency and are not dependent upon the assay pH [Reference 28; S. Vandevoorde, K.-O. Jonsson, C.J. Fowler and D. Lambert, unpublished data]. For the present compounds, the maximum inhibition attainable and the pI_{50} values for the inhibitable component, respectively, were: AA-*N*-5HT, 100% and 5.93 ± 0.02;[28] AA-*N*-T, 81 ± 2% and 5.57 ± 0.04; Lin-*N*-T 83 ± 3% and 5.34 ± 0.05; AA-*O*-T, 61 ± 7% and 4.61 ± 0.11; AA-*N*-Fur (UCM707) 100% and 4.53 ± 0.04; Lin-*N*-Fur, 85 ± 3% and 5.36 ± 0.05. The mean IC_{50} values normalised to AA-*N*-5HT (= 1) are: AA-*N*-T, 2.3; Lin-*N*-T, 3.9; AA-*O*-T, 21; AA-*N*-Fur, 25; and Lin-*N*-Fur, 3.8 (the higher the value, the lower the potency).

AA-*N*-T and Lin-*N*-Fur were shown further to act as mixed-type inhibitors of [^{3}H]AEA metabolism (Figure 3), a result consistent with the data of Bisogno *et al.*[23] for AA-*N*-5HT, which also inhibited [^{3}H]AEA hydrolysis by N18TG2 neuroblastoma cell membranes in a mixed-type manner. Consistent with previous reports for AA-*N*-Fur,[25] AA-*N*-T, Lin-*N*-Fur and Lin-*N*-T interacted weakly with CB_1, CB_2 and VR1 receptors, with K_i values >1 μM in each case (data not shown). An additional compound, where the arachidonic side chain of AA-*N*-Fur was replaced by an oleic side chain, was also assessed as an FAAH inhibitor and gave maximum inhibition and pI_{50} values of 55 ± 4% and 5.25 ± 0.10, respectively (IC_{50} value relative to AA-*N*-5HT = 4.8; data not shown).

A number of conclusions can be drawn from the present data.

1. In their original paper, Bisogno *et al.*[23] suggested on the basis of comparisons with dopamine and histamine derivatives that the 5-hydroxy group of AA-*N*-5HT was an important factor determining the potency of this compound. The AA-*N*-T analogue shown here is indeed a weaker inhibitor of AEA metabolism, but the difference in potency is 0.36 log units, i.e. a factor of 2.3. A similar reduction in potency was seen by Bisogno *et al.*[23] when the arachidonic side chain of AA-*N*-5HT was replaced by an icosapentaenoic (20:5) side chain. This would suggest that the contribution of the 5-hydroxy group is not a vital determinant of potency.
2. It is well known that the composition of the acyl side chain can affect not only the ability of ethanolamide, amide and *p*-nitroanilide derivatives to act as FAAH substrates[18,30–33] but also the potency of substrate analogues as

FAAH inhibitors.[22,23,34–37] In the present study, we found that replacement of the arachidonic side chain of AA-*N*-T with a linolenic hydrophobic tail has no effect upon the potency of the compound to prevent AEA metabolism. In contrast, for the three furan derivatives, the arachidonic derivative is ~6 fold less potent than the linolenic or oleic analogues. This is in contrast to the situation with acyl *N*-dopamine inhibitors, where the 18:3 linolenoyl-dopamine was a three-fold weaker inhibitor of AEA metabolism by N18TG2 membranes than either the 20:4 arachidonoyl-, the 18:3 pinolenoyl- or the 18:4-stearidonoyl- analogues.[36] The present results are thus consistent with the suggestion that the side chain length and degree of saturation affect the observed affinity towards FAAH in a manner that is also dependent upon the nature of the head group.

3. Replacement of the amide linkage of AA-*N*-T with an ester linkage reduces the potency towards FAAH nine-fold. With the exception of 2-arachidonoyl glycerol (which can be metabolised by FAAH although the preferred enzyme is a monoglycerol lipase[38]), relatively little is known concerning the interaction of ester linked arachidonoyl compounds with FAAH, although arachidonoyl ethylene glycol was reported to inhibit the breakdown of AEA by N18TG2 membrane preparations with an IC_{50} value of 23.5 μM.[23] However, the discovery of virodhamine (arachidonoyl-*O*-ethanolamine) as an endogenous cannabinoid receptor ligand with a lower efficacy than AEA[39] highlights the need to investigate such compounds. The data shown here demonstrate a reduced potency for inhibition of AEA metabolism by the ester compound. We have also seen this effect in separate studies using both arachidonoyl- and palmitoyl- based compounds. Thus, for arachidonic derivatives, a reduction in potency following replacement of the amide linkage by an ester linkage was seen for *N*-(fur-3-ylmethyl) [3.3], *N*-(fur-2-ylmethyl) [1.5], *N*-[1-(fur-2-yl)ethyl] [>2.5], (±)-*N*-(tetrahydrofur-2-ylmethyl) [>2.5] and *N*-(thien-3-ylmethyl) [7] compounds (values in parentheses show the IC_{50} values of the carboxylate derivatives relative to their isosteric carboxamide). In contrast, the *N*-(thien-2-ylmethyl) [0.11] and *N*-(2-thien-2-ylethyl) [0.24] compounds showed the reverse potency (present authors, unpublished results), again suggesting that the nature of the head group affects this interaction with FAAH. For compounds with a saturated 16 carbon side chain, the methanolamide compound showed the same potency as its corresponding ester (pI_{50} values of 4.57 and 4.75, respectively), whereas the ester equivalent of the -isopropylamide compound (pI_{50} value 4.89[28]) is inactive (S. Vandevoorde, K.-O. Jonsson, C.J. Fowler and D. Lambert, unpublished data).

In conclusion, the focus of the investigation has been to explore further the structure–activity relationships of FAAH substrate analogues related to AA-*N*-5HT, rather than to obtain highly potent compounds. Nevertheless, the lack of direct effect of the present compounds upon cannabinoid receptors may make them useful as templates for the design of more potent FAAH inhibitors in the future.

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