Bioorganic & Medicinal Chemistry 21 (2013) 5188–5197

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Macamides and their synthetic analogs: Evaluation of in vitro FAAH inhibition

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ARTICLE INFO

Article history:
Received 29 January 2013
Revised 5 June 2013
Accepted 14 June 2013
Available online 27 June 2013

Keywords:
Maca
Lepidium meyenii
Macamide
Fatty acid amide
FAAH
3-Methoxybenzylamine
Oleamide
Anandamide
Urea
Carbamate
OL-135
PF-750

ABSTRACT

Maca (*Lepidium meyenii*), a traditional food crop of the Peruvian Andes is now widely touted as a dietary supplement. Among the various chemical constituents isolated from the plant are a unique series of non-polar, long-chain fatty acid *N*-benzylamides known as macamides. We have synthesized 11 of the 19 reported macamides and have tested each as potential inhibitors of the human enzyme, fatty acid amide hydrolase (FAAH). The five most potent macamides were FAAH inhibitors (IC_{50} = 10–17 μM). These amides were derivatives of oleic, linoleic and linolenic acids and benzylamine or 3-methoxybenzylamine. Of the three compounds evaluated in a pre-incubation time study, two macamides were not irreversible inhibitors of FAAH. The third, a carbamate structurally related to macamides, was shown to be an irreversible inhibitor of FAAH (IC_{50} = 0.153 μM).

1. Introduction

1.1. History of Maca as a medicinal agent

The plant known as Maca (*Lepidium meyenii* (Walp.)) has for centuries been cultivated by the indigenous people in the Peruvian Andes as a staple food crop.[1] This herb in the Brassicaceae family grows at 4000 m elevation during a 9-month season (Oct–July).[2,3] In recent times a variety of preparations obtained by grinding or extracting the dried Maca hypocotyls have become commercial naturopathic medicines touted around the world for a variety of indications ranging from being a general tonic to acting as a promoter of sex drive in men and fertility in women. Some authors term Maca 'the ginseng of the Andes'.[4] A number of studies in animals[3,5,6] and humans[7–9] give credence to these and other biological activities for Maca. Clinical trials employing Maca root powders have shown a diminution of sexual dysfunction in women and suggest a lessening of menopausal symptoms.[10,11]

1.2. Macamides: Maca constituents previously associated with biological activity

Previous studies demonstrated that the active principals of Maca reside in the non-polar extracts of the plant.[5,12–15] Neuroprotective properties of the pentane extract were revealed in previous studies, but the mechanism of action was unknown.[13] Known compounds unique to Maca which best fit this solubility profile are the *N*-benzylamides of long-chain fatty acids frequently referred to as macamides and macaenes.[†] While the macamide most frequently isolated in the literature is *N*-benzylpalmitamide, **3a**, nineteen of these macamides have been identified in Maca extracts in the last decade.[4,16–18]

All macamides are *N*-benzylamides. Some are amides of saturated (C_8 and C_{15-18}) fatty acids, while others are derivatives of the common C_{18} unsaturated fatty acids, oleic, linoleic and linolenic acid. In six of the known macamides (also C_{18}), the fatty acid

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[†] The term 'macaenes' is frequently found in the chemical literature on Maca, but no alkene hydrocarbons have ever been isolated. The only alkene functions that have been isolated from the non-polar fractions of the plant extract are the free fatty acids from which the amides are formed, for example, oleic, linoleic, linolenic, and **9ii**. As such, we suggest that use of the name macaene be discontinued.

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http://dx.doi.org/10.1016/j.bmc.2013.06.034

chain is modified to contain ketone functions. In four of these ketomacamides the ketone function is conjugated to *trans* double bonds. In four reported non-ketone macamides, the *N*-benzyl group of the amide bears a single *m*-methoxy substituent. All four of these 3-methoxybenzyl compounds have been synthesized in this study.

1.3. Macamides bear structural similarity to the endogenous substrates of fatty acid amide hydrolase (FAAH)

Macamides **5a** and **5b** (see Fig. 1) are *N*-benzyl derivatives of FAAH substrate oleamide **1**.[19,20] Anandamide (AEA) **2a**, the principal endocannabinoid acting as an agonist at the CB1 and CB2 receptors,[21–24] contains four *cis* double bonds in its acyl chain. Macamides **6a** and **6b** contain two and **7a** and **7b** contain three *cis* double bonds. Indeed, we reported earlier that the crude nonpolar (pentane) extracts of Maca contained inhibitors of FAAH (IC_{50} = 7.5 ± 3.1 mg/mL).[13] In a subsequent study of the enzyme kinetics of FAAH testing **6b** as an inhibitor, a Lineweaver–Burk plot indicated that **6b** acts as a mixed inhibitor of FAAH.[25] We continue our study of the chemical basis of the biological activity of Maca by comparing individual macamides as inhibitors of FAAH. To this end we have synthesized eleven of the nineteen reported macamides, as well as, a series of structurally related amides, ureas, and a carbamate. The structures of all synthetic compounds and known FAAH inhibitors used as standards in this paper are presented in Figures 1–3.

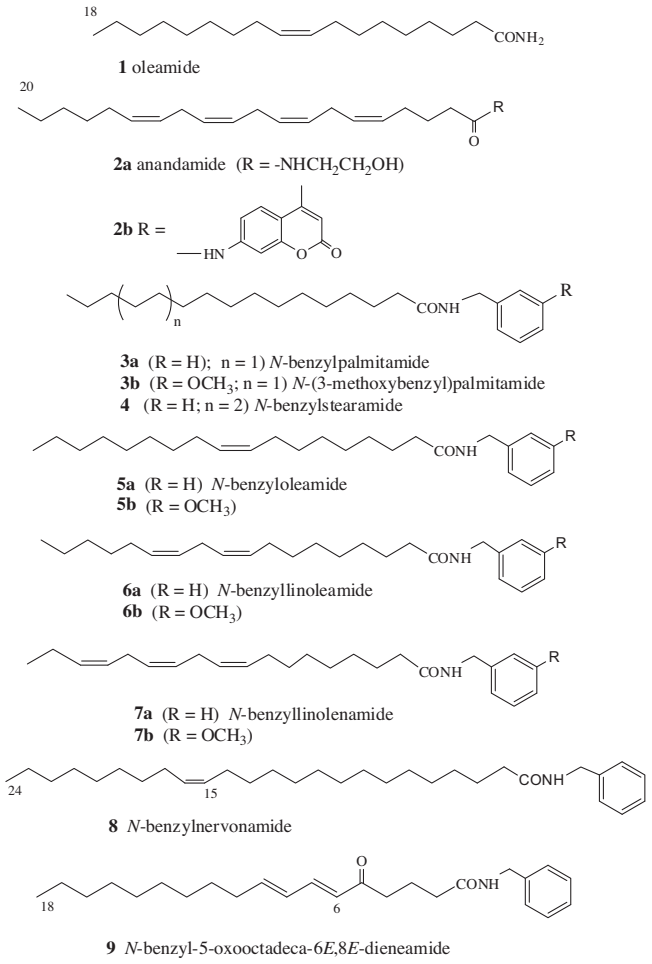


Figure 1. Oleamide **1**, AEA **2**, and synthesized macamides **3**–**9**.

2. Results

2.1. Chemistry

The synthesis of oleamide **1**, macamides **3–8**, and synthetic derivatives **10–13** was carried out by reacting the parent carboxylic acid with 1,1′-carbonyldiimidazole (CDI). Solutions of the resulting *N*-acylimidazole intermediates were added to ammonia or the appropriate benzylamine. The structures of these compounds are presented in Figures 1 and 2.

From among the six reported ketone-containing macamides, we chose the dienone **9** for synthesis. The three-step sequence shown in Scheme 1 began with the *syn*-addition of DiBALH to 1-undecyne. When the resulting *E*-vinylalane was coupled with a large excess of *trans*-1,2-dibromoethylene (in a *cis* > *trans* mixture) with a Pd catalyst, it gave exclusively *E,E*-1-bromo-1,3-tridecadiene **9i**. This selective reactivity for *trans*-1,2-dibromoethylene is reported in the literature.[26,27] Lithium exchange in **9i** at very low temperature gave the dienyllithium which when added to glutaric anhydride[28] gave the 5-ketoacid **9ii** directly. Coupling with HATU yielded the desired N-benzylamide, **9**.

In a synthetic modeling study, we prepared 5-ketostearamides, by the low temperature acylation of 1-tridecyl Grignard with methyl 5-chloro-5-oxovalerate to yield the methyl ester of 5-ketostearic acid **14i**.[29] Hydrolysis to the acid and coupling with HATU gave the *N*-benzyl 5-ketostearamides **14a**/**b** as shown in Scheme 2.

From oleamide, **1**, we synthesized *Z*-9-octadecenylamine by LAH reduction. Reaction of this amine with commercial isocyanates gave ureas **15a–c**. From oleic acid by a modified Curtius rearrangement with diphenylphosphoryl azide, we prepared 8-heptadecenyl isocyanate **16i** which was reacted with benzylamines to give ureas **16a**/**b**, with 3-pyridylmethylamine to give urea **17a**, and with 3-pyridylmethanol to give carbamate **17b**.

Early in the studies of synthetic FAAH inhibitors a series of ketonic 2-oleyloxazoles were reported along with their SAR.[30] Subsequent work led to the preparation of a significantly more inhibitory set of 2-acyloxazoles in which the oleyl group was

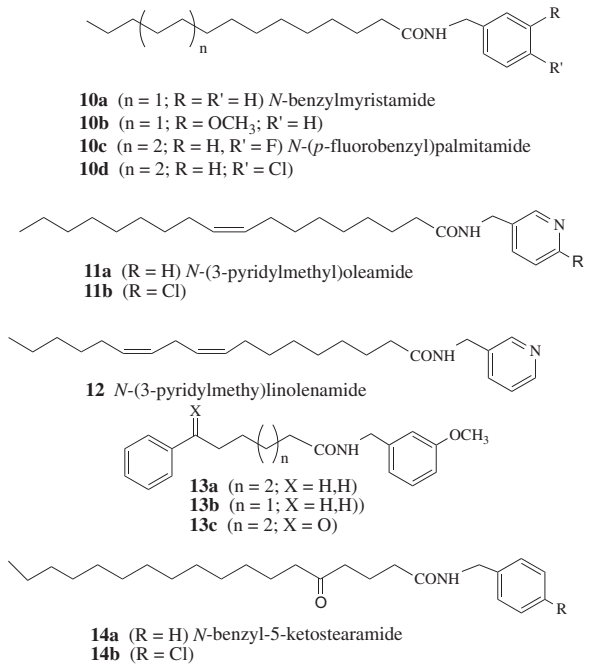


Figure 2. Synthetic amides resembling macamides.

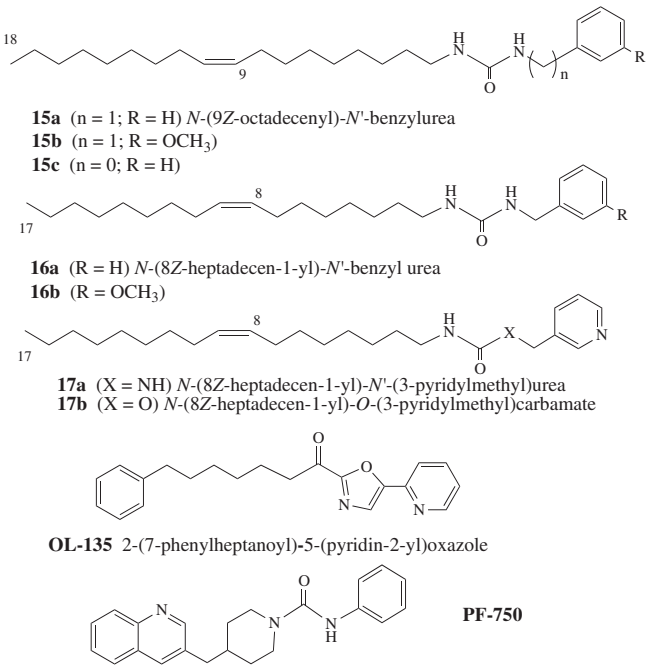
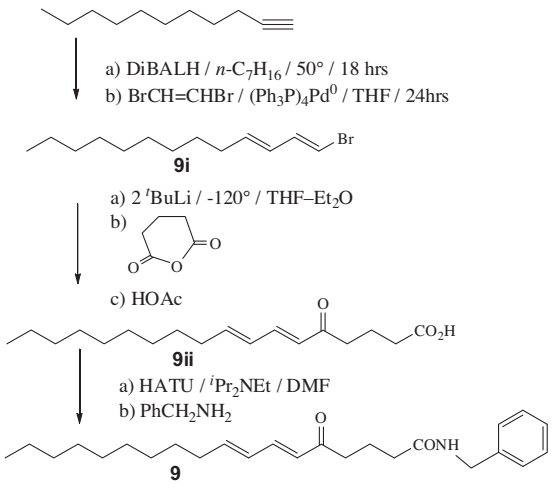


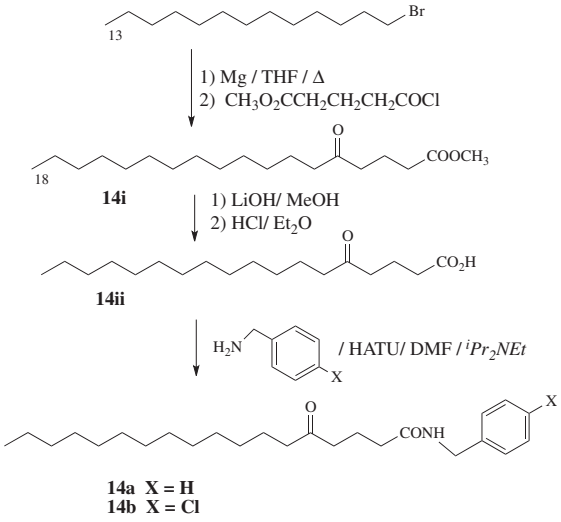
Figure 3. Long chain ureas, a carbamate, and standard inhibitors of FAAH.



Scheme 1. Synthesis of *N*-benzyl-5-oxooctadeca-6*E*,8*E*-dieneamide, **9**.

replaced with phenyl-terminated, shorter acyl chains.[20] The most widely studied of these second-generation FAAH inhibitors has been the compound named **OL-135** which contains a 3-pyridyl substituent on C-5 of the oxazole ring (see Fig. 3). We synthesized this compound for use as a standard of FAAH inhibitory activity. Additionally, we prepared three macamide analogs (**11a**/**b** and **12**) in which the *N*-(3-methoxybenzyl) groups of **5b** and **6b** were replaced with *N*-(3-pyridylmethyl) groups. We also prepared simple amides **13a**–**c** combining substructures from both macamides and **OL-135**.

In the literature there are many reports of irreversible FAAH inhibitors that are ureas[31–33] or carbamates.[34,35] To expand our study to include examples of these functional groups, we chose to prepare the series of oleic acid derivatives which are ureas, **15**–**17a**, and one carbamate, **17b**.



Scheme 2. Synthesis of 5-ketostearamides **14a** and **14 b**.

2.2. Measurement of enzyme inhibitory activity

Data for the inhibitory activity of synthesized macamides **3**–**9** is recorded in Table 1. Data for the synthetic analogues **10**–**17** and known reversible inhibitor **OL-135** and irreversible inhibitor **PF-750** is listed in Table 2. In order to identify appropriate concentration ranges for IC_{50} measurements, all compounds were first tested against FAAH at 10 and 500 μM concentrations. Compounds failing to achieve ~70% enzyme inhibition at the higher concentration were not tested further. The more inhibitory compounds were subjected to an additional assay done in triplicate at 5–7 concentrations. IC_{50} values were calculated from the sigmoidal plots of concentration versus % inhibition, and the maximum value of enzyme inhibition achieved was listed in the second column of Tables 1 and 2. IC_{50} values not determined for compounds of inhibitory potency 50–70% at 500 μM are listed as *n.d*, and weakly active compounds with inhibitory potency <10% are listed as ~0 in the Tables 1 and 2.

Table 3 shows the comparison of IC_{50} values for the macamides, **5b**, **6b**, and carbamate **17b** when the standard preincubation time of the test compound (20 min.) was lengthened to 60 min. before substrate **2b** was added.

3. Discussion

3.1. Fatty acid amide hydrolase (FAAH) controls chemical signaling

The enzyme fatty acid amide hydrolase (FAAH) is a membrane-bound mammalian enzyme responsible for the destruction of a number of endogenous signaling amides by hydrolysis. Among the amides hydrolyzed by FAAH are the primary amide oleamide, **1**, a sleep-inducing lipid, and the ethanolamide, anandamide, **2**, an endogenous cannabinoid agonist.[20] Various studies have been carried out on FAAH preparations from rat (rFAAH), cloned-human (hFAAH), and hybridized human-rat (h-rFAAH) sources. The latter has the active site of hFAAH, but the solubility and crystallizing properties of rFAAH.[36]

In FAAH, an amidase signature enzyme with a unique ser, ser, lys catalytic triad, the deprotonated ser-241 residue nucleophilically attacks the carbonyl group of the substrate or inhibitor. The resulting tetrahedral oxyanion from amide substrates goes on to

Table 1
The inhibitory potency of macamides in the FAAH assay[a]

Compound	IC_{50} (μM) (±SEM)	Max. % inhibition from IC_{50} curve	10 μM % inhibition (±SEM)	500 μM %inhibition (±SEM)
3a	—		24.8 ± 1.9	43.8 ± 2.0
3b	*n.d.*		40.0 ± 2.1	70.1 ± 1.0
4	—		~0	26.2 ± 4.0
5a	16.7 ± 2.9	76.1	49.7 ± 4.1	76.7 ± 1.1
5b	11.0 ± 2.3	76.4	46.8 ± 2.5	78.4 ± 1.3
6a	10.8 ± 1.3	83.2	50.9 ± 1.3	82.0 ± 1.9
6b	10.3 ± 1.3	87.5	48.4 ± 1.2	86.1 ± 0.6
7a	41.8 ± 2.4	79.7	14.5 ± 1.1	75.6 ± 0.3
7b	13.7 ± 1.3	82.8	39.7 ± 1.3	82.4 ± 0.4
8	—		~0	49.2 ± 1.9
9	—		~0	26.4 ± 4.5

[a] Values indicated as *n.d.* were not determined; —indicates that IC_{50} values would likely be >500 μM.

Table 2
The FAAH inhibitory potency of synthetic compounds structurally similar to macamides and two standard inhibitors[a]

Compound	IC_{50} (μM) (±SEM)	Max. % inhibition from IC_{50} curve	10 μM % inhibition (±SEM)	500 μM % inhibition (±SEM)
10a	—		15.9 ± 3.3	27.2 ± 3.1
10b	—		29.3 ± 1.1	38.7 ± 1.8
10c	—		33.9 ± 3.0	41.9 ± 2.9
10d	*n.d.*		16.0 ± 2.8	65.0 ± 1.9
11a	7.57 ± 1.07	90	56.8 ± 3.9	81.2 ± 1.4
11b	15.3 ± 1.9	79	43.5 ± 2.8	76.1 ± 1.2
12	8.41 ± 0.71	94	52.2 ± 2.2	81.9 ± 1.4
13a	—		~0	16.1 ± 2.9
13b	—		~0	12.4 ± 1.6
13c	—		~0	~0
14a	—		11.0 ± 2.4	11.0 ± 3.0
14b	—		~0	~0
15a	*n.d.*		~0	59.9 ± 1.6
15b	—		10.8 ± 2.6	48.5 ± 1.9
15c	—		~0	~0
16a	*n.d.*		14.2 ± 3.2	61.3 ± 2.9
16b	*n.d.*		31.3 ± 3.4	66.0 ± 1.7
17a	—		22.0 ± 3.8	42.6 ± 2.0
17b	0.153 ± 0.034	~100	*n.d.*	*n.d.*
OL-135	0.033 ± 0.006	~100	*n.d.*	*n.d.*
PF-750	1.09 ± 0.18	~100	*n.d.*	*n.d.*

[a] Values indicated as *n.d.* were not determined; — indicates that IC_{50} values would likely be >500 μM.

Table 3
Variations in IC_{50} values at two preincubation times for macamides **5b**, **6b**, and carbamate **17b**

Preincubation time (min)	IC_{50} (μM) macamide **5b** (±SEM)	IC_{50} (μM) macamide **6b** (±SEM)	IC_{50} (μM) carbamate **17b** (±SEM)
20	11.0 ± 2.3	10.3 ± 1.3	0.153 ± 0.034
60	11.1 ± 2.0	16.1[a]	0.0596 ± 0.0063

[a] Value taken from Ref. 25.

hydrolyze to protonated ammonia (or amines) and a carboxylate anion. With urea and carbamate inhibitors, the loss of the amine from the oxyanion intermediate leads to an inactivated, O-acylated enzyme (which is a carbamate) and free amines.[37] With ketone inhibitors the oxyanion substrate has been observed to sit in the active site, and well-resolved X-ray structures with various ketones as oxyanions have been reported.[38,39] Acylation of the enzyme by urea and carbamate inhibitors has been shown to be irreversible.[36,37] The ketone inhibitors are known to be reversible inhibitors since enzyme activity has been recovered after exposure to a large excess of the ketones.[40]

3.2. FAAH topology

PF 750 and **OL-135** and several analogs have been crystallized in the active site of FAAH, and X-ray structures reveal the presence of three channels in the enzyme by which substrates enter and products leave: the membrane access channel (MAC), the acyl binding pocket (ABP), and the cytosolic port. The active site with the catalytic triad is located at the juncture of the ABP and the cytosolic port. The 7-phenylheptanoyl chain of **OL-135** in extended form fills the ABP. Longer oleyl chains fill the APB to the region of the $\Delta^{9,10}$ *cis*-double bond and then extend further into the MAC in a bent conformation facilitated by the *cis* stereochemistry of the double bond. The oxazole and the appended 2-pyridyl ring of **OL-135** lie in the cytosolic port.[38,39] One key structural feature in the latter channel is the thr-236 side chain which makes a flexible, water-mediated hydrogen bond to the 2-pyridyl nitrogen of **OL-135**. Through its electron withdrawing properties, the oxazole ring uniquely contributes to the binding which makes **OL-135** and its analogs inhibitors at nanomolar concentrations.[38,39]

3.3. Synthetic compounds as FAAH inhibitors

Among the macamides, **3**–**9** in Table 1, the most inhibitory compounds were the unsaturated C-18 amides **5**–**7**. Less active were the C-16 palmitamides **3a** and **3b**. Essentially inactive were stearamide **4**, the C-24 *N*-benzylnervonamide **8**, and the C-18 dienoneamide **9**. Macamides **5**–**7** would be expected to fit into the ABP and extend into the MAC channels of FAAH. The chain length of **8** and the stiff *trans*-dieneone chain of **9** would not be expected to fit well into enzyme channels. But the saturated C-16 chain of compounds **3** apparently made for a better fit in the same enzyme channels than the C-18 compound **4**.

Five of the six unsaturated macamides showed inhibition of FAAH (IC_{50} = 10–17 μM). The presence of the 3-methoxy substituent on the *N*-benzyl group lowered IC_{50} values in the oleamide **5b** and the linolenamide **7b**, but did not change the value for the linoleamide **6b**. This order would suggest that the $\Delta^{9,12}$ octadienoyl group had the most stabilizing interactions with the ABP and MAC channels, and that the *N*-(3-methoxybenzyl) oxygen provided further association with the enzyme by accepting a hydrogen bond (most likely mediated by a water molecule) from the thr-236 residue present in the cytosolic port of FAAH. Further evidence of the proposed effects of the inhibitor-enzyme hydrogen bonding was provided by the data from the synthetic *N*-(3-pyridylmethyl) analogs of the natural macamides, **11a**–**12** in Table 2. With the preferred oleyl and linoleyl groups, compounds **11a** and **12** showed greater inhibition (IC_{50} = 8 μM). Placement of an electronegative 2-chloro substituent next to the nitrogen in **11a** gave **11b** which clearly showed a diminished inhibition (IC_{50} = 15 μM) as would be expected from the lowered basicity of the nitrogen atom in **11b**. These inhibitory potencies are in agreement with the hypothesis that macamides are at least in part substrates for FAAH and as such, are hydrolyzed by the enzyme.

Recognizing that the 6-phenylhexyl group of **OL-135** is reported to be an exact fit for the MAC channel of FAAH,[38] we prepared compound **13a** containing that hydrocarbon tail attached to the *N*-(3-methoxyphenylcarboxamido) head group found in the most active macamides. The resulting compound **13a** had no significant activity as an FAAH inhibitor. As model compounds for the synthetic pathway that led to **9**, we prepared **14a**/**b** with 5-keto functions in a saturated C-18 chain. These ketoamides showed no significant inhibition of FAAH.

The final set of macamide-inspired analogs reported here were a series of ureas and a single carbamate each derived from oleic acid. Only weak inhibition of FAAH was observed for ureas **15a**/**c** in which the double bond is one methylene further away from the carbonyl group than it is in the macamides **5**–**7**. That extra methylene would probably cause a difficult fit for the hydrocarbon tails of these ureas in the ABP of the enzyme. In ureas **16a**/**b** and **17a** and carbamate **17b** the carbonyl group is restored to the relationship with the double bond that pertains in macamides **5**–**7** allowing a typical oleyl fit in the ABP and the MAC channels. The three ureas produced only modest inhibitory activity against FAAH, but the carbamate **17b** with an IC_{50} = 0.153 μM was almost two orders of magnitude more potent as an inhibitor of FAAH than any other compound prepared in this study.

Time-dependent preincubation studies have been used to characterize the type of inhibition of both FAAH inhibitors[37] and other enzyme inhibitors.[41,42] Decreases in IC_{50} values after a longer preincubation time are taken to mean that a compound is inhibiting the enzyme irreversibly since the concentration of active enzyme is reduced irreversibly during the preincubation period. Little or no change in IC_{50} is equated with reversible inhibition. Urea **PF-750** has been identified as an irreversible inhibitor in this way.[37]

We applied this method to two typical macamides **5b** and **6b**, and to the synthetic carbamate, **17b**; the resulting IC_{50} values are shown in Table 3. The results are in agreement with macamides **5b** and **6b** acting as reversible inhibitors and carbamate **17b** acting as an irreversible inhibitor of FAAH.

3.4. Conclusion

This study shows that macamides exhibit moderate FAAH inhibition. The more potent natural products showed IC_{50} values of about 10 μM. As chemically neutral lipids in a plant rich in carbohydrates, macamides should easily cross the intestinal wall and the blood–brain barrier. While these in vitro values of macamide inhibition are not especially high, the cumulative effect of regular consumption of Maca might well alter the balance of the signaling amide system. While we have not demonstrated that macamides are fully reversible, competitive inhibitors, we have reported that inhibitory potencies of natural macamides **3–9** and their synthetic structural analogs (**10–17**) vary roughly in accord with the size and placement of the residues lining the enzyme channels leading to the active site. We report one synthetic carbamate with an oleyl chain, modified by substitution of an NH group for the α-CH_2, **17b**, that is a much more potent inhibitor of FAAH (IC_{50} = 0.153 μM). A time-dependent preincubation study was in agreement with the interpretation that carbamate **17b** was an irreversible inhibitor and that inhibition by macamides **5b** and **6b** was reversible.

Further study should investigate whether the macamides are indeed responsible for the varied medicinal properties attributed to Maca. Clinical studies using powders or extracts from the plant should be carried out only on plant samples or extracts in which the macamide concentrations have been predetermined. We will report elsewhere the methodology necessary to assay macamide content.[43]

4. Experimental

4.1. Synthesis

All reagents, solvents, anandamide (**2a**), and urea PF-750 were used as received from commercial sources. 1,2-Dibromoethylene was synthesized by addition of aliquots of iron powder to prewarmed (90°) solution of *sym*-tetrabromoethane in DMF (successive exotherms kept the temperature between 95° and 125°) and was distilled as a (3:2 mixture of *cis*/*trans* isomers).[44] Compound OL-135 was synthesized by the literature method.[20] Thin-layer chromatography (TLC) was performed on silica-coated glass plates (EMD silica gel 60 F_{254}). LC/MS analysis was performed on a Shimadzu HPLC/UV (214 nm and/or 254 nm wavelength) system coupled to ELSD (Sedex 75, Sedere) and MS (ZQ Micromass) detectors. NMR spectrawere recorded on a Varian Mercury NMR spectrometer operating at 400 MHz and chemical shifts are reported in ppm. Melting points were measured in a Fisher-Johns apparatus between glass cover slips of samples that solidified after chromatography; no final products were recrystallized. All temperatures are recorded in degrees Centigrade. The 96-well plates used in the fluorescent assay were read by a Biotek Synergy HT Microplate Reader.

4.1.1. N-benzylpalmitamide (3a)

To a solution of palmitic acid (300 mg, 1.17 mmol) in dichloromethane (DCM, 10 mL) was added 1,1′-carbonyldiimdazole (209 mg, 1.29 mmol). The reaction was stirred at room temperature for 2 h. The reaction mixture was slowly added to a solution of benzylamine (153 μL, 1.40 mmol) and 4-dimethylaminopyridine (14 mg, 0.12 mmol) in dichloromethane (5 mL). The solution was stirred at room temperature for 18 h. DCM (100 mL) and saturated

aqueous $NaHCO_3$ (30 mL) were added to the reaction mixture. The organic layer was separated and washed with H_2O (30 mL), brine (30 mL), dried over anhyd sodium sulfate, filtered, and concentrated to dryness under reduced pressure. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc (3:1, v/v) to give the title compound as a white solid (578 mg, 86%). Mp 85–87° [lit.[45] mp 94.5–95°, and[46] fp 95.1°]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.36 (m, 2H), 7.26–7.30 (m, 3H), 5.72 (br s, 1H), 4.45 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.2 Hz, 2H), 1.61–1.67 (m, 2H), 1.25–1.34 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.21, 138.66, 128.92, 128.05, 127.71, 43.80, 37.06, 32.16, 29.92, 29.89, 29.84, 29.73, 29.59, 29.56, 26.01, 22.93, 14.36; LCMS, $C_{23}H_{39}NO$, [M+H]: 346.

4.1.2. *N*-(3-Methoxybenzyl)palmitamide (3b)

It was prepared as described for **3**a. Yield 73%. White powder, mp 58–60°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.27 (m, 1H), 6.85 (d, J = 7.6 Hz, 1H), 6.80–6.82 (m, 2H), 5.68 (br s, 1H), 4.42 (d, J = 6.0 Hz, 2H), 3.80 (s, 3H), 2.21 (t, J = 7.2 Hz, 2H), 1.61–1.69 (m, 2H), 1.25–1.34 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.18, 160.13, 140.23, 129.98, 120.25, 113.60, 113.19, 55.46, 43.78, 37.07, 32.16, 29.92, 29.90, 29.89, 29.84, 29.73, 29.59, 29.57, 26.01, 22.92, 14.35; LCMS, $C_{24}H_{41}NO_2$, [M+H]: 376.

4.1.3. *N*-Benzylstearamide (4)

Prepared from stearic acid by the method for **3a**. Yield 85%. White powder, mp 84–86° [lit.[46] fp 99.2°]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.36 (m, 2H), 7.26–7.30 (m, 3H), 5.68 (br s, 1H), 4.45 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.65–1.69 (m, 2H), 1.25–1.29 (m, 28H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.20, 138.64, 128.94, 128.07, 127.73, 43.82, 37.06, 32.16, 29.93, 29.84, 29.73, 29.60, 26.01, 22.93, 14.36; LCMS, $C_{25}H_{43}NO$, [M+H]: 374.

4.1.4. *N*-Benzyloleamide (5a)

It was prepared from oleic as described for **3a**. White powder. Yield 82%. Mp 48–50° [lit.[46] fp 58.8°]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.36 (m, 2H), 7.26–7.29 (m, 3H), 5.72 (br s, 1H), 5.32–5.37 (m, 2H), 4.44 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.98–2.03 (m, 4H), 1.63–1.67 (m, 2H), 1.26–1.30 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.20, 138.63, 130.23, 129.98, 128.94, 128.06, 127.74, 43.81, 37.03, 32.13, 30.00, 29.93, 29.76, 29.56, 29.52, 29.49, 29.37, 27.45, 27.40, 26.00, 22.92, 14.36; LCMS, $C_{25}H_{41}NO$, [M+H]: 372.

4.1.5. *N*-(3-Methoxybenzyl)oleamide (5b)

It was prepared as described for **3a**. Yield 49%. Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.27 (m, 2H), 6.86 (d, J = 7.2 Hz, 1H), 6.81–6.83 (m, 3H), 5.67 (br s, 1H), 5.32–5.36 (m, 2H), 4.42 (d, J = 6.0 Hz, 2H), 3.80 (s, 1H), 2.21 (t, J = 7.2 Hz, 2H), 2.03–1.88 (m, 4H), 1.61–1.68 (m, 2H), 1.26–1.30 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.12, 160.11, 140.21, 130.23, 129.99, 129.98, 120.25, 113.61, 113.19, 55.46, 43.78, 37.06, 32.13, 30.00, 29.93, 29.76, 29.56, 29.53, 29.49, 29.37, 27.45, 27.40, 26.00, 22.92, 14.36; LCMS, $C_{26}H_{43}NO_2$, [M+H]: 402.

4.1.6. (9*Z*,12*Z*)-*N*-Benzyloctadeca-9,12-dienamide (6a)

It was prepared from linoleic acid as described for **3a**. Yield 84%. White powder, mp 30–32° [lit.[46] fp 35.8°]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.36 (m, 5H), 5.73 (br s, 1H), 5.30–5.40 (m, 4H), 4.44 (d, J = 5.6 Hz, 2H), 2.77 (t, J = 6.4 Hz, 2H), 2.21 (t, J = 8.0 Hz, 2H), 2.02–2.07 (m, 4H), 1.63–1.67 (m, 2H), 1.26–1.37 (m, 14H), 0.89 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.15, 138.64, 130.46, 130.27, 128.94, 128.27, 128.13, 128.06, 127.74, 43.82, 37.03, 31.75, 29.82, 29.57, 29.51, 29.48, 29.37, 27.42, 25.98, 25.85, 22.81, 14.31; LCMS, $C_{25}H_{39}NO$, [M+H]: 370.

4.1.7. (9*Z*,12*Z*)-*N*-(3-Methoxybenzyl)octadeca-9,12-dienamide (6b)

It was prepared as described for **3a**. Yield 75%. Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.27 (m, 1H), 6.86 (d, J = 7.2 Hz, 1H), 6.81–6.83 (m, 2H), 5.67 (br s, 1H), 5.29-5.40 (m, 4H), 4.42 (d, J = 5.6 Hz, 2H), 3.80 (s, 3H), 2.77 (t, J = 6.8 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 2.02–2.07 (m, 4H), 1.61–1.68 (m, 2H), 1.27–1.39 (m, 14H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.10, 160.13, 140.23, 130.46, 130.27, 129.98, 128.28, 128.13, 120.25, 113.62, 113.18, 55.46, 43.78, 37.05, 31.75, 29.83, 29.57, 29.52, 29.49, 29.37, 27.42, 25.98, 25.85, 22.81, 14.31; LCMS, $C_{26}H_{41}NO_2$, [M+H]: 400.

4.1.8. (9*Z*,12*Z*,15*Z*)-*N*-Benzyloctadeca-9,12,15-trienamide (7a)

It was prepared from linolenic acid as described for **3a**. Yield 85%. Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.35 (m, 5H), 5.73 (br s, 1H), 5.30–5.41 (m, 6H), 4.44 (d, J = 5.2 Hz, 2H), 2.80 (t, J = 6.4 Hz, 4H), 2.21 (t, J = 7.6 Hz, 2H), 2.02–2.11 (m, 4H), 1.62–1.67 (m, 2H), 1.30–1.36 (m, 8H), 0.97 (t, J = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.14, 138.64, 132.20, 130.49, 128.94, 128.51, 128.48, 128.06, 127.95, 127.74, 127.34, 43.82, 37.03, 29.80, 29.51, 29.47, 29.36,27.43, 25.98, 25.85, 25.75, 20.78, 14.51; LCMS, $C_{25}H_{37}NO$, [M+H]: 368.

4.1.9. (9*Z*,12*Z*,15*Z*)-*N*-(3-Methoxybenzyl)octadeca-9,12,15-trienamide (7b)

It was prepared as described for **3a**. Yield 80%. Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.27 (m, 1H), 6.86 (d, J = 7.2 Hz, 1H), 6.81–6.83 (m, 2H), 5.67 (br s, 1H), 5.29–5.41 (m, 6H), 4.42 (d, J = 6.0 Hz, 2H), 3.80 (s, 3H), 2.80 (t, J = 6.8 Hz, 4H), 2.21 (t, J = 7.6 Hz, 2H), 2.02–2.10 (m, 4H), 1.61–1.68 (m, 2H), 1.30–1.36 (m, 8H), 0.97 (t, J = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.10, 160.13,140.22, 132.21, 130.50, 129.98, 128.52, 128.48, 127.96, 127.34, 120.26, 113.63, 113.18, 55.46, 43.78, 37.05, 29.81, 29.52, 29.48, 29.36, 27.43, 25.98, 25.85, 25.75, 20.78, 14.51; LCMS, $C_{26}H_{39}NO_2$, [M+H]: 398.

4.1.10. (*Z*)-*N*-Benzyltetracos-15-enamide (8)

It was prepared from nervonic acid as described for **3a**. Yield 94%. White powder, mp 64–66°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.36 (m, 5H), 5.68 (br s, 1H), 5.31–5.39 (m, 2H), 4.44 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.98–2.04 (m, 4H), 1.62–1.69 (m, 2H), 1.25–1.29 (m, 32H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.17, 138.64, 130.13, 128.94, 128.07, 127.74, 43.83, 37.07, 32.13, 30.01, 29.89, 29.84, 29.80, 29.75, 29.73, 29.58, 29.55, 27.44, 26.01, 22.91, 14.35; LCMS, $C_{31}H_{53}NO$, [M+H]: 456.

4.1.11. (1*E*,3*E*)-1-Bromotrideca-1,3-diene (9i)

To neat 1-undecyne (2.28 g, 15.0 mmol) was added slowly diisobutylaluminum hydride (1.0 M in heptane; 15 ml, 15.0 mmol) at room temperature. The reaction was heated at 50° for 18 h. The heptane was removed under reduced pressure to give (1*E*)-diisobutyl-1-undecenylaluminum as an oil. The oil was diluted with dry THF (6 ml) and with stirring was slowly added to a mixture of tetrakis(triphenylphosphine)palladium(0) (347 mg, 0.3 mmol) and dibromoethylenes (13.8 g, 75.0 mmol, 60% *cis* and 40% *trans*) in dry THF (4 ml) under N_2. After 24 h at room temperature the reaction mixture was quenched with 3 N HCl (5 ml) and extracted with diethyl ether (60 ml). The organic layer was washed with satd $NaHCO_3$ (20 ml), H_2O (20 mL) and brine (20 ml), dried over sodium sulfate and concentrated to dryness. The crude product (oil) was purified by flash chromatography on silica gel eluting with hexanes

to yield **9i** as a colorless oil (2.25 g, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (dd, J = 13.2 Hz, J = 10.4 Hz, 1H), 6.17 (d, J = 14.0 Hz, 1H), 5.95 (dd, J = 15.6 Hz, J = 10.8 Hz, 1H), 5.73 (dt, J = 15.2 Hz, J = 6.8 Hz, 1H). 2.01–2.07 (m, 2H), 1.26–1.40 (m, 14 H), 0.88 (t, J = 6.8 Hz, 3H).

4.1.12. (6*E*,8*E*)-5-Oxooctadeca-6,8-dienoic acid (9ii)

A solution of **9i** (500 mg, 1.93 mmol) in THF (2 ml)/ether (5 ml) was cooled to −120° under N_2 in a bath containing ethanol and liquid N_2 and treated with *t*-butyllithium (1.7 M in pentane) (2.33 ml, 3.96 mmol). The reaction was kept between −120° and −100 °C for 1 h, then allowed to warm to room temperature. The crude product solution (**9ii**) was slowly added to a solution of glutaric anhydride (208 mg, 1.82 mmol) in THF (2.5 ml)/ether (2.5 ml) at −90 °C. After warming to room temperature, the reaction was quenched by pouring into a separatory funnel containing 10 mmoles of acetic acid, brine (10 ml) and DCM (50 ml). The organic layer was dried over sodium sulfate and concentrated to dryness to give crude (**9ii**).

4.1.13. (6*E*,8*E*)-*N*-Benzyl-5-oxooctadeca-6,8-dienamide (9)

To a solution of **9ii** (100 mg, 0.34 mmol) in dry DMF (3 mL) was added HATU (258 mg, 0.68 mmol) and diisopropylethylamine (118 μL, 0.68 mmol). After stirring for 10 min, benzylamine (56 μL, 0.52 mmol) was added. The reaction was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and the residue was taken up into EtOAc (80 mL). The organic layer was washed with sat. $NaHCO_3$ (30 mL), H_2O (30 mL), brine (30 mL), and dried over Na_2SO_4 and concentrated to dryness. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc (2:1, v/v) to yield a white solid (20 mg, 15%). Mp 95–97°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.35 (m, 5H), 7.14 (dd, J = 16.0 Hz, J = 10.0 Hz, 1H), 6.15–6.20 (m, 2H), 6.05 (d, J = 15.6 Hz, 1H), 5.88 (br s, 1H), 4.44 (d, J = 5.6 Hz, 2H), 2.64 (t, J = 7.2 Hz, 2H), 2.27 (t, J = 7.2 Hz, 2H), 2.15–2.20 (m, 2H), 1.97–2.03 (m, 2H), 1.41–1.45 (m, 2H), 1.27 (br s, 12H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.64, 172.54, 146.54, 143.83, 138.50, 128.99, 128.95, 128.05, 127.88, 127.74, 43.83, 39.19, 35.75, 33.41, 32.10, 29.75, 29.66, 29.53, 29.42, 28.92, 22.90, 20.55, 14.35; LCMS, $C_{25}H_{37}NO_2$, [M+H]: 384.

4.1.14. *N*-Benzyltetradecanamide (10a)

It was prepared from myristic acid as described for **3a**. White powder. Yield 89%. Mp 78–80° [lit.[45] mp 89–90°]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.36 (m, 5H), 5.71 (br s, 1H), 4.44 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.61–1.69 (m, 2H), 1.20–1.34 (m, 20H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.19, 138.65, 128.94, 128.07, 127.74, 43.82, 37.07, 32.15, 29.91, 29.88, 29.83, 29.73, 29.58, 29.55, 26.01, 22.93, 14.36; LCMS, $C_{21}H_{35}NO$, [M+H]: 317.

4.1.15. *N*-(3-Methoxybenzyl)tetradecanamide (10b)

It was prepared using 3-methoxybenzylamine as described for **3a**. Yield 78%. White powder, mp 52–54°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.27 (m, 1H), 6.85 (d, J = 7.6 Hz, 1H), 6.80–6.82 (m, 2H), 5.72 (br s, 1H), 4.41 (d, J = 5.6 Hz, 2H), 3.80 (s, 3H), 2.21 (t, J = 7.6 Hz, 2H), 1.61–1.69 (m, 2H), 1.25–1.34 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.19, 160.12, 140.24, 129.98, 120.25, 113.59, 113.18, 55.48, 43.76, 37.07, 32.16, 29.91, 29.89, 29.85, 29.73, 29.60, 26.02, 22.93, 14.36; LCMS, $C_{22}H_{37}NO_2$, [M+H]: 348.

4.1.16. *N*-(4-Fluorobenzyl)palmitamide (10c)

It was prepared using palmitic acid and 4-fluorobenzylamine as described for **3a**. Yield 68%. White powder, mp 86–88°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.26 (m, 2H), 6.99–7.04 (m, 2H), 5.70 (br s, 1H), 4.41 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.61–1.69 (m, 2H), 1.25–1.30 (m, 24H), 0.88 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.24, 163.61,161.17, 134.50, 134.47, 129.74, 129.66, 115.85, 115.64, 43.05, 37.01, 32.15, 29.92, 29.88, 29.83, 29.72, 29.59, 29.57, 29.54, 25.98, 22.92, 14.35; LCMS, $C_{23}H_{38}FNO$, [M+H]: 364.

4.1.17. *N*-(4-Chlorobenzyl)palmitamide (10d)

It was prepared using palmitic acid and 4-chlorobenzylamine as described for **3a**. Yield 68%. White powder, mp 80–82°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.30 (d, J = 8.0 Hz, 2H), 7.20–7.22 (d, J = 8 Hz, 2H), 5.76 (br s, 1H), 4.40 (d, J = 6.0 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.61–1.68 (m, 2H), 1.29–1.20 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.29, 137.25, 133.50, 129.36, 129.04, 43.06, 37.01, 32.16, 29.92, 29.91, 29.89, 29.83, 29.72, 29.59, 29.57, 29.54, 25.97, 22.93, 14.36; LCMS, $C_{23}H_{38}ClNO$, [M+H]: 380.

4.1.18. *N*-(Pyridin-3-ylmethyl)oleamide (11a)

It was prepared as described for **3a**. Yield 57%. White powder, mp 41–43°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 2H), 7.63 (d, J = 8.0 Hz, 1H), 7.25–7.28 (m, 1H), 5.93 (br s, 1H), 5.29–5.39 (m, 2H), 4.46 (d, J = 6.0 Hz, 2H), 2.22 (t, J = 7.2 Hz, 2H), 1.98–2.03 (m, 4H), 1.61–1.67 (m, 2H), 1.26–1.30 (m, 20H), 0.88 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.39, 149.36, 149.11, 135.87, 134.40, 130.24, 129.94, 123.82, 41.18, 36.90, 32.13, 29.99, 29.92, 29.75, 29.55, 29.50, 29.47, 29.34, 27.45, 27.39, 25.92, 22.91, 14.35; LCMS, $C_{24}H_{40}N_2O$, [M+H]: 373. Anal. Calcd for $C_{24}H_{40}N_2O$: C, 77.37; H, 10.82; N, 7.52. Found: C, 77.18; H, 10.76; N, 7.53.

4.1.19. *N*-[(2-Chloropyridin-5-yl)methyl]oleamide (11b)

It was prepared as described for **3a**. Yield 36%. White powder, mp 62–64°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 2.4 Hz, 1H), 7.62 (dd, J = 8.0 Hz, J = 2.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 5.96 (br s, 1H), 5.32–5.36 (m, 2H), 4.42 (d, J = 6.4 Hz, 2H), 2.22 (t, J = 7.6 Hz, 2H), 1.98–2.03 (m, 4H), 1.60–1.67 (m, 2H), 1.26–1.30 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.52, 150.80, 149.10, 138.82, 133.51, 130.27, 129.92, 124.53, 40.40, 36.84, 32.13, 29.98, 29.92, 29.75, 29.55, 29.48, 29.46, 29.34, 27.45, 27.39, 25.87, 22.91, 14.35; LCMS, $C_{24}H_{39}ClN_2O$, [M+H]: 407

4.1.20. (9*Z*,12*Z*)-*N*-(Pyridin-3-ylmethyl)octadeca-9,12-dienamide (12)

It was prepared as described for **3a**. Yield 49%. White powder, mp 29–31°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48–8.50 (m, 2H), 7.62 (dt, J = 8.0 Hz, J = 1.6 Hz, 1H), 7.25 (dd, J = 8 Hz, J = 4.8 Hz, 1H), 6.27 (br s, 1H), 5.30–5.41 (m, 4H), 4.43 (d, J = 6.0 Hz, 2H), 2.77 (t, J = 6.8 Hz, 2H), 2.22 (t, J = 7.2 Hz, 2H), 2.02–2.07 (m, 4H), 1.61–1.67 (m, 2H), 1.24-1.39 (m, 14H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.48, 149.33, 149.01, 135.84, 134.50, 130.45, 130.23, 128.28, 128.10, 123.79, 41.12, 36.84, 31.74, 29.82, 29.56, 29.50, 29.47, 29.34, 27.41, 25.92, 25.85, 22.79, 14.31; LCMS, $C_{24}H_{38}N_2O$, [M+H]: 371.

4.1.21. *N*-(3-Methoxybenzyl)-7-phenylheptanamide (13a)

To a solution of 7-phenylheptanoic acid (500 mg, 2.42 mmol) in dichloromethane ((10 mL) was added 1.1′-carbonyldiimdazole (432 mg, 2.67 mmol). The reaction was stirred at room temperature for 2 h. The reaction mixture was slowly added to a solution of 3-methoxybenzylamine (372 μL, 2.91 mmol) and 4-dimethylaminopyridine (30 mg, 0.24 mmol) in dichloromethane (5 mL). The reaction was carried out at room temperature for 18 h. DCM (100 mL) and saturated aqueous $NaHCO_3$ (30 mL) were added to the reaction mixture. The organic layer was separated and washed with H_2O (30 mL), brine (30 mL), dried over sodium sulfate and concentrated to dryness under reduced pressure. The crude was

purified by flash chromatography on silica gel eluting with hexane/EtOAc (3:1, v/v) to give the title compound as a white solid (457 mg, 58%, mp 68–70°); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.28 (m, 3 H), 7.15–7.18 (m, 3H), 6.85 (d, J = 7.2 Hz, 1H), 6.80–6.82 (m, 2H), 5.69 (br s, 1H), 4.41 (d, J = 6.0 Hz, 2H), 3.79 (s, 3H), 2.59 (t, J = 7.6 Hz, 2H), 2.20 (t, J = 7.6, 2H), 1.59–1.68 (m, 4H), 1.33-1.37 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.08, 160.11, 142.92, 140.21, 129.99, 128.63, 128.47, 125.85, 120.26, 113.63, 113.16, 55.47, 43.77, 36.98, 36.09, 31.52, 29.38, 29.18, 25.91; LCMS, $C_{21}H_{27}NO_2$, [M+H]: 326.

4.1.22. *N*-(3-Methoxybenzyl)-6-phenylhexanamide (13b)

It was prepared using 3-methoxybenzylamine as described for **13a**. Colorless oil. Yield 53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.29 (m, 3 H), 7.15–7.19 (m, 3H), 6.81–6.86 (m, 3H), 5.66 (br s, 1H), 4.41 (d, J = 6.0 Hz, 2H), 3.80 (s, 3H), 2.61 (t, J = 7.6 Hz, 2H), 2.20 (t, J = 7.6, 2H), 1.60–1.74 (m, 4H), 1.35–1.42 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.01, 160.11, 142.75, 140.19, 129.99, 128.62, 128.50, 125.91, 120.26, 113.64, 113.16, 55.47, 43.78, 36.92, 35.97, 31.39, 29.13, 25.82; LCMS, $C_{20}H_{25}NO_2$, [M+H]: 312.

4.1.23. *N*-(3-Methoxybenzyl)-7-oxo-7-phenylheptanamide (13c)

It was prepared as described for **13a**. Yield 54%. White powder, mp 50–52°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92–7.95 (m, 2 H), 7.54–7.57 (m, 1H), 7.44–7.47 (m, 2H), 7.23 (t, J = 8.0 Hz, 1H), 6.79–6.86 (m, 3H), 5.85 (br s, 1H), 4.41 (d, J = 6.0 Hz, 2H), 3.78 (s, 3H), 2.97 (t, J = 6.8 Hz, 2H), 2.24 (t, J = 7.6, 2H), 1.68–1.80 (m, 4H), 1.39–1.47 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 200.51, 172.97, 160.10, 140.20, 137.19, 133.21, 129.97, 128.81, 128.25, 120.26, 113.64, 113.13, 55.45, 43.79, 38.47, 36.66, 29.04, 25.73, 23.95; LCMS, $C_{21}H_{25}NO_3$, [M+H]: 340.

4.1.24. Methyl 5-oxooctadecanoate (14i)

Magnesium power (465 mg, 19.37 mmol) in dry THF (2 mL) was mechanically activated under N_2 for 4 h. A solution of 1-bromotridecane (5.0 g, 18.99 mmol) in dry THF (10 mL) was added dropwise at room temperature. The mixture was refluxed for 4 h until the disappearance of the Mg power to yield tridecylmagnesium bromide solution (~1.5 M). To a mixture of methyl 5-chloro-5-oxovalerate (2.84 g, 17.26 mmol) and copper (I) iodide (362 mg, 1.90 mmol) in dry THF (20 mL) was slowly added the prepared Grignard reagent solution at −78 °C under N_2. The mixture was stirred for 4 h at −78° and was allowed to warm up to room temperature. The mixture was quenched by a saturated aqueous solution of ammonium chloride and was extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc (2:1, v/v) to yield a white solid (3.6 g, 67%, mp 54–56°); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 (s, 3H), 2.47 (t, J = 7.6 Hz, 2H), 2.38 (t, J = 7.6 Hz, 2H), 2.34 (t, J = 7.6 Hz, 2H), 1.86–1.93 (m, 2H), 1.53–1.57 (m, 2H), 1.25–1.32 (m, 20H), 0.88 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.67, 173.88, 51.77, 43.12, 41.67, 33.29, 32.14, 29.89, 29.86, 29.82, 29.70, 29.63, 29.57, 29.47, 24.08, 22.91, 19.10, 14.34; LCMS, $C_{19}H_{36}O_3$, [M+H]: 313.

4.1.25. 5-Oxooctadecanoic acid (14ii)

To a solution of methyl 5-oxooctadecanoate (800 mg, 2.56 mmol) in THF (5 mL) and methanol (5 mL) was added lithium hydroxide (108 mg, 2.56 mmol) solution in H_2O (2 mL). The reaction was stirred at room temperature for 18 h until completion of the hydrolysis. The solvent was removed under reduced pressure. Dichloromethane (8 mL) was added to the residue, followed by addition of 2 M HCl (in ethyl ether) (1.28 mL, 2.56 mmol). The mixture was stirred for half an hour. After filtration, the filtrate was concentrated to dryness and dried in high vacuum to a white solid (762 mg, 100%). Mp 85–87°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.50 (t, J = 7.6 Hz, 2H), 2.39 (t, J = 6.8 Hz, 4H), 1.86–1.94 (m, 2H), 1.53–1.58 (m, 2H), 1.25–1.32 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.65, 178.87, 43.15, 41.50, 33.11, 32.15, 29.90, 29.87, 29.83, 29.70, 29.63, 29.58, 29.47, 24.07, 22.92, 18.77, 14.35; LCMS, $C_{18}H_{34}O_3$, [M+H]: 299.

4.1.26. *N*-Benzyl-5-oxooctadecanamide (14a)

To a solution of 5-oxooctadecanoic acid (**14ii**,146 mg, 0.49 mmol) in dry DMF (8 mL) was added HATU (186 mg, 0.49 mmol) and diisopropylethylamine (180 μL, 1.0 mmol). After stirring for 10 min, benzylamine (59 μL, 0.54 mmol) was added to the mixture. The reaction was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and the residue was taken up into EtOAc (80 mL). The organic layer was washed with sat. $NaHCO_3$ (30 mL), H_2O (30 mL), brine (30 mL), and dried over Na_2SO_4 and concentrated to dryness. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc (2:1, v/v) to yield a white solid (142 mg, 75%, mp 98–100°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26–7.36 (m, 5H), 5.81 (br s, 1H), 4.43 (d, J = 5.6 Hz, 2H), 2.49 (t, J = 6.8 Hz, 2H), 2.37 (t, J = 7.6 Hz, 2H), 2.24 (t, J = 7.6 Hz, 2H), 1.88–1.96 (m, 2H), 1.53–1.57 (m, 2H), 1.25–1.32 (m, 20H), 0.88 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.29, 172.46, 138.47, 128.96, 128.06, 128.05, 127.78, 43.85, 43.14, 41.63, 35.69, 32.15, 29.90, 29.88, 29.84, 29.71, 29.64, 29.58, 29.47, 24.08, 22.92, 19.97, 14.35; LCMS, $C_{25}H_{41}NO_2$, [M+H]: 388.

4.1.27. *N*-(4-Chlorobenzyl)-5-oxooctadecanamide (14b)

From **14ii** following the procedure for **14a** but using 4-chlorobenzylamine was isolated a white powder (152 mg, 73%). Mp 93–95°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 5.88 (br s, 1H), 4.39 (d, J = 5.6 Hz, 2H), 2.49 (t, J = 6.8 Hz, 2H), 2.37 (t, J = 7.6 Hz, 2H), 2.24 (t, J = 7.2 Hz, 2H), 1.90-1.95 (m, 2H), 1.52–1.57 (m, 2H), 1.25–1.32 (m, 20H), 0.88 (t, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 211.33, 172.56, 137.10, 133.55, 129.37, 129.06, 43.15, 43.09, 41.58, 35.66, 32.15, 29.91, 29.88, 29.85, 29.71, 29.64, 29.59, 29.47, 24.08, 22.92, 19.94, 14.36; LCMS, $C_{25}H_{40}ClNO_2$, [M+H]: 423.

4.1.28. Oleyl chloride

To a solution of oleic acid (3.0 g, 10.63 mmol) in dry DCM (30 mL) was added slowly oxalyl chloride (13.3 mL, 26.57 mmol) at 0°. The reaction was allowed to warm up to room temperature and stirred for 4 h. The reaction mixture was concentrated under reduced pressure. The resulting sticky liquid was used for the next step.

4.1.29. Oleamide (1)

The oleyl chloride solution in dry DCM (2 mL) was added slowly to aqueous ammonia solution (28–30%, w/w) (30 mL) with vigorous stirring at 0°. The mixture was stirred at 0° for half an hour. EtOAc (150 mL) and H_2O (30 mL) were added to the reaction mixture. The organic layer was separated and washed sequentially with saturated aq $NaHCO_3$ (50 mL), H_2O (50 mL), brine (50 mL). The EtOAc layer was dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc (2:1) to give a white solid (2.35 g, 79%). Mp 71–73° [lit.[47] mp 72–73°]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (br s, 2H), 5.32–5.39 (m, 2H), 2.22 (t, J = 7.2 Hz, 2H), 1.98–2.03 (m, 4H), 1.60–1.67 (m, 2H), 1.26–1.31 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.73, 130.26, 130.19, 130.02, 129.91, 36.14, 32.13, 29.99, 29.92, 29.76, 29.55, 29.47, 29.34, 27.45, 27.39, 25.75, 22.91, 14.35; LCMS, $C_{18}H_{35}NO$, [M+H]: 282.

4.1.30. Oleylamine (1i)

To a solution of **1** (2.1 g, 7.47 mmol) in dry THF (10 mL) was added 10.5 ml (10.46 mmol) of 1.0 M LAH solution in THF so that gentle reflux was maintained. The reaction mixture was allowed to stir at room temperature overnight. It was then hydrolyzed by cautiously adding dropwise aqueous NaOH (2 N, 10 mL), extracted with ethyl ether (2 × 80 mL). The combined organic layers was washed with H_2O (50 ml), brine (50 mL), dried over Na_2SO_4 and evaporated in vacuo to yield a colorless oil (1.9 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33–5.36 (m, 2H), 2.68 (t, J = 7.2 Hz, 2H), 1.99–2.04 (m, 4H), 1.42–1.45 (m, 2H), 1.20–1.29 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H); LCMS, $C_{18}H_{37}N$, [M+H]: 268.

4.1.31. (*Z*)-1-(Octadec-9-en-1-yl)-3-benzylurea (15a)

To a solution of **1i** (200 mg, 0.75 mmol) in dry DMA (3 mL) was added benzyl isocyanate (102 μL, 0.82 mmol). The reaction was stirred at room temperature overnight. Ethyl acetate (80 mL) was added and the solution was washed with saturated aq. $NaHCO_3$ (30 ml), brine (30 ml), and was dried over Na_2SO_4 and concentrated to dryness. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc (4:1) to yield a white solid (152 mg, 51%). Mp 74–76°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.35 (m, 5H), 5.33–5.36 (m, 2H), 4.68 (br s, 1H), 4.36 (s, 3H), 3.14 (t, J = 6.8 Hz,2H), 1.98-2.03 (m, 4H), 1.43–1.47 (m, 2H), 1.26–1.32 (m, 22H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.33, 139.43, 130.26, 130.20, 130.15, 130.07, 129.97, 128.89, 127.70, 44.86, 40.93, 32.13, 30.40, 29.98, 29.76, 29.70, 29.55, 27.45, 27.08, 22.91, 14.32; LCMS, $C_{26}H_{44}N_2O$, [M+H]: 401.

4.1.32. (*Z*)-1-(Octadec-9-en-1-yl)-3-(3-methoxybenzyl)urea (15b)

It was prepared using 3-methoxybenzyl isocyanate as described for **15a**. Yield 40%. White solid, mp 42–44°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (t, J = 8.4 Hz, 1H), 6.77–6.87 (m, 3H), 5.30–5.39 (m, 2H), 4.82 (br s, 1H), 4.48 (br s, 1H), 4.31 (s, 2H), 3.78 (s, 3H), 3.13 (t, J = 7.2 Hz, 2H), 1.98–2.03 (m, 4H), 1.43–1.47 (m, 2H), 1.26–1.32 (m, 22H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.10, 158.44, 141.15, 130.20, 130.07, 129.97, 119.86, 113.16, 113.00, 55.49, 55.36, 44.76, 40.88, 32.13, 30.42, 30.00, 29.76, 29.72, 29.56, 29.50, 27.45, 27.10, 22.92, 14.35; LCMS, $C_{27}H_{46}N_2O_2$, [M+H]: 431.

4.1.33. (*Z*)-1-Isocyanatoheptadec-8-ene (16i)

To a solution of oleic acid (215 mg, 0.76 mmol) and triethylamine (212 μL, 1.52 mmol) in toluene (1.5 mL) was added diphenylphosphoryl azide (DPPA, 181 μL, 0.84 mmol). After the reaction was heated at 80° overnight, it was ready for use in the next step.

4.1.34. (*Z*)-1-Benzyl-3-(heptadec-8-en-1-yl)urea (16a)

To the above solution was added benzylamine (83 μL, 0.76 mmol). The reaction was again heated at 80° overnight. Ethyl acetate (80 mL) and H_2O (30 mL) were added to the reaction mixture. The organic layer was separated and washed with brine (30 mL), dried over Na_2SO_4, and concentrated to dryness under reduced pressure. The crude was purified by flash chromatography on silica gel eluting with hexane/EtOAc (3:1) to give a white solid (162 mg, 56%, mp 58–60°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23–7.34 (m, 5H), 5.29–5.39 (m, 2H), 4.71 (br s, 1H), 4.36 (s, 3H), 3.14 (t, J = 6.8 Hz, 2H), 1.98–2.03 (m, 4H), 1.43–1.47 (m, 2H), 1.26–1.32 (m, 20H), 0.88 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.36, 139.43, 130.25, 129.95, 128.88, 127.70, 127.57, 44.85, 40.91, 32.13, 30.39, 29.99, 29.92, 29.76, 29.55, 29.47, 29.44, 27.45, 27.40, 27.06, 22.91, 14.35; LCMS, $C_{25}H_{42}N_2O$, [M+H]: 387.

4.1.35. (*Z*)-1-(Heptadec-8-en-1-yl)-3-(3-methoxybenzyl)urea (16b)

It was prepared from **16i** using 3-methoxybenzylamine as described for **16a**. Yield 37%. White solid, mp 34–36°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (t, J = 7.6 Hz, 1H), 6.75–6.84 (m, 3H), 5.32–5.36 (m, 2H), 5.12 (br s, 1H), 4.79 (br s, 1H), 4.27 (s, 2H), 3.76 (s, 3H), 3.09 (t, J = 6.4 Hz, 2H), 1.97–2.02 (m, 4H), 1.39–1.44 (m, 2H), 1.26–1.32 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.06, 158.70, 141.26, 130.23, 129.95, 129.82, 119.79, 113.10, 112.90, 55.41, 55.36, 44.63, 40.79, 32.14, 30.45, 30.01, 29.95, 29.76, 29.57, 29.53, 29.48, 27.46, 27.42, 27.10, 22.92, 14.35; LCMS, $C_{26}H_{44}N_2O_2$, [M+H]: 417. Anal. Calcd for $C_{26}H_{44}N_2O_2$: C, 74.53; H, 10.65; N, 6.69. Found: C, 74.95; H, 10.64; N, 6.72.

4.1.36. (*Z*)-1-(Heptadec-8-en-1-yl)-3-(3-pyridylmethyl)urea (17a)

It was prepared using 3-pyridylmethylamine as described for **16a**. Yield 43%. White solid, mp 68–70°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50–8.53 (m, 2H), 7.67 (d, J = 8.0 Hz, 1H), 7.24–7.27 (m, 1H), 5.13–5.39 (m, 2H), 4.70 (br s, 1H), 4.40 (s, 1H), 4.40 (d, J = 6.0 Hz, 2H), 3.14–3.19 (m, 2H), 1.98–2.03 (m, 4H), 1.46–1.50 (m, 2H), 1.26–1.30 (m, 20H), 0.88 (t, J = 6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.30, 149.11, 148.84, 135.70, 135.43, 130.26, 129.91, 123.78, 42.09, 40.91, 32.13, 30.42, 29.98, 29.92, 29.75, 29.55, 29.47, 29.44, 27.45, 27.39, 27.09, 22.90, 14.35; LCMS, $C_{24}H_{41}N_3O$, [M+H]: 388.

4.1.37. (*Z*)-1-(Heptadec-8-en-1-yl)-3-(pyridin-3-ylmethyl) carbamate (17b)

It was prepared using 3-pyridylmethanol as described for **16a**. White solid. Yield 26%. White solid, mp 22–24°; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H), 8.57 (d, J = 3.6 Hz, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.29 (dd, J = 7.6, 4.4 Hz, 1H), 5.29–5.38 (m, 2H), 5.11 (s, 2H), 4.74 (br s, 1H), 3.16–3.21 (m, 2H), 1.98-2.05 (m, 4H), 1.46–1.50 (m, 2H), 1.26–1.30 (m, 20H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.25, 149.63, 136.19, 132.57, 130.27, 129.93,123.65, 64.20, 41.40, 32.13, 30.15, 29.98, 29.89, 29.75, 29.55, 29.40, 27.45, 27.38, 26.93, 22.91, 14.35; LCMS, $C_{24}H_{40}N_2O_2$, [M+H]: 389. Anal. Calcd for $C_{24}H_{40}N_2O_2$: C, 74.64; H, 10.38; N, 7.16. Found: C, 74.18; H, 10.38; N, 7.21.

4.2. Measurement of inhibitory activity of macamides

To assay the activity of macamides and related compounds as FAAH inhibitors, we employed the first available commercial assay kit for FAAH inhibition. The assay is carried out by preincubating each compound tested with human recombinant hFAAH for 20 min at 25° before **2b**, the non-fluorescent enzyme substrate, *N*-arachidonyl-7-amino-4-methylcoumarin is added, and the temperature is immediately increased to 37°. Enzyme-catalyzed hydrolysis of **2b** liberates the fluorescent 7-amino-4-methylcoumarin.[48] After a 30-minute incubation the fluorescence intensity in each well is read.

By this assay method all compounds **3a–17b** were measured at the two concentrations noted in the Table 1. For most compounds showing 65% or greater enzyme inhibition at a concentration of 500 μmol, complete IC_{50} curves were measured at 5–8 concentrations of inhibitor in separate experiments. The values calculated from these curves are shown in Table 1.

Acknowledgment

We greatly appreciate the financial support of our employers, MCPHS including the summer undergraduate research fellowship (SURF) for H.D.V. and Arqule, Inc. We gratefully acknowledge

discussions with Dr. Jitendra Belani and email exchanges with Dr. Lida Sahakyan for references to the dibromoethylene preparation.

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