Inhibition of Oleamide Hydrolase Catalyzed Hydrolysis of the Endogenous Sleep-Inducing Lipid *cis*-9-Octadecenamide

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Abstract: Oleamide (**1**, *cis*-9-octadecenamide) is a naturally occurring brain constituent that has been shown to accumulate and disappear under conditions of sleep deprivation and sleep recovery, respectively. Synthetic **1** has been found to induce sleep in a structurally specific manner at nanomolar quantities. Hydrolysis of **1** by an enzyme (oleamide hydrolase) present in the cell membrane rapidly degrades oleamide to oleic acid (*cis*-9-octadecenoic acid). Such observations suggest **1** may constitute a prototypical member of a class of fatty acid primary amide biological signaling molecules in which the diversity and selectivity of function are derived from the length of the alkane chain as well as the position, stereochemistry, and degree of unsaturation. A series of inhibitors of oleamide hydrolase were designed and prepared which were expected to derive their properties through interactions with the putative active site cysteine residue within oleamide hydrolase. This approach yielded a series of rapid, selective, and highly potent inhibitors (K_i = 13 μM to 1 nM) which in addition to their potential therapeutic value may serve as useful tools to define the biological role of oleamide.

Oleamide (**1**, *cis*-9-octadecenamide) is a naturally occurring brain constituent that has been shown to accumulate and disappear under conditions of sleep deprivation and sleep recovery, respectively.[1−3] In a structurally specific manner, **1** has been shown to induce physiological sleep in animals at nanomolar quantities when injected intravascularly.[1] In an effort to isolate a regulatory agent responsible for controlling endogenous concentrations of **1**, an integral membrane protein, oleamide hydrolase, was found to catalyze the hydrolytic degradation of oleamide to give oleic acid (*cis*-9-octadecenoic acid) and ammonia (Figure 1), neither of which demonstrate somnolescent activity.[1] To further explore and define the roles of **1** as a prototypical member of a new class of biological signaling agents and oleamide hydrolase as a potentially important factor in its regulation, we describe the preparation and evaluation of a series of potent transition-state mimetic and mechanism-based oleamide hydrolase inhibitors, **2**−**22**.

Oleamide hydrolase could be inhibited by phenylmethanesulfonyl fluoride, 4,4′-dithiodipyridyl disulfide (a potent disulfide-forming reagent), and $HgCl_2$ (IC_{50} = 700 nM, $K_{i,app}$ = 37 nM), but not 1 mM EDTA, suggesting that a thiol is intimately involved in the catalytic process and that the enzyme may be a cysteine amidase or possibly a serine amidase with an active site cysteine residue. A variety of tight binding or irreversible inhibitors of serine and cysteine proteases have been described. These include irreversible inhibitors such as halomethyl ketones,[4−8] Michael acceptors,[9] epoxides,[10] *O*-acylhydroxylamines,[11] and diazomethyl ketones,[12] as well as reversible transition state mimetic inhibitors[13] such as ketones,[14] aldehydes,[15] cyclopropenones,[16] and electron-deficient carbonyl compounds such as trifluoromethyl ketones,[17−20] α-keto acid derivatives,[21−28] and tricarbonyl compounds.[29] Only one pos-

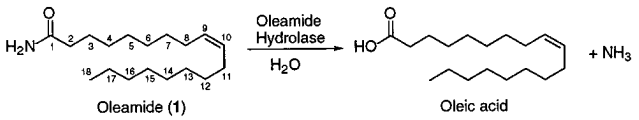

Figure 1. Oleamide hydrolase catalyzes the hydrolysis of **1**, a biological signaling agent with sleep-inducing properties, to produce oleic acid and ammonia.

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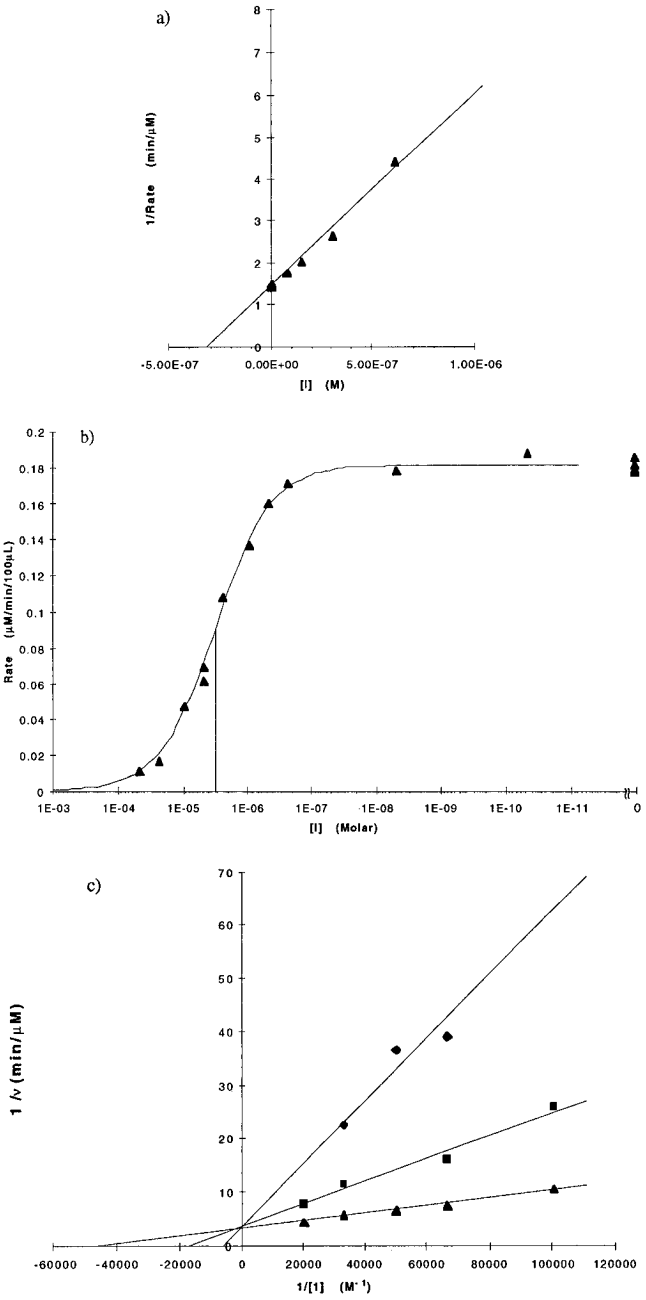

Figure 2. (a) Dixon plot of the activity of **6** against oleamide hydrolase catalyzed degradation of **1**, (b) Effect of **11** against oleamide hydrolase. (c) Lineweaver−Burke plot of competitive oleamide hydrolase inhibition by **12**.

sibly specific inhibitor of oleamide hydrolase has been reported (IC_{50} = 3 μM at [S] = 0.26K_m),[30] and only one report of an investigation of inhibitors of related fatty acid amidases has been disclosed to date.[20]

The potency of the inhibitors was determined using an ion selective electrode to measure the production of ammonia as

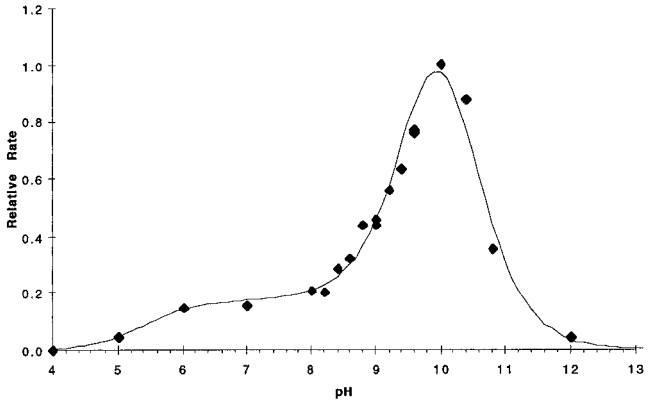

Figure 3. pH−rate dependence of oleamide hydrolase cleavage of **1**, with the fit showing apparent active site pK_a values of 5.4, 9.7, and 10.3. The rate maximum occurs at pH 10.0.

the result of the hydrolysis of 100 μM oleamide (~20K_m) by a membrane bound preparation of oleamide hydrolase. The K_m of oleamide was found to be 5 ± 2 μM. Inhibition constants were determined by the Dixon method (Figure 2). Subject to solubility limitations, all inhibitors that were tested were able to achieve 100% inhibition at high concentrations, and no inhibitor exhibited polymodal inhibition behavior characteristic of two or more separate active sites with different K_i values. Since the likelihood of two or more different enzymes binding twenty-two separate inhibitors with nearly identical affinity is remote, this strongly suggests that a single enzyme in the preparation is responsible for greater than 90% of the observed oleamide hydrolase activity.

The rate of enzyme-catalyzed oleamide hydrolysis was found to be pH dependent (Figure 3) with apparent active site pK_a values of 5.4, 9.7, and 10.3. Our unusual pH−rate dependence profile, oleamide K_m, and inhibition results for PMSF are consistent with results by Maurelli,[30,31] suggesting that the oleamide hydrolase presented here (from rat liver membrane fractions) and the anandamide amidohydrolase (from mouse neuroblastoma cell culture membrane fractions) may be the same enzyme, subject to interspecies variation. However, in the absence of sequence data or purified enzyme, the latter of which is often difficult to achieve with integral membrane proteins, this remains to be proven. However, our results are quite different from those of another report of anandamide amidohydrolase activity which exhibits rate maxima at pH 6 and 8,[32] so there is also evidence to support a many-enzyme model of fatty acid amide hydrolysis *in vivo*. The inhibitors were assayed at pH 10.0, the pH at which oleamide hydrolase activity is at its maximum under our assay conditions.

The most potent inhibitors (Table 1) possess an electrophilic carbonyl group capable of reversibly forming a (thio)hemiacetal or (thio)hemiketal to mimic the transition state of a serine or cysteine protease catalyzed reaction (Figure 4). The relative potencies of the inhibitors were found to follow the expected electrophilic character of the reactive carbonyl cumulating in the tight binding α-keto ethyl ester **8** (1.4 nM) and the trifluoromethyl ketone inhibitor **12** (1.2 nM). A similar correlation between carbonyl electrophilicity and binding constant has been observed in inhibitors of insect juvenile hormone esterase[33] and anandaminase.[20] However, the most electrophilic member of the set, the tricarbonyl inhibitor **11**, bound relatively

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Table 1. Binding Affinities

#	Inhibitor	$K_{i,app}$ (μM)	#	Inhibitor	$K_{i,app}$ (μM)
1	oleamide	K_m = 5 ± 2 μM		R = $C_{16}H_{32}$	
Oleic acid		6.0	12		0.0012 ± 0.0004
2		5 ± 1	13		0.002 ± 0.0006
3		0.7 ± 0.3	14	R (*trans*)	0.009 ± 0.003
4		0.19 ± 0.06	15	R (*saturated*)	0.023 ± 0.007
5		0.04 ± 0.01	16		> 300
6		0.016 ± 0.005	17		> 100
7		0.017 ± 0.006	18		5 ± 2
8		0.0014 ± 0.0005	19		> 100
9		0.009 ± 0.003	20		0.3 ± 0.1
10	R (*saturated*)	0.012 ± 0.003	21		13 ± 4
11	$(CH_2)_{14}CH_3$	0.150 ± 0.040	22		Insoluble

poorly at 150 nM. This behavior may be the result of destabilizing steric interactions between the bulky *tert*-butyl ester and the enzyme or may be in part due to the sp^2 character at C-3, which is uncharacteristic of the natural substrate.

The extent of hydration and the relative electrophilic character of the inhibitor carbonyls could be easily and accurately assessed by NMR analysis, and they were found to follow the expected trends (*e.g.*, **11** > **12** > **8** > **6** ≥ **4**). The central carbonyl of the tricarbonyl inhibitor **11** was fully hydrated upon preparation and characterization. The remaining agents were isolated and characterized as their carbonyl structures without hydration including the reactive trifluoromethyl ketones. ^{1}H NMR and ^{13}C NMR were used to establish and quantitate the addition of CD_3OD or D_2O to the electrophilic carbonyl in CD_3OD and acetone-d_6, respectively (Table 2). Representative of these trends, **11** and **12** were fully converted to their hemiacetals in CD_3OD, and the remaining agents showed diminished hemiacetal formation consistent with their expected electrophilic character: **11** (100%), **12** (100%), **8** (75%), **6** (48%), and **4** (47%).

While the trifluoromethyl ketones **12**, **13**, **14**, and **15** exist in aqueous solution almost entirely as hydrates, these compounds are thought to bind to the enzyme as reversible covalent enzyme−inhibitor hemiketal complexes as shown in structural studies of elastase[34] and α-chymotrypsin[35] and kinetic studies of a series of serine proteases[19] bound to peptidyl trifluoromethyl ketones. Also, though the α-keto amides **6** and **7** are likely to exist at least in part as the sp^2 keto species in solution, α-keto amides have been observed in protease active sites to be completely sp^3.[19] Similarly, aldehydes in the active site of the cysteine protease papain bind as thiohemiacetals.[31,36] The hypothesis that these inhibitors bind as (thio)hemiketals rather than *gem*-diols (hydrated ketones) is further supported by the poor inhibiton of oleamide hydrolase by **16**, **17**, and **18**, despite their structural similarity to the *gem*-diol.

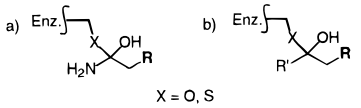


Figure 4. (a) A common intermediate found in papain and other cysteine or serine proteases[53] involving the formation of a (thio)-hemiacetal during the catalysis of peptide hydrolysis. (b) The possible mode of action for inhibitors **3**−**15**.

We note that though electrophilicity of the reactive carbonyl seems to play a large role in dictating the affinity with which these inhibitors bind to oleamide hydrolase, there are likely other factors which also exert influence on the affinity of these compounds for oleamide hydrolase. While the aldehydes **4** and α-keto amide **7** appear to be equally electrophilic, the α-keto amide binds more tightly, suggesting that there are additional favorable interactions being made between the enzyme and the amide functionality, possibly an additional hydrogen bond(s). Similarly, the α-keto ester **8** and the trifluoromethyl ketone **12** bind equally tightly despite the higher electrophilicity of the trifluoromethyl ketone.

Interestingly, aldehyde **5** which incorporates a carbonyl at a position analogous to C-2 of oleamide was found to bind 5 times more tightly than **4**, which incorporates the aldehyde carbonyl in the position analogous to the C-1 of oleamide. This was also observed with the α-keto ester series of inhibitors, where the incorporation of the electrophilic carbonyl at C-2 vs C-1 of oleamide (**8** vs **9**) resulted in a 6-fold increase in binding affinity. This distinction was not seen in the α-keto amides or trifluoromethyl ketones. In these inhibitor classes the placement of the electrophilic carbonyl at the C-2 vs C-1 of oleamide position provided equally effective inhibitors. This suggests the possibility of subtly different binding modes for carbonyl positional analogs of C-1 versus C-2 of oleamide.

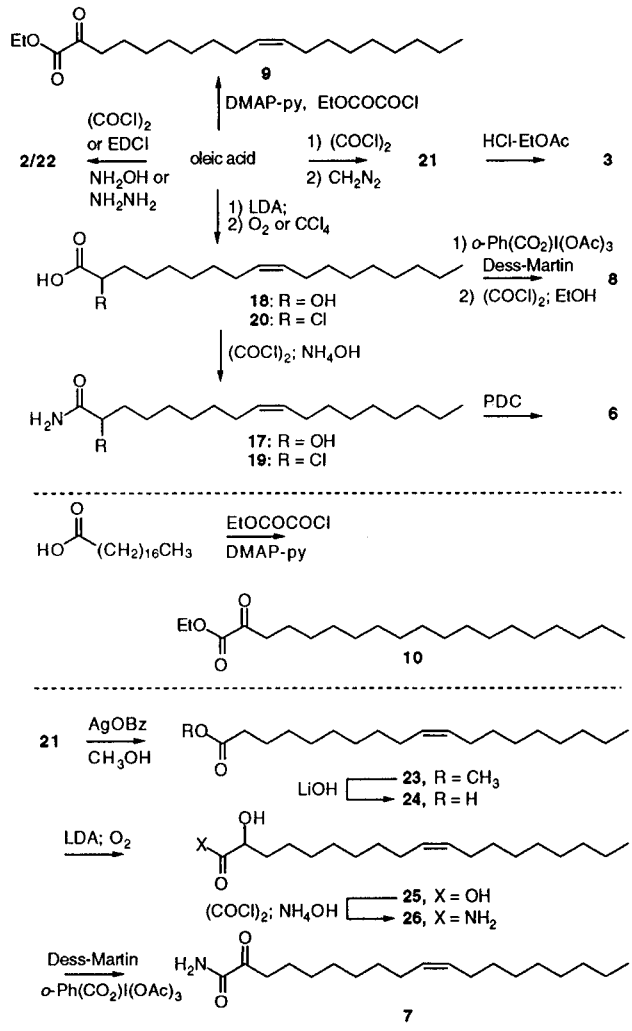
These studies also reveal that oleamide hydrolase displays an approximately 10-fold preference for fatty acid inhibitors which contain a *cis* double bond stereochemistry at the 9 position similar to the natural substrate. This trend is seen most clearly in the trifluoromethyl ketone series where the *cis* double bond

Table 2. Electrophilic Carbonyl Hydration Studies, Diagnostic NMR Signals

agent	^{1}H or ^{13}C NMR signal	$CDCl_3$ (δ)	CD_3OD (δ)	acetone-d_6 (δ)	acetone-d_6 + D_2O (δ)[a]
4	^{1}H (α-CH_2)	2.40	2.42, 1.56	2.41	no change
	^{1}H (CHO)	9.73	9.69 (53%), 4.46 (47%)	9.71	no change
	^{13}C (C═O)	202.8	204.7, 99.9	202.5	no change
6	^{1}H (α-CH_2)	2.89	2.81 (52%), 1.75 (48%)	2.83	no change
	^{13}C (1C═O)	161.9	165.2, 176.2	163.4	no change
	^{13}C (2C═O)	198.6	200.0, 100.1	200.0	no change
8	^{1}H (α-CH_2)	2.81	2.81 (25%), 1.78 (75%)	2.79	no change
	^{13}C (1C═O)	161.3	162.5, 172.4	162.1	no change
	^{13}C (2C═O)	194.8	195.8, 100.0	195.1	no change
12	^{1}H (α-CH_2)	2.68	− (0%), 1.65 (100%)	2.87 (91%), 1.75 (9%)	2.82 (9%), 1.60 (91%)
	^{13}C (C═O)	191.6	97.3	192.3, 94.2	93.8
	^{13}C (CF_3)	115.6	125.2	116.5, 125.2	125.0

[a] 7% D_2O in acetone-d_6.

Scheme 1

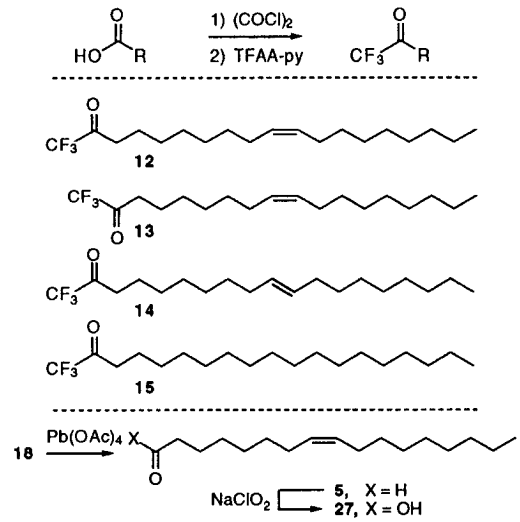


containing **12** is bound approximately an order of magnitude more tightly than the *trans* double bond containing **14** or the saturated derivative **15**.

Most of the potential irreversible inhibitors (**3, 19**−**21**) demonstrated no measurable time dependent inhibitory activity over the first 15 min of incubation at concentrations up to their solubility limits. The chloromethyl ketone **3** gave time independent but moderate inhibition (K_i = 0.7 μM) which is consistent with the formation of a reversible (thio)hemiketal between the putative active site cysteine and the ketone,[37] or a reversible and noncovalent enzyme−inhibitor complex. The presence of an adjacent chloro substituent augments the electrophilicity of the carbonyl, favoring nucleophilic attack. 2-Chlorooleic acid (**20**; K_i = 0.3 μM) also appeared to bind reversibly, with its binding mode possibly similar to that of oleic acid (K_i = 6 μM). Diazomethyl ketone **21** bound more weakly (K_i = 18 μM).

Inhibitor Synthesis. Many of the inhibitors were prepared from oleic acid by known procedures or adaption of known procedures (Scheme 1). Reaction of the acid chloride derived from oleic acid (3 equiv of $(COCl)_2$, CH_2Cl_2, 25 °C, 3 h) with hydroxylamine or diazomethane provided **2** and **21**, and direct condensation of oleic acid with hydrazine (1.1 equiv; 2.2 equiv of EDCI, 0.2 equiv of DMAP, CH_2Cl_2, 25 °C, 19 h) provided **22**. Treatment of **21** with anhydrous 1 N HCl−EtOAc (25 °C, 10 min, 92%) cleanly provided **3**. The aldehyde **4**[38] along with the dimethyl acetal **16**[39] could be prepared directly from oleic acid as described. Trapping of the enolate derived from oleic acid (LDA, THF) with CCl_4 or O_2 provided **20**[40] and **18**,[41] respectively, which were converted to the corresponding primary amides **19** and **17** via acid chloride generation (3 equiv of $(COCl)_2$, CH_2Cl_2, 25 °C, 3 h) and condensation with aqueous NH_4OH.

Scheme 2



The C-18 oleic acid based α-keto amide **6** and α-keto ester **8** bearing an electrophilic carbonyl at the position analogous to C-2 of oleamide rather than C-1 were prepared by oxidation of the α-hydroxy amide **17** (PDC) and α-hydroxy acid **18** (Dess−Martin) followed by ethyl ester formation (Scheme 1). The corresponding α-keto esters **9** and **10**, bearing an electrophilic carbonyl at the position analogous to C-1 of oleamide were prepared directly from the corresponding 18-carbon carboxylic acids, oleic and stearic acids, employing a modified Dakin−West reaction[21,42] (Scheme 1). The α-keto amide **7** of similar length was prepared by one-carbon extension of oleic acid available through Wolff rearrangement of **21** (catalytic AgOBz, CH_3OH, 25 °C, 2.5 h, 82%) to provide the methyl ester **23**. Hydrolysis of the methyl ester followed by conversion of the C-19 carboxylic acid **24** to the α-keto amide followed the approach detailed for **6** (Scheme 1).

The trifluoromethyl ketone inhibitors **12**, **13**, which incorporates the electrophilic carbonyl at the C-2 position of a C-18 lipid containing a 9-*cis*-olefin, **14**, and **15** were prepared in one operation by conversion of the corresponding carboxylic acids to their respective acid chlorides and subsequent treatment with TFAA−pyridine[43] (6 equiv/8 equiv, Et_2O, 0.75−2 h, 54−79%) (Scheme 2). Oxidative cleavage of α-hydroxy acid **18** ($Pb(OAc)_4$, 1.1 equiv, 25 °C, benzene, 50 mL) yielded aldehyde **5**. This was further oxidized ($NaClO_2$) to give acid **27** which was used to prepare **13**.

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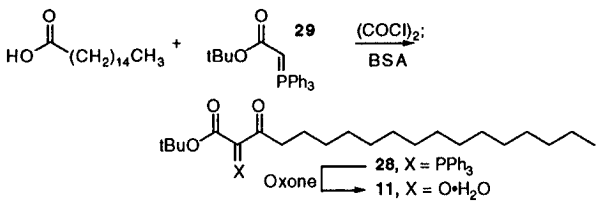
Scheme 3



The tricarbonyl inhibitor **11** was prepared following the procedures detailed by Wasserman.[44] Treatment of the acid chloride derived from palmitic acid with *tert*-butyl (triphenylphosphoranylidene)acetate (**29**) in the presence of bis(trimethylsilyl)acetamide (BSA) followed by oxone oxidation provided **11** (Scheme 3).

In summary, several potent inhibitors of the enzyme oleamide hydrolase, responsible for the hydrolysis of an endogenous sleep-inducing lipid (**1**, *cis*-9-octadecenamide), have been developed, providing insights into the mechanism of the enzyme and the fundamental basis for the development of agents for the control and regulation of sleep. One inhibitor, **6**, also induces sleep in a dose dependent manner analogous to oleamide (**1**) itself and may function both as an inhibitor of oleamide hydrolase and as an agonist of **1**. The behavior of **6** and related effects of the inhibitors described herein will be described in due time. Work is in progress to prepare the pure enzyme for further specificity and inhibition studies.

Experimental Section

The agents **1**,[1] **4**,[38] **15**,[20] **16**,[39] **18**,[41] **20**,[40] and **29**[45] were prepared as detailed.

***N*-Hydroxy-9(*Z*)-octadecenamide (2).** Oleic acid (250 μL, 0.79 mmol, 1 equiv) was dissolved in anhydrous CH_2Cl_2 (4 mL) and cooled to 0 °C under N_2. Oxalyl chloride (2 M in CH_2Cl_2, 1.2 mL, 2.4 mmol, 3 equiv) was added slowly. The solution was warmed to 25 °C and allowed to stir for 3 h in the dark. The solvent was removed in vacuo and the flask cooled to 0 °C. Excess hydroxylamine in EtOAc (the hydrochloride salt was extracted into EtOAc from a 50% NaOH solution before use) was added slowly. The solvent was removed in vacuo, and chromatography (SiO_2, 1.5 × 13 cm, 33−66% EtOAc−hexane gradient elution) afforded **2** as a white solid (104 mg, 45%): mp 61−62 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ 5.28−5.20 (m, 2H), 2.00−1.91 (m, 6H), 1.50 (p, 2H, J = 6.8 Hz), 1.22−1.19 (m, 20H), 0.80 (t, 3H, J = 6.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.0, 130.9, 130.8, 33.8, 33.1, 30.9(2), 30.6, 30.5, 30.4, 30.33(2), 30.26, 30.19, 28.2, 26.8, 23.8, 14.5; IR (film) ν_{max} 3276, 2999, 2917, 2849, 1665, 1621, 1463, 1428, 1117, 1067, 968 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 320.2577 ($C_{18}H_{35}NO_2$ + Na^+ requires 320.2565).

1-Chloro-10(*Z*)-nonadecen-2-one (3). A sample of **21** (347 mg, 1.13 mmol, 1 equiv) was treated with 1 M HCl in EtOAc (4.0 mL, 4.0 mmol, 3.5 equiv) for 10 min at 25 °C before the mixture was concentrated in vacuo. Chromatography (SiO_2, 3 × 13 cm, 5% EtOAc−hexane) afforded **3** (328 mg, 92%) as a clear oil: ^{1}H NMR (CD_3OD, 400 MHz) δ 5.29−5.21 (m, 2H), 4.18 (s, 2H), 2.48 (t, 2H, J = 7.3 Hz), 1.93 (m, 4H), 1.50 (p, 2H, J = 7.1 Hz), 1.31−1.21 (m, 20H), 0.81 (t, 3H, J = 6.8 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 204.5, 130.9, 130.8, 49.3, 40.3, 33.1, 30.9, 30.8, 30.6, 30.5, 30.40, 30.37, 30.19, 30.17(2), 28.1, 24.6, 23.8, 14.5; IR (film) ν_{max} 2925, 2854, 1722, 1463, 1403, 1260, 1101, 796, 723 cm^{-1}; FABHRMS (NBA) *m/z* 315.2468 ($C_{19}H_{35}OCl$ + H^+ requires 315.2455).

8(*Z*)-Heptadecenal (5). A solution of **18** (120 mg, 0.40 mmol, 1 equiv) in anhydrous benzene (1.6 mL) at 25 °C under N_2 was treated with $Pb(OAc)_4$ (197 mg, 0.44 mmol, 1.1 equiv), and the reaction mixture was stirred for 50 min. Water (2 mL) was added, and the aqueous layer was extracted with EtOAc (6 × 2 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 2 × 13 cm, 1−5% EtOAc−hexane gradient elution) afforded **5** (68 mg, 67%) as a clear oil. Spectral properties agree with those described in the literature.[46,47]

2-Oxo-9(*Z*)-octadecenamide (6). A solution of **17** (8 mg, 0.027 mmol, 1 equiv) in anhydrous DMF (0.13 mL) under Ar was treated with PDC (51 mg, 0.13 mmol, 5 equiv), and the reaction mixture was stirred for 1 h at 25 °C. The crude reaction was treated with H_2O (2 mL), and the aqueous layer was extracted with Et_2O (4 × 2 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 1 × 3 cm, 20−66% EtOAc−hexane gradient elution) afforded **6** (6 mg, 70%) as a white solid and some recovered starting material (2 mg, 26%). Data for **6**: mp 85−86 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (br, 1H), 5.47 (br, 1H), 5.37−5.28 (m, 2H), 2.89 (t, 2H, J = 7.4 Hz), 2.02−1.93 (m, 4H), 1.59 (p, 2H, J = 7.2 Hz), 1.39−1.24 (m, 20H), 0.86 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 198.6, 161.9, 130.1, 129.6, 36.5, 31.9, 29.7, 29.5(2), 29.3(2), 28.9(2), 27.2, 27.1, 23.1, 22.7, 14.1; IR (film) ν_{max} 3391, 2915, 2850, 1716, 1668, 1470, 1400, 1108 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 428.1547 ($C_{18}H_{33}NO_2$ + Cs^+ requires 428.1566).

2-Oxo-10(*Z*)-nonadecenamide (7). A solution of **26** (42 mg, 0.14 mmol, 1 equiv) in anhydrous CH_2Cl_2 (2.8 mL) at 25 °C was treated with *o*-Ph(CO_2)I$(OAc)_3$ (174 mg, 0.41 mmol, 3 equiv), and the reaction mixture was stirred for 1.5 h. The mixture was treated with 10% aqueous NaOH (30 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 1.5 × 13 cm, 10−20% EtOAc−hexane gradient elution) afforded **7** (24 mg, 57%) as a white solid: mp 69−70 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.82 (br, 1H), 5.68 (br, 1H), 5.36−5.28 (m, 2H), 2.88 (t, 2H, J = 7.4 Hz), 1.98 (m, 4H), 1.58 (p, 2H, J = 7.0 Hz), 1.28−1.24 (m, 20H), 0.85 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 198.7, 162.0, 130.0, 129.7, 36.5, 31.9, 29.74, 29.66, 29.5, 29.3(2), 29.2, 29.1, 29.0, 27.2, 27.1, 23.1, 22.7, 14.1; IR (film) ν_{max} 3395, 3217, 2922, 2850, 1718, 1672, 1601, 1469, 1406, 1115 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 332.2570 ($C_{19}H_{35}NO_2$ + Na^+ requires 332.2565).

Ethyl 2-Oxo-9(*Z*)-octadecenoate (8). A solution of **18** (102 mg, 0.34 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1.1 mL) at 25 °C under N_2 was treated with *o*-Ph(CO_2)I$(OAc)_3$ (287 mg, 0.68 mmol, 2 equiv) and stirred for 1 h. The reaction mixture was treated with 10% aqueous NaOH (20 mL) and extracted with EtOAc (3 × 20 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in anhydrous CH_2Cl_2 (1.5 mL) and cooled to 0 °C under N_2. Oxalyl chloride (2 M in CH_2Cl_2, 0.5 mL, 1.0 mmol, 3 equiv) was added slowly. The reaction mixture was warmed to 25 °C and was stirred in the dark for 3 h before the solvent was removed in vacuo and absolute EtOH (5 mL) was added. Chromatography (SiO_2, 2 × 10 cm, 1−5% EtOAc−hexane) afforded **8** (36 mg, 33%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.37−5.27 (m, 2H), 4.29 (q, 2H, J = 7.2 Hz), 2.81 (t, 2H, J = 7.3 Hz), 1.98 (m, 4H), 1.61 (p, 2H, J = 7.1 Hz), 1.36−1.24 (m, 21H), 0.86 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.8, 161.3, 130.1, 129.6, 62.4, 39.3, 31.9, 29.8, 29.5(2), 29.3(2), 28.92, 28.86, 27.2, 27.1, 22.9, 22.7, 14.1, 14.0; IR (film) ν_{max} 2925, 2854, 1729, 1462, 1260, 1056 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 457.1706 ($C_{20}H_{36}O_3$ + Cs^+ requires 457.1719).

Ethyl 2-Oxo-10(*Z*)-nonadecenoate (9). A solution of oleic acid (100 μL, 0.32 mmol, 1 equiv) in anhydrous THF (0.2 mL) at 25 °C under Ar was treated with DMAP (4 mg, 0.03 mmol, 0.1 equiv), anhydrous pyridine (77 μL, 0.95 mmol, 3 equiv), and ethyl oxalyl chloride (71 μL, 0.64 mmol, 2 equiv). The reaction mixture was stirred for 24 h before additional DMAP (46 mg, 0.37 mmol, 1.1 equiv), pyridine (80 μL, 0.95 mmol, 3 equiv), ethyl oxalyl chloride (80 μL, 0.64 mmol, 2 equiv), and THF (0.5 mL) were added. The reaction mixture was stirred at 25 °C for an additional 24 h and then was warmed to 40 °C for 48 h before the solvent was concentrated in vacuo. Chromatography (SiO_2, 2 × 13 cm, 0−10% EtOAc−hexane) afforded **9** (46 mg, 43%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.36−5.28 (m, 2H), 4.29 (q, 2H, J = 7.1 Hz), 2.80 (t, 2H, J = 7.3 Hz), 1.99 (m, 4H), 1.60 (m, 2H), 1.36−1.20 (m, 23H), 0.85 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.8, 161.2, 130.0, 129.7, 62.4, 39.3,

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31.9, 29.7, 29.6, 29.5, 29.3(2), 29.2, 29.0, 28.9, 27.2, 27.1, 22.9, 22.7, 14.1, 14.0; IR (film) ν_{max} 2925, 2854, 1730, 1465, 1260, 1059 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 471.1875 ($C_{21}H_{38}O_3$ + Cs^+ requires 471.1888).

Ethyl 2-Oxo-nonadecanoate (10). A solution of stearic acid (101 mg, 0.36 mmol, 1 equiv) in anhydrous THF (0.2 mL) at 25 °C under Ar was treated with DMAP (4 mg, 0.03 mmol, 0.1 equiv), anhydrous pyridine (85 μL, 1.1 mmol, 3 equiv), and ethyl oxalyl chloride (79 μL, 0.71 mmol, 2 equiv). The reaction mixture was stirred for 24 h before the solvent was concentrated in vacuo. Chromatography (SiO_2, 2 × 13 cm, 5−10% EtOAc−hexane) afforded **10 (**35 mg, 30%) as a white solid: mp 43−44 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.29 (q, 2H, J = 7.2 Hz), 2.80 (t, 2H, J = 7.4 Hz), 1.60 (p, 2H, J = 7.2 Hz), 1.35 (t, 3H, J = 7.1 Hz), 1.33−1.23 (m, 28H), 0.86 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.8, 161.2, 62.4, 39.3, 31.9, 29.7(7), 29.6, 29.40, 29.35, 29.28, 28.9, 23.0, 22.7, 14.1, 14.0; IR (film) ν_{max} 2916, 2848, 1733, 1472, 1463, 723 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 363.2885 ($C_{21}H_{40}O_3$ + Na^+ requires 363.2875).

***tert*-Butyl 3-Oxo-2,2-dihydroxyoctadecanoate (11).** A solution of **28** (161 mg, 0.26 mmol, 1 equiv) in THF−H_2O (2:1; 3 mL) was treated with Oxone (249 mg, 0.41 mmol, 1.6 equiv), and the reaction mixture was stirred at 25 °C for 7 h. Water (30 mL) was added, and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were combined, dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 2 × 15 cm, 10−20% EtOAc−hexane gradient elution) afforded **11** (65 mg, 64%) as a white solid: mp 49−51 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.96 (s, 2H), 2.17 (t, 2H, J = 7.4 Hz), 1.49−1.38 (m, 11H), 1.22 (s, 24H), 0.84 (t, 3H, J = 6.8 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 205.6, 174.5, 94.2, 81.5, 35.6, 33.6, 31.3, 29.0(3), 28.9, 28.8, 28.72, 28.70, 28.53, 28.46, 27.4(2), 24.5, 22.9, 22.1, 13.9; IR (film) ν_{max} 3440, 2914, 2849, 1728, 1471, 1371, 1260, 1122, 831, 718 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 409.2925 ($C_{22}H_{42}O_5$ + Na^+ requires 409.2930).

1,1,1-Trifluoro-10(*Z*)-nonadecen-2-one (12). Oleic acid (100 μL, 0.32 mmol, 1 equiv) was dissolved in anhydrous CH_2Cl_2 (1.5 mL) and cooled to 0 °C under N_2. Oxalyl chloride (2 M in CH_2Cl_2, 0.47 mL, 0.94 mmol, 3 equiv) was added slowly. The reaction mixture was warmed to 25 °C and was stirred in the dark for 3 h before the solvent was removed in vacuo. Anhydrous Et_2O (2.2 mL), trifluoroacetic anhydride (270 μL, 1.9 mmol, 6 equiv), and pyridine (0.2 mL, 2.5 mmol, 8 equiv) were added at 25 °C, and the solution was stirred for 45 min before being cooled to 0 °C. The reaction was quenched with the addition of H_2O (30 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 1.5 × 13 cm, 1% Et_3N in 5% EtOAc−hexane) afforded **8** (75 mg, 71%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.37−5.28 (m, 2H), 2.68 (t, 2H, J = 7.3 Hz), 1.98 (m, 4H), 1.65 (p, 2H, J = 7.1 Hz), 1.29−1.25 (m, 20H), 0.86 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.6 (d, J = 17 Hz), 130.0, 129.5, 115.6 (q, J = 145 Hz), 36.3, 31.9, 29.8, 29.6, 29.5, 29.3(2), 29.1, 29.0, 28.7, 27.2, 27.1, 22.7, 22.4, 14.1; IR (film) ν_{max} 2926, 2855, 1766, 1467, 1404, 1261, 1208, 1153, 1039, 802, 709 cm^{-1}; ESIMS *m/z* (M^+) 334.

1,1,1-Trifluoro-9(*Z*)-octadecen-2-one (13). A solution of **27** (101 mg, 0.38 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1.8 mL) was cooled to 0 °C under N_2 and treated dropwise with oxalyl chloride (2 M in CH_2Cl_2, 0.56 mL, 1.1 mmol, 3 equiv). The reaction mixture was warmed to 25 °C and stirred for 3 h before the solvent was removed in vacuo. Anhydrous Et_2O (2.5 mL), trifluoroacetic anhydride (0.32 mL, 2.3 mmol, 6 equiv), and anhydrous pyridine (0.12 mL, 1.5 mmol, 4 equiv) were added at 25 °C, and the solution was stirred for 2 h before being cooled to 0 °C. The reaction mixture was treated with H_2O (30 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 2 × 15 cm, 1% Et_3N in 10% EtOAc−hexane) afforded **13** (65.5 mg, 54%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.39−5.26 (m, 2H), 2.69 (t, 2H, J = 7.2 Hz), 1.99 (m, 4H), 1.66 (m, 2H), 1.35−1.24 (m, 18H), 0.86 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.4, 130.5, 129.1, 115.6 (q, J = 146 Hz), 36.3, 31.9, 29.7, 29.5, 29.3(3), 29.2, 28.3, 27.2, 26.8, 22.7, 22.3, 14.1; IR (film) ν_{max} 2926, 2855, 1765, 1462, 1209, 1154, 1024 cm^{-1}; ESIMS *m/z* (M + Na^+) 343.

1,1,1-Trifluoro-10(*E*)-nonadecen-2-one (14). A solution of elaidic acid (204 mg, 0.72 mmol, 1 equiv) in anhydrous CH_2Cl_2 (3.5 mL) was cooled to 0 °C under N_2 and treated with oxalyl chloride (2 M in CH_2Cl_2, 1.1 mL, 2.2 mmol, 3 equiv). The reaction mixture was warmed to 25 °C and stirred for 3 h before the solvent was removed in vacuo. Anhydrous Et_2O (5 mL), trifluoroacetic anhydride (0.6 mL, 4.3 mmol, 6 equiv), and anhydrous pyridine (0.23 mL, 2.8 mmol, 4 equiv) were added at 25 °C, and the solution was stirred for 1 h before being cooled to 0 °C. The mixture was treated with H_2O (30 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 2 × 13 cm, 1% Et_3N in 5−10% EtOAc−hexane gradient elution) afforded **14 (**190 mg, 79%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.41−5.31 (m, 2H), 2.68 (t, 2H, J = 7.3 Hz), 1.94 (m, 4H), 1.65 (p, 2H, J = 6.9 Hz), 1.28−1.24 (m, 20H), 0.86 (t, 3H, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.5 (q, J = 35 Hz), 130.6, 130.1, 115.6 (q, J = 291 Hz), 36.3, 32.6, 32.5, 31.9, 29.7, 29.5(2), 29.3, 29.2, 29.1, 28.8, 28.7, 22.7, 22.4, 14.0; IR (film) ν_{max} 2925, 2855, 1765, 1466, 1208, 1152, 967, 709 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 334.2475 ($C_{19}H_{33}OF_3$ − H^+ requires 334.2484).

2-Hydroxy-9(*Z*)-octadecenamide (17). A solution of **18** (52 mg, 0.18 mmol, 1 equiv) in anhydrous CH_2Cl_2 (0.7 mL) cooled to 0 °C under N_2 was treated with oxalyl chloride (2 M in CH_2Cl_2, 0.22 mL, 0.44 mmol, 3 equiv). The solution was allowed to warm to 25 °C and stirred for 3 h in the dark. The solvent was removed in vacuo, and the acid chloride was cooled to 0 °C. The sample was treated with excess concentrated aqueous NH_4OH. Chromatography (SiO_2, 1.5 × 10 cm, 66−100% EtOAc−hexane gradient elution) afforded **17** (31 mg, 60%) as a white solid: mp 103−104 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.37 (br, 1H), 5.64 (br, 1H), 5.36−5.28 (m, 2H), 4.12 (t, 1H, J = 3.8 Hz), 2.66 (br, 1H), 2.02−1.94 (m, 4H), 1.86−1.77 (m, 1H), 1.68−1.59 (m, 1H), 1.43−1.24 (m, 20H), 0.86 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.6, 130.0, 129.7, 71.9, 34.8, 31.9, 29.7, 29.6, 29.5, 29.3(2), 29.2, 29.1, 27.2, 27.1, 24.9, 22.7, 14.1; IR (film) ν_{max} 3381, 3289, 2917, 2848, 1637, 1461, 1417, 1331, 1074 cm^{-1}; FABHRMS (NBA) *m/z* 298.2760 ($C_{18}H_{35}NO_2$ + H^+ requires 298.2746).

2-Chloro-9(*Z*)-octadecenamide (19). A solution of **20** (48 mg, 0.15 mmol, 1 equiv) in anhydrous CH_2Cl_2 (0.7 mL) cooled to 0 °C under N_2 was treated with oxalyl chloride (2 M in CH_2Cl_2, 0.23 mL, 0.46 mmol, 3 equiv). The solution was allowed to warm to 25 °C and was stirred for 3 h in the dark before the solvent was removed in vacuo. The crude acid chloride was cooled to 0 °C and treated with excess concentrated aqueous NH_4OH. Chromatography (SiO_2, 1.5 × 10 cm, 20−33% EtOAc−hexane gradient elution) afforded **19** (37 mg, 78%) as a yellow solid: mp 49−50 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.49 (br, 1H), 5.92 (br, 1H), 5.36−5.27 (m, 2H), 4.29 (m, 1H), 2.12−1.86 (m, 6H), 1.53−1.16 (m, 20H), 0.85 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.9, 130.1, 129.6, 60.6, 35.5, 31.9, 29.7, 29.6, 29.5, 29.3(2), 29.0, 28.7, 27.2, 27.1, 25.8, 22.7, 14.1; IR (film) ν_{max} 3383, 3183, 3001, 2921, 2850, 1657, 1465, 1412, 1240, 1100 cm^{-1}; FABHRMS (NBA) *m/z* 316.2415 ($C_{18}H_{34}NOCl$ + H^+ requires 316.2407).

1-Diazo-10(*Z*)-nonadecen-2-one (21). Oleic acid (1.0 mL, 3.2 mmol, 1 equiv) was dissolved in anhydrous CH_2Cl_2 (15 mL) under N_2. The solution was cooled to 0 °C, and oxalyl chloride (2 M in CH_2Cl_2, 4.8 mL, 9.6 mmol, 3 equiv) was added. The reaction mixture was allowed to warm to 25 °C and was stirred for 3 h in the dark. The solvent was removed in vacuo before the acid chloride was transferred to a flask with no ground glass joints and cooled to 0 °C. Excess diazomethane in Et_2O (prepared from *N*-nitrosomethylurea in 50% aqueous KOH and drying over KOH pellets) was added. The reaction was stirred at 0 °C for 1 h before warming to 25 °C overnight. The solution was diluted with EtOAc (60 mL) and washed with saturated aqueous $NaHCO_3$ (60 mL) and saturated aqueous NaCl (60 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 4.0 × 16 cm, 5−10% EtOAc−hexane gradient elution) afforded **21 (**0.89 g, 92%) as a yellow oil: ^{1}H NMR (CD_3OD, 400 MHz) δ 5.72 (br, 1H), 5.29−5.21 (m, 2H), 2.23 (m, 2H), 1.94 (m, 4H), 1.50 (p, 2H, J = 6.9 Hz), 1.23−1.20 (m, 20H), 0.81 (t, 3H, J = 6.9 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 198.8, 130.9, 130.8, 41.6, 33.1, 30.9, 30.8(2), 30.6, 30.5, 30.4(2), 30.3, 30.2, 28.1(2), 26.5, 23.8,

14.5; IR (film) ν_{max} 3083, 2924, 2854, 2102, 1644, 1463, 1372, 1144 cm^{-1}; FABHRMS (NBA) *m/z* 307.2738 ($C_{19}H_{34}N_2O + H^+$ requires 307.2749).

***N*-Amino-9(*Z*)-octadecenamide (22).** A solution of oleic acid (250 μL, 0.79 mmol, 1 equiv) and hydrazine monohydrate (42 μL, 0.87 mmol, 1.1 equiv) in anhydrous CH_2Cl_2 (12 mL) under N_2 at 0 °C was treated with EDCI (267 mg, 0.90 mmol, 1.1 equiv) and DMAP (20 mg, 0.16 mmol, 0.21 equiv) before the reaction mixture was allowed to stir at 25 °C for 7 h. Another portion of EDCI (269 mg, 0.91 mmol, 1.1 equiv) was added, and the reaction was stirred at 25 °C for an additional 12 h before the solvent was removed in vacuo. Chromatography (SiO_2, 3 × 18 cm, 20−100% EtOAc−hexane gradient elution) afforded **22** (123 mg, 52%) as a white solid: mp 95−96 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.94 (s, 1H), 5.36−5.27 (m, 2H), 2.23 (t, 2H, J = 7.6 Hz), 1.98 (m, 4H), 1.63 (p, 2H, J = 7.0 Hz), 1.27−1.24 (m, 20H), 0.86 (t, 3H, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.7, 130.0, 129.7, 34.1, 31.9, 29.8, 29.7, 29.5, 29.3(2), 29.23, 29.19, 29.12, 27.21, 27.17, 25.4, 22.7, 14.1; IR (film) ν_{max} 3201, 2917, 2848, 1595, 1410, 1184, 1090, 927, 717, 671 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 297.2916 ($C_{18}H_{36}N_2O + H^+$ requires 297.2906).

Methyl 10(*Z*)-Nonadecenoate (23). A solution of silver benzoate (21.8 mg, 0.095 mmol, 0.1 equiv) and anhydrous Et_3N (0.19 mL, 1.36 mmol, 1.4 equiv) was added dropwise to a solution of 1-diazo-10(*Z*)-nonadecen-2-one (**21**; 298 mg, 0.97 mmol, 1 equiv) in anhydrous CH_3OH (1.5 mL) under N_2, and the reaction was stirred at 25 °C for 2.5 h. The reaction mixture was diluted with EtOAc (30 mL) and washed with 1 N aqueous HCl (30 mL) and saturated aqueous $NaHCO_3$ (30 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 3 × 15 cm, 1−5% EtOAc−hexane gradient elution) afforded **23** (246 mg, 82%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.35−5.27 (m, 2H), 3.63 (s, 3H), 2.27 (t, 2H, J = 7.6 Hz), 1.97 (m, 4H), 1.59 (p, 2H, J = 7.3 Hz), 1.26−1.24 (m, 22H), 0.85 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.3, 129.9, 129.8, 51.4, 34.1, 31.9, 29.74, 29.70, 29.5, 29.3(2), 29.2(2), 29.1(2), 27.2(2), 24.9, 22.6, 14.1; IR (film) ν_{max} 2925, 2854, 1744, 1465, 1436, 719 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 311.2969 ($C_{20}H_{38}O_2 + H^+$ requires 311.2950).

10(*Z*)-Nonadecenoic Acid (24). A solution of **23** (620 mg, 2.0 mmol, 1 equiv) in THF−CH_3OH−H_2O (3:1:1; 7 mL) at 25 °C was treated with $LiOH\cdot H_2O$ (250 mg, 5.96 mmol, 3 equiv), and the reaction mixture was stirred for 3 h. The reaction mixture was acidified with the addition of 1 N aqueous HCl (60 mL), and the aqueous layer was extracted with EtOAc (2 × 60 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 4 × 15 cm, 10−100% EtOAc−hexane gradient elution) afforded **24** (510 mg, 86%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.37−5.28 (m, 2H), 2.32 (t, 2H, J = 7.5 Hz), 1.98 (m, 4H), 1.61 (p, 2H, J = 7.3 Hz), 1.27−1.25 (m, 22H), 0.86 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.4, 130.0, 129.8, 34.1, 31.9, 29.8, 29.7, 29.5, 29.3(2), 29.2(2), 29.0(2), 27.20, 27.17, 24.6, 22.7, 14.1; IR (film) ν_{max} 2925, 2854, 1711, 1466, 1412, 1260, 1093, 1019, 938, 801, 722 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 319.2605 ($C_{19}H_{36}O_2 + Na^+$ requires 319.2613). This compound can alternatively be prepared by the method of Doleshall.[48]

2-Hydroxy-10(*Z*)-nonadecenoic Acid (25). A fresh solution of LDA was prepared at −55 °C under Ar from diisopropylamine (0.4 mL, 2.9 mmol, 4.5 equiv), and *n*-BuLi (2.3M, 1.1 mL, 2.5 mmol, 4 equiv) in anhydrous THF (2 mL). A solution of 10(*Z*)-nonadecenoic acid (**24**; 188 mg, 0.63 mmol, 1 equiv) and anhydrous HMPA (0.11 mL, 0.63 mmol, 1 equiv) in THF (0.5 mL) was added dropwise to the LDA solution at −55 °C. The reaction mixture was allowed to warm gradually to 25 °C and then was warmed to 50 °C for 30 min. After the reaction mixture was recooled to 25 °C, O_2 was bubbled through the solution for 20 min. The mixture was treated with 1 N aqueous HCl (30 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 2 × 13 cm, 50−100% EtOAc−hexane gradient elution) afforded **25** (96 mg, 49%) as a white solid: mp 53−54 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.36−5.28 (m, 2H), 4.24 (dd, 1H, J = 7.5 Hz, 7.6 Hz), 1.98 (m, 4H), 1.83 (m, 1H), 1.67 (m, 1H), 1.47−1.24 (m, 22H), 0.86 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.8, 130.0, 129.7, 70.2, 34.2, 31.9, 29.8, 29.7, 29.5, 29.33, 29.31(2), 29.22, 29.19, 27.20, 27.16, 24.8, 22.7, 14.1; IR (film) ν_{max} 3512, 2917, 2849, 1704, 1467, 1293, 1274, 1251, 1212, 1143, 1079, 1041, 918, 726, 648 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 335.2574 ($C_{19}H_{36}O_3 + Na^+$ requires 335.2562).

2-Hydroxy-10(*Z*)-nonadecenamide (26). A solution of **25** (71 mg, 0.23 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1.5 mL) under N_2 was cooled to 0 °C and treated dropwise with oxalyl chloride (2 M in CH_2Cl_2, 0.34 mL, 0.68 mmol, 3 equiv). The reaction mixture was allowed to warm to 25 °C and was stirred for 3 h in the dark. The solvent was removed in vacuo, the residue was cooled to 0 °C, and excess concentrated aqueous NH_4OH (2 mL) was added. Chromatography (SiO_2, 1.5 × 13 cm, 50−66% EtOAc−hexane gradient elution) afforded **26** (53 mg, 75%) as a white solid: mp 101−102 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.36 (br, 1H), 5.65 (br, 1H), 5.36−5.28 (m, 2H), 4.12 (dd, 1H, J = 7.9 Hz, 8.0 Hz), 1.99 (m, 4H), 1.81 (m, 1H), 1.63 (m, 1H), 1.43−1.24 (m, 22H), 0.86 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 176.6, 130.0, 129.8, 71.9, 34.8, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3(3), 29.2, 27.20, 27.16, 24.9, 22.7, 14.1; IR (film) ν_{max} 3383, 3290, 2917, 2849, 1644, 1467, 1426, 1331, 1075 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 334.2731 ($C_{19}H_{37}NO_2 + Na^+$ requires 334.2722).

8(*Z*)-Heptadecenoic acid (27). A solution of **5** (66 mg, 0.26 mmol, 1 equiv) and 2-methyl-2-butene (1.6 mL, 15.1 mmol, 58 eq) in tBuOH (6.5 mL) at 25 °C under N_2 was treated dropwise with a solution of $NaClO_2$ (80%, 208 mg, 2.3 mmol, 9 equiv) and $NaH_2PO_4\cdot H_2O$ (250 mg, 1.8 mmol, 7 equiv) in deionized H_2O (2.5 mL). The reaction mixture was allowed to stir for an additional 15 min before being concentrated in vacuo. The residue was treated with water (30 mL), and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo. Chromatography (SiO_2, 2 × 13 cm, 10−20% EtOAc−hexane gradient elution) afforded **27** (66 mg, 95%) as a clear oil. Spectral properties agree with those described in the literature.[49,50]

***tert*-Butyl 3-Oxo-2-(triphenylphosphoranylidene)octadecanoate (28).** A solution of palmitic acid (103 mg, 0.40 mmol, 1 equiv) in anhydrous CH_2Cl_2 (2 mL) under N_2 was cooled to 0 °C and treated with oxalyl chloride (2 M in CH_2Cl_2, 0.6 mL, 1.2 mmol, 3 equiv). The solution was allowed to stir at 25 °C for 3 h before the solvent was removed in vacuo. A solution of *tert*-butyl (triphenylphosphoranylidene)acetate[45] (**29**; 167 mg, 0.44 mmol, 1.1 equiv) and bis(trimethylsilyl)-acetamide (195 μL, 0.79 mmol, 2 equiv) in anhydrous benzene (3 mL) at 5 °C was treated dropwise with a solution of the crude acid chloride in benzene (3 mL). The reaction mixture was allowed to warm to 25 °C and was stirred for 1.5 h before the solvent was removed in vacuo. Chromatography (SiO_2, 2 × 15 cm, 10 −20% EtOAc−hexane gradient elution) afforded **28** (193 mg, 78%) as a clear oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67−7.61(m, 6H), 7.49−7.37 (m, 9H), 2.82 (t, 2H, J = 7.6 Hz), 1.55 (p, 2H, J = 7.0 Hz), 1.23−1.21 (m, 24H), 1.04 (s, 9H), 0.86 (t, 3H, J = 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.9 (d, J = 6 Hz), 167.3 (d, J = 13 Hz), 132.9 (d, 6C, J = 9 Hz), 131.3 (3), 128.4 (d, 6C, J = 12 Hz), 127.4 (d, 3C, J = 96 Hz), 78.4, 71.2 (d, J = 114 Hz), 40.0, 31.9, 29.70(8), 29.66, 29.3, 28.1(3), 25.9, 22.7, 14.1; IR (film) ν_{max} 3426, 2923, 2852, 1665, 1551, 1438, 1363, 1302, 1173, 1106, 1081, 746, 690 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 615.3959 ($C_{40}H_{55}O_3P + H^+$ requires 615.3967).

Determination of Binding Constants. The potency of the these compounds against oleamide hydrolysis was evaluated using an ion-selective ammonia electrode (ATI/Orion) to directly measure ammonia formation as the product of the reaction. All K_i values except for that of oleic acid were determined by the Dixon method. (*x* intercepts of weighted linear fits of [I] vs 1/rate plots at a constant substrate concentration were converted to K_i values using the formula $K_i = -X_{int} / [1 + [S]/K_m]$.) The oleic acid K_i was obtained from a nonlinear weighted least-squares fit of rate vs substrate and inhibitor concentrations. The assays were done with constant stirring in 10 mL of 50 mM CAPS buffer (Sigma) adjusted to pH 10.0, the pH at which the rate of enzymatically catalyzed oleamide hydrolysis is maximal. In all cases which involved Dixon analysis, the substrate concentration

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was 100 μM. The oleic acid K_i was determined over a range of substrate concentrations from 10 to 100 μM. Substrate and inhibitors were dissolved in DMSO prior to addition to the 50 mM CAPS buffer, generating a final DMSO assay concentration of 1.67%. Concentrations of up to 20% DMSO exhibited only minor effects upon the rate. The enzyme concentration was adjusted to produce a rate of roughly 0.2 μM/min in the absence of inhibitor and the rate of ammonia production observed over a period of 7−10 min.

The enzyme was used as a crude, heterogenous, membrane-containing preparation from rat liver. Enzyme boiled for 5 min demonstrated no activity. Within solubility limits, all inhibitors achieved 100% inhibition of activity at concentrations greater than 100K_i. No detectable activity was found in the absence of added oleamide. Likewise, only a very minimal rate of ammonia production from 100 μM oleamide was detected in the absence of enzyme at this pH. This suggests that the catalytic oleamide hydrolysis activity observed in this crude enzyme preparation arises from a single protein.

The K_m for oleamide was determined as the average K_m obtained from four independent assays. Each independent K_m was obtained from a weighted linear fit of data in a Lineweaver−Burke plot. A fifth concurring K_m was obtained as a result of the determination of oleic acid inhibition by nonlinear methods. The rate data were fit with the standard Michaelis−Menten kinetic equation. (The equation for the rate of Ping Pong Bi Bi kinetics collapses to the simple Michaelis−Menten-like equation when the concentration of the second substrate, in this case water, is constant.) In the range 30−100 μM, the reaction rate has essentially a zero-order dependence on substrate concentration.

Because we have not yet been able to determine the amount of oleamide hydrolase present in the enzyme sample, we do not present values for V_{max} here. Our inhibition data suggest that the enzyme concentration is lower than 2 nM, since higher enzyme concentrations would have caused significant depletion of inhibitor in solution, causing the apparent K_i to be measured as [E]/2 in the limiting case of [E] $\gg$ K_i. Since 1 nM was the lowest inhibition constant measured, [E] < 2 nM.

Error values presented with K_i values should be considered goodness-of-fit estimates derived from propagation of errors treatment of data. They are not necessarily an indication of reproducibility. However, in cases where experiments were repeated, results were within statistical agreement as predicted by the apparent error values given here.

pH−Rate Dependence. Crude enzyme was added to a solution of 200 μM oleamide (approximately the solubility limit) **1** in 20 mL of buffer at the appropriate pH, containing 5% DMSO. (Concentrations of up to 20% DMSO had only a minimal effect on enzyme rates.) A 50 mM sodium citrate/Bis-Tris buffer was used for data points in the pH 4−9 range. A 50 mM Bis-Tris/CAPS buffer was used for data points in the 8−11 range. At pH 12, the solution was assumed to be self-buffering. At periodic time intervals, 1 mL aliquots were removed and diluted with 9 mL of pH 14 buffer. Ammonia concentrations were measured with an ion-selective ammonia electrode (Orion) connected to a 720A meter (Orion), calibrated against known standards. The rate was obtained from the linear portion of the curve which was fit using a standard least-squares procedure. These rates were replotted against pH and fit with the equation[51] below by a weighted nonlinear least-squares method.[52]

$$\text{rate} = \frac{[H^+]^3\nu_{H_3E} + [H^+]^2K_1\nu_{H_2E} + [H^+]K_1K_2\nu_{HE} + K_1K_2K_3\nu_E}{[H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3}$$

$$H_3E \overset{K_1}{\rightleftharpoons} H_2E + H \overset{K_2}{\rightleftharpoons} HE + 2H \overset{K_3}{\rightleftharpoons} E + 3H$$

In cases where two pK_a values are close together (less than one unit) there will be substantial mixing of various species of enzyme present in solution. Under such conditions, manifestation of the theoretical rate maximum for that species may never actually be observed because the most active species of enzyme may never reach a high degree of abundance. It is for this reason that simple graphical methods of determination of pK_a values may disagree with the values of 9.7 and 10.3 pH units presented here.

Liver Plasma Membrane Prep, Large Scale (12−14 Rat Livers). Twelve to fourteen rat livers were sectioned and placed in 300 mL of 1 mM $NaHCO_3$. The solution of diced liver was strained and washed with additional 300 mL of 1 mM $NaHCO_3$. Any conspicuous connective tissue was removed. The liver was transferred to a fresh 800 mL of 1 mM $NaHCO_3$, stirred, and then transferred in 400 mL aliquots to a blender. Blended liver aliquots were combined and filtered through eight layers of cheesecloth. This was diluted to 1.0 L with 1 mM $NaHCO_3$ and centrifuged at 6000 rpm for 20 min at 4 °C (Beckman JA-17 rotor). The supernatant was decanted and the pellets resuspended in 1 mM $NaHCO_3$, combined, and dounce homogenized. Centrifugation, decantation, and resuspension/homogenization were repeated to give a final volume of approximately 90 mL. The homogenate was added to 2 volume equivalents of 67% sucrose, mixed thoroughly, and transferred to ultracentrifuge compatible tubes. The tubes were topped with 30% sucrose and spun at 27 000 rpm for 2 h (SW-28 rotor). The middle yellow band was removed from the sucrose gradient, combined, resuspended in 1 mM $NaHCO_3$, and dounce homogenized. The sample was further centrifuged at 17 000 rpm for 45 min at 4 °C (JA-17 rotor). The supernatant was removed and the pellets resuspended in 100 mM Na_2CO_3, dounce homogenized, and left on ice for 30 min. The solution was centrifuged at 27 000 rpm for 1 h (SW-28 rotor), the supernatant was decanted, and the pellet was resuspended in 15 mL of 50 mM Tris−HCl, pH 7.4, with 1 mM EDTA, and homegenized with a dounce homogenizer. This material was divided into multiple aliquots and frozen at −78 °C until use. Each enzyme sample was only frozen once.

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