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Brief Article

Bis(dialkylaminethiocarbonyl)disulfides as Potent and Selective Monoglyceride Lipase Inhibitors

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Monoglyceride lipase (MGL) inhibition may offer an approach in treating diseases in which higher 2-arachidonoylglycerol activity would be beneficial. We report here the synthesis and pharmacological evaluation of bis(dialkylaminethiocarbonyl)disulfide derivatives as irreversible MGL inhibitors. Inhibition occurs through interactions with MGL C208 and C242 residues, and these derivatives exhibit high inhibition selectivity over fatty acid amide hydrolase, another endocannabinoid-hydrolyzing enzyme.

Introduction

The endocannabinoid system (ECS[a]), consisting in a set of endogenous lipid derivatives, endocannabinoids (eCBs), their GPCR targets, and proteins for their biosynthesis and degradation, has been implicated in several physiopathological functions both in the central nervous system and in peripheral organs.[1] More importantly, modulating the activity of the ECS turned out to hold therapeutic promise for instance, in growth inhibition of prostate and breast cancer cells. As expected for neuromodulators, eCBs, i.e., 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA), are efficiently metabolized to ensure rapid signal inactivation.[2] As a consequence, one way of modulating the ECS is the inhibition of enzymes responsible for eCBs degradation. To date, four enzymes have been characterized at the molecular level: fatty acid amide hydrolase (FAAH),[3] type-2 fatty acid amide hydrolase,[4] *N*-acylethanolamine-hydrolyzing acid amidase (NAAA),[5] and monoglyceride lipase (MGL).[6,7] Strong evidence suggests that MGL is the main enzyme responsible for 2-AG hydrolysis in the brain,[8−10] but only a few inhibitors have been reported to date.[11−13] In addition, they lack enzyme selectivity, except for *N*-arachidonylmaleimide[14] and the recently described 4-nitrophenyl-4-(di(benzo[*d*][1,3]dioxol-5-yl)(hydroxyl)methyl)piperidine-1-carboxylate (JZL184).[15] We previously reported that human purified MGL (hMGL) is sensitive to inhibition by bis(diethylaminethiocarbonyl)-disulfide (disulfiram, **1**), an aldehyde dehydrogenase (ALDH) inhibitor.[16,17]

Therefore, the aim of the present study was to determine the structure−activity relationships (SAR) within a series of synthesized bis(dialkylaminethiocarbonyl)disulfide derivatives, in order to improve activity and selectivity for hMGL inhibition, by studying the sensitivity of this enzyme toward disulfiram analogues possessing different *N*-substitutions. Furthermore, to underline the role of disulfide functionality and/or thiocarbonyl groups for hMGL inhibition, analogues lacking these functionalities were synthesized and assayed for their inhibition of hMGL. Finally, the reversibility of hMGL inactivation, as well as the potential involvement of a redox process involving the enzyme sulfhydryl residues and resulting in disulfide bond formation inactivating the enzyme, were investigated using both pharmacological and molecular (mutant hMGL lacking specific cysteine residues) approaches.

Results and Discussion

Chemistry. Bis(dialkylaminethiocarbonyl)disulfide derivatives (**1**−**22**) were synthesized as reported by Neelakantan,[18] (Scheme 1). The second goal of this study was to evaluate the importance of the thiocarbonyl and disulfide moieties by modifying one or two thiocarbonyl groups and/or the disulfide moiety. We obtained {[(dialkylamino)-carbothioyl]thio} methylene (dialkylamino)methanedithioate (**23**−**25**), dithiocarbamate (**26**−**27**), (dialkylcarbamoyl)methyl-enedialkylcarbamodithioate (**28**−**29**), (dialkylthiocarbamoyl) methylenedialkylcarbamodithioate (**30**−**31**), bis(dialkylthio-carbamoyl)sulfide (**32**−**33**), and dithiobisamines (**34**−**36**) derivatives (Scheme 2).

Pharmacological Evaluation. To determine the inhibitory potential of the synthesized compounds (**2**−**36**) toward MGL and FAAH, human recombinant enzymes developed in our laboratory have been used.[16,19]

As preliminary experiment, the inhibitory potential of **1** (disulfiram) against hMGL and hFAAH has been measured and compared to six previously reported MGL inhibitors (see Supporting Information Table 1): benzyl 4-(5-methoxy-2-oxo-1,3,4-oxadiazol-3(2*H*)-yl)-2-methylphenylcarbamate (CAY10499),[13,20] *N*-arachidonylmaleimide,[14] methyl arachidonyl fluorophosphonate (MAFP),[7] arachidonyl trifluoromethyl

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[a] Abbreviations: ALDH, aldehyde dehydrogenase; AEA, *N*-arachidonoylethanolamine; [^{3}H]-AEA, tritiated *N*-arachidonoylethanolamine; 2-AG, 2-arachidonoylglycerol; DTT, 1,4-dithio-DL-threitol; eCBs, endocannabinoids; ECS, endocannabinoid system; FAAH, fatty amide hydrolase; hFAAH, human fatty acid amide hydrolase; GPCR, G protein coupled receptors; hMGL, human monoglyceride lipase; MGL, monoglyceride lipase; NAAA, *N*-acylethanolamine-hydrolyzing acid amidase; [^{3}H]-2-OG, tritiated 2-oleoylglycerol; SAR, structure−activity retationships.

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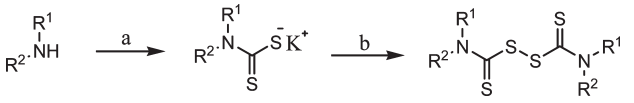
ketone (ATFMK),[7] biphenyl-3-ylcarbamic acid cyclohexylester (URB602),[21] and 5-biphenyl-4-ylmethyl-tetrazole-1-carboxylic acid dimethylamide (LY2183240).[22] This study confirms **1** as an interesting starting point due to its hMGL inhibitory activity and selectivity when compared with hFAAH inhibition (see Table 1). The synthesized bis(dialkylaminethiocarbonyl)-disulfide derivatives (**2**−**22**) were first screened at 100 and 10 μM on both enzymes. All the compounds showed more than 50% of hMGL activity inhibition at 10 μM and thus were further characterized by determining their pIC_{50} values. Compounds **2**−**6**, **8**−**12**, **14**−**16**, **19**, and **22** presented higher pIC_{50} values compared to **1**. In addition, these compounds exhibit a selective hMGL inhibition profile as their selectivity ratio (hFAAH versus hMGL) ranges from 45 (**2**) to over 6000 (**11**). Taken together, these data show that these bis-(dialkylaminethiocarbonyl)disulfide derivatives are potent and selective hMGL inhibitors. The most potent inhibitor in this series was bis(4-methyl-1-piperazinylthiocarbonyl)disulfide (**10**) with a pIC_{50} value of 6.97. It is noteworthy that amines with bulky substituents led to compounds exhibiting a weaker activity (e.g., compounds **7**, **13**, **20**, and **21** showing $pIC_{50} <$ 5.3), probably due to an unfavorable accommodation in the active site of the enzyme. On the other hand, a high steric hindrance on the nitrogen may alter the inhibitory properties by preventing interactions, for instance, between the disulfide group and residues in the active site of enzyme (e.g., compound 18, pIC_{50} = 5.39). Indeed, rigidification of **18**, which reduces steric hindrance by immobilizing the ethyl group, increases activity (compound **19**, pIC_{50} = 6.46). Derivatives bearing a cyclohexyl group also exhibited a good activity compared to those bearing a phenyl ring. This is apparent when comparing compounds **11** and **12** (pIC_{50} of 6.78 and 6.31, respectively) or compounds **14** and **15** (pIC_{50} of 6.85 and 6.13, respectively). One possible explanation for this is that the spatial arrangement of cyclohexyl allows for a better positioning in the active site of the enzyme. For several cyclic amines, it appears that the presence of a heteroatom in the cycle decreases selectivity for hFAAH inhibition while not affecting hMGL inhibition. For instance, compounds **4**, **8**, and **9** all inhibit hMGL with pIC_{50} value around 6.50, while the inhibition of FAAH by **8** and **9** is at least 1 order of magnitude higher when compared to **4**. This enhancement of hFAAH inhibition by these compounds is probably due to the establishment of a hydrogen bond interaction with a residue in hFAAH active site, but further studies will be needed to confirm this hypothesis.

In our hands, disulfiram (**1**) inhibits hMGL, in vitro, with a pIC_{50} value of 5.9. Because it was reported to inhibit rat liver mitochondrial ALDH activity with a pIC_{50} value of 4.4,[17] **10** weakly inhibited rat liver mitochondrial ALDH (pIC_{50} 4.8).

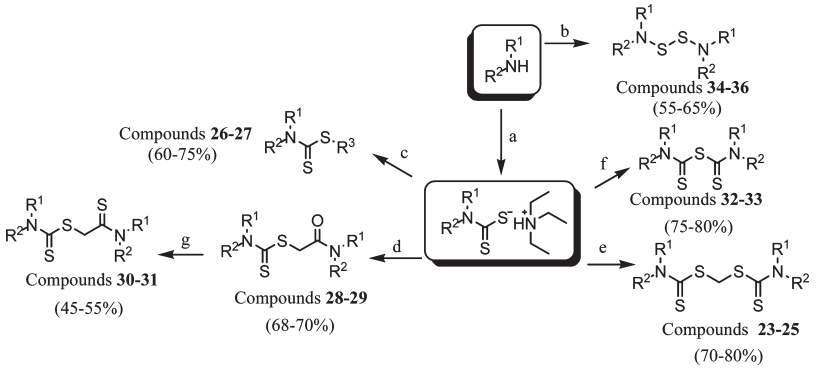
We next investigated the effect of introducing modifications to the thiocarbonyl and disulfide moieties of disulfiram by preparing compounds **23**−**36**. These compounds exhibited a weaker inhibitory potential toward hMGL, with pIC_{50} values comprised between 3 and 5.19 (Table 2). It is apparent from the data summarized in Table 2 that the high inhibition of hMGL activity by these series of compounds is dependent on the presence of both disulfide moiety and thiocarbonyl group. Indeed, compound **23**, possessing a similar structure to disulfiram (**1**) but lacking the disulfide bond, is more than 1 order of magnitude less active (pIC_{50} values of 5.9 and 4.7 for **1** and **23**, respectively). This is also apparent for derivatives **30** (pIC_{50} = 4.72) and **31** (pIC_{50} = 4.81), lacking one sulfur of the disulfide bond, and for the disulfide derivatives **34** (pIC_{50} = 4.95) and **36** (pIC_{50} = 5.10), lacking the thiocarbonyls, when compared to the thiocarbonyl disulfide derivatives **8** (pIC_{50} = 6.66) and **1** (pIC_{50} = 5.90), respectively. Finally, the dithiocarbamate derivatives **26** and **27** (i.e., the pseudo monomers of **8** and **1**, respectively) were unable to significantly inhibit hMGL. These data support the inhibition mechanism suggested for **1** in a previous report by our group.[16] Indeed, the sulfhydryl moieties of C208 and/or C242, located in the vicinity of hMGL active site, likely react with disulfide-bearing derivatives, to form either a mix adduct or an intramolecular disulfide bond.[16] However, the fact that analogues lacking disulfide moiety (e.g., **23**, **30**, **31**) are also able to inhibit hMGL indicates the possibility of other inhibition mechanisms. Nevertheless, due to the lower activity of those derivatives, these additional mechanisms of inhibition likely contribute to a lesser extent to disulfiram-derivatives activity. Besides the importance of the disulfide

Scheme 1. Synthesis of Bis(dialkylaminethiocarbonyl)disulfide Derivatives (**1**−**22**)



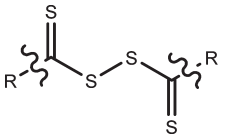
Reagents: (a) R^1NHR^2 (50 mmol), CS_2 (51 mmol), H_2O (250 mL), KOH (110 mol); (b) $NaNO_2$ (3 g), CH_3OH (3 mL), HCl 37% (10 mL).

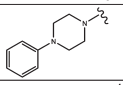
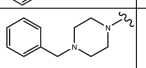
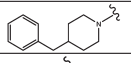
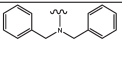
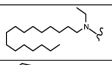
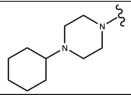
Scheme 2. Modifications of Thiocarbonyl and Disulfide Moieties



Reagents: (a) R^1NHR^2 (10 mmol), CS_2 (12 mmol), $N(C_2H_5)_3$ (30 mmol); (b) R^1NHR^2 (0.5 mmol), S_2Cl_2 (0.125 mmol); (c) dithiocarbamic acid salt (20 mmol), RI (22 mmol); (d) dithiocarbamic acid salt (0.01 mol), 2-chloro-1-dialkylaminoethanone (12 mmol); (e) dithiocarbamic acid salt (22 mmol), CH_2I_2 (10 mmol), dithiocarbamic acid salt (10 mmol), dimethylthiocarbamoyl chloride (12 mmol); (g) (dialkylcarbamoyl) methylene dialkylcarbamodithioate (2.5 mmol), P_4S_{10}/Al_2O_3 (1 g, 0.85 mmol).

Table 1. Influence of the Modification of Nitrogen Substituents of **1**: hMGL and hFAAH Inhibition by Bis(dialkylaminethiocarbonyl)disulfide Derivatives (Results are Expressed as pIC_{50} Values ± SEM)



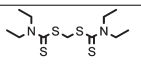
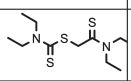
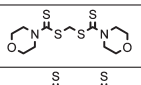
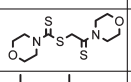
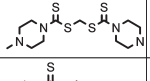
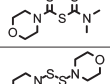
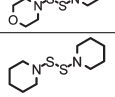
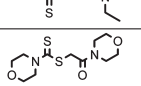
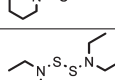
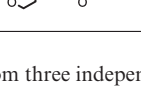
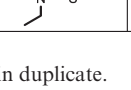
N°	R	pIC_{50} hMGL	pIC_{50} hFAAH	N°	R	pIC_{50} hMGL	pIC_{50} hFAAH
1		5.90±0.04	3.44±0.05	**12**		6.31±0.08	3.87±0.05
2		6.01±0.05	4.35±0.05	**13**		5.30±0.05	3.77±0.06
3		6.04±0.09	<3	**14**		6.85±0.05	5.32±0.09
4		6.50±0.06	<3	**15**		6.13±0.04	4.47±0.06
5		6.57±0.08	<3	**16**		6.13±0.07	<3
6		6.32±0.09	<3	**17**		6.36±0.07	<3
7		5.16±0.04	<3	**18**		5.39±0.10	<3
8		6.66±0.08	4.10±0.03	**19**		6.46±0.05	<3
9		6.50±0.06	4.60±0.04	**20**		5.02±0.07	<3
10		6.96±0.05	3.70±0.07	**21**		4.98±0.09	<3
11		6.78±0.03	<3	**22**		6.09±0.09	<3

moiety, our data provide good evidence for the influence of the thiocarbonyl moieties on the inhibition of the enzyme. Again, this is apparent when comparing the activities of **34** with **8**, as well as the activity of **28** with that of **30** or activity of **29** with that of **31**. In one of our earlier reports, we already showed the importance of the thiocarbonyl moiety because thioamide derivatives were able to inhibit hMGL whereas amines and amides of similar structures were devoid of hMGL inhibition activity.[23] One explanation can be found in the higher size and polarizability of the sulfur atom of thiocarbonyl moiety, when compared with a carbonyl group, which in turn will interfere with steric and electronic interactions at the active site of the enzyme.

The reversibility of the inhibition was investigated by performing a high dilution (300×) of the enzyme−inhibitor complex prior to the substrate addition. This method constitutes a classical way to distinguish irreversible from reversible enzyme inhibitors.[13,24] Compound **10** was incubated, at three concentrations (30, 10, and 3 μM), with the enzyme prior to dilution and measurement of hMGL activity. These concentrations are at least 20 times the IC_{50} observed for this compound in hMGL inhibition, and after dilution (300×) of the enzyme−inhibitor complex, they become respectively 100, 33.3, and 10 nM. hMGL activity was measured up to 1 h postdilution. The absence of substrate hydrolysis, at all time points, in the inhibitor-preincubated condition compared to a robust substrate hydrolysis in the DMSO-preincubated condition confirms the irreversible nature of the inhibition (Figure 1A). Note that at the 300× diluted concentrations (e.g., 10 nM), **10** demonstrated no enzyme inhibition, confirming that the reduction in activity observed here is due to the irreversible binding of **10** to the enzyme (Figure 1A).

To test the hypothesis that a redox mechanism involving sulfhydryl groups and leading to disulfide bond formation inactivating hMGL, we investigated whether hMGL inhibition, by **10**, could be reversed by a reducing reagent, namely 1,4-dithio-DL-threitol (DTT). We therefore incubated the enzyme in the presence of derivative **10** to allow for the formation of disulfide bonds and then added DTT (10^{-2} M) prior to substrate addition and measurement of hMGL activity.[16] Because DTT was able to restore the activity of the enzyme (Figure 1B), we believe that hMGL inhibition by these compounds indeed occurs through disulfide bond formation (Figure 1B). Finally, to further establish the relevance of the cysteine residues in the inhibition mechanism, we used mutant hMGL enzymes. According to the proposed hMGL model, C208 and C242 are located in the vicinity of the active site,[14] thus we compared inhibitory potency in the wild-type enzyme and in three hMGL mutants in which these cysteines were changed to alanine. With the single mutants C208A and C242A, the pIC_{50} values measured after inhibition by **10** were respectively of 5.08 and 6.52, whereas when using the double mutant C208A/C242A, **10** was unable to inhibit the substrate hydrolysis. These results constitute strong evidence that binding of **10** to the

Table 2. Influence of the Modification of Thiocarbonyl Groups and Disulfide Moiety[a]

N°	Structure	pIC_{50}		N°	Structure	pIC_{50}	
		hMGL	hFAAH			hMGL	hFAAH
23		4.68±0.07	<3	**30**		4.72±0.07	<3
24		3.99±0.10	<3	**31**		4.81±0.09	<3
25		4.02±0.04	<3	**32**		4.91±0.06	<3
26		<3	<3	**33**		5.19±0.07	<3
27		<3	<3	**34**		4.95±0.11	<3
28		3.20±0.06	<3	**35**		5.02±0.08	<3
29		3.66±0.03	<3	**36**		5.10±0.10	<3

[a] Values are the mean ± SEM from three independent experiments performed in duplicate.

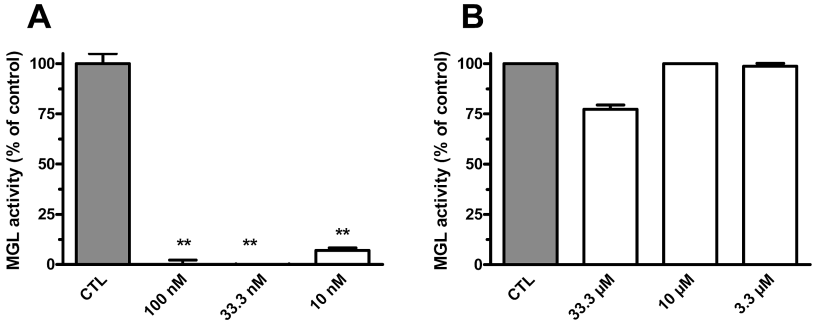


Figure 1. Study of the mechanism of inhibition of hMGL by derivative **10**. (A) Inhibition of MGL by compound **10** is not sensitive to the high dilution of the enzyme−inhibitor solution, showing that **10** binds irreversibly to the enzyme. Preincubation concentrations were 30, 10, and 3 μM, resulting in concentrations of 100, 33.3, and 10 nM during the assessment of MGL activity (i.e., 300× dilution). (B) hMGL inhibition by **10** is reversed by the reducing agent DTT (10^{-2} M), suggesting the implication of a disulfide bond in the inhibition of MGL by **10**. With these concentrations, no significant inhibition was found when compared to the robust inhibition observed in the absence of DTT (data not shown).

C208 residue takes place, and this event is responsible for the observed inhibitory potency. However, the lack of inhibition in the double mutant also indicates that an alternative mechanism involving C242 is also possible, albeit this mechanism appears less determinant. In conclusion, we have demonstrated that bis(dialkylaminethiocarbonyl)disulfide derivatives are hMGL inhibitors with a high selectivity profile regarding FAAH and their activity is closely related to the presence of both the thiocarbonyl groups and disulfide moiety which interact with sulfhydryls residues of C208 and C242 of hMGL.

Experimental Section

General Synthesis of Bis(dialkylaminethiocarbonyl)disulfide Derivatives. (1−22). A mixture of secondary amine (50 mmol), carbon disulfide (51 mmol), potassium hydroxide (110 mmol), and water (250 mL) was heated (50 °C) under stirring for 6 h. To this solution was added 3 g of sodium nitrite in 3 mL of methanol, and under cooling (0 to 5 °C) and stirring concentrated HCl (10 mL) was added dropwise. The precipitated product was collected and crystallized from ethanol to yield 55−65% of the desired compound.[18]

Bis(4-methyl-1piperazinylthiocarbonyl)disulfide (10). ^{1}H NMR ($CDCl_3$) δ(ppm) 4.29 (t, 8H, $4CH_2$ J = 7.04 Hz), 2.57 (t, 8H, $4CH_2$, J = 4.36 Hz), 2.34 (s, 6H, $2CH_3$). ^{13}C NMR ($CDCl_3$) δ (ppm) 191.38, 52.37, 49.18, 43.42. MS: m/z = 349.83. IR (KBr) cm^{-1} 2936, 2792, 1596, 1474, 1426, 1288, 1246, 1226, 1141, 1029, 979. Elemental analysis ($C_{12}H_{22}N_2S_4$) calculated: C, 41.11%; H, 6.32%; N, 15.98%; S, 36.58%. Found: C, 40.87%; H, 6.09%; N, 15.69%; S, 36.41%.

Synthesis of {[(Dialkylamino)carbothioyl]thio}methylene(dialkylamino)methanedithioate (23−25), Dithiocarbamates (26−27), (Dialkylcarbamoyl)methylenedialkyl Carbamodithioate (28−29), and Bis(dialkylaminethiocarbonyl)monosulfide Derivatives (32−33). To the dithiocarbamic acid salt (22 mmol) in acetonitrile (20 mL) was added diiodomethane (10 mmol) (**23−25**) or methyl iodide (24 mmol) (**26−27**) or 2-chloro-1-dialkylaminoethanone (22 mmol) (**28−29**) or dimethylcarbamothioic chloride (24 mmol) (**32−33**), and the mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was diluted with ethyl acetate (50 mL) and washed with water (3 × 20 mL). The organic layer was recovered, washed with brine, and dried over sodium sulfate. The residue was purified over a silica gel column with ethyl acetate−hexane 2:8 (v/v).[25]

(Diethylcarbamoyl)methylene Diethylcarbamodithioate (28). ^{1}H NMR ($CDCl_3$) δ (ppm) 4.30 (s, 2H, CH_2), 4.01 (q, 2H, CH_2, J = 6.84 Hz), 3.78 (q, 2H, CH_2, J = 7.48 Hz), 3.44 (q, 2H, CH_2, J = 7.88 Hz), 3.38 (q, 2H, CH_2, J = 7.52 Hz), 1.25 (t, 6H, $2CH_3$, J = 7.24 Hz), 1.11 (t, 6H, $2CH_3$, J = 7.08 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm) 194.47, 166.46, 49.78, 46.93, 42.62, 41.15, 40.82, 14.44, 12.91, 12.51, 11.53. MS: m/z = 262.03. IR (KBr) (cm^{-1}) 2970, 1654, 1490, 1413, 1357, 1299, 1268, 1208, 1136, 1007, 986, 919.

Synthesis of (Dialkylthiocarbamoyl)methylenedialkyl Carbamodithioates (30−31). One g of P_4S_{10}/Al_2O_3 (6 g of P_4S_{10}, 10 g of

Al_2O_3) was suspended in a solution of the (dialkylcarbamoyl)-methylenedialkylcarbamodithioate (**28**−**29**) (2.5 mmol) in 15 mL of anhydrous dioxane. The reaction was stirred under reflux for 1 h and filtered. The filtrate was poured onto ice (150 g), and the resulting mixture was stirred for 30 min. The precipitate was filtered and recrystallized from 2-propanol-cyclohexane 1:4. A silica gel column was used for purification with ethyl acetate−hexane 1:4 as eluent.[26]

(Diethylthiocarbamoyl)methylene Diethylcarbamodithioate (30). ^{1}H NMR ($CDCl_3$) δ (ppm) 4.48 (s, 2H, CH_2), 3.78 (q, 4H, $2CH_2$, J = 7.20 Hz), 3.74 (q, 4H, $2CH_2$, J = 7.20 Hz), 1.10 (t, 6H, $2CH_3$, J = 7.52 Hz), 1.03 (t, 6H, $2CH_3$, J = 6.60 Hz). ^{13}C NMR ($CDCl_3$) δ (ppm) 194.57, 193.60, 49.91, 48.39, 47.24, 46.90, 45.31, 13.88, 12.61, 11.53, 10.98. MS: m/z = 278.98. IR (KBr) (cm^{-1}) 2968, 1502, 1487, 1415, 1357, 1286, 1268,1208, 1141, 1119, 1010, 984, 834.

Synthesis of Dithiobisamine Derivatives (34−36). A solution of secondary amine (0.5 mmol) in petroleum ether (40 mL) was precooled to −78 °C before disulfur dichloride (10 μL, 0.125 mmol) was added. The solution was vigorously stirred for 15 min at −78 °C and another 30 min at room temperature. Water 0.20 mL) was added, and the desired compound was extracted into the organic phase using diethyl ether (3 × 10 mL). The combined organic phase was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The compound was purified using silica gel column and ethyl acetate−hexane 2:8 (v/v) as eluent.[27]

***N*-(2-(Diethylamino)disulfanyl)-*N*-ethylethanamine (36).** ^{1}H NMR ($CDCl_3$) δ (ppm) 2.78−2.73 (m, 8H, $4CH_2$), 1.29−1.15 (m, 12H, $4CH_3$). ^{13}C NMR ($CDCl_3$) δ (ppm) 52.12, 51.88, 51.72, 51.20, 14.20, 13.81, 13.25, 13.18. MS: m/z = 208.32. IR (KBr) (cm^{-1}) 2974, 1467, 1375, 1360, 1290, 1159, 1060, 901.

Pharmacological Evaluation. hMGL and hFAAH assays were performed as previously described.[16,19] The reversibility assays (a) by high dilution of the enzyme−inhibitor complex and (b) by adding the reducing agent DTT were performed as previously reported.[13,16] The plasmids encoding MGL mutants (C208A, C242A, and C208A/C242A) were obtained following standard molecular biology procedures, and the expression of these modified enzymes was performed as described previously.[16]

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Supporting Information Available: pIC_{50} values of previously reported MGL inhibitors obtained using recombinant human MGL and FAAH. description of the synthesized compounds and pharmacological protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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