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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Search for monoglyceride lipase inhibitors: synthesis and screening of arylthioamides derivatives

Coco N. Kapanda · Giulio G. Muccioli · Geoffray Labar · Nihed Draoui · Didier M. Lambert · Jacques H. Poupaert

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Abstract Monoglyceride lipase (MGL) is the enzyme responsible for the termination of 2-arachidonoylglycerol (2-AG) signalling, an endogenous ligand for the G-protein coupled cannabinoid receptors CB_1 and CB_2. Its known abundance and physiological roles emphasize the interest of MGL as an attractive therapeutic target. Search for MGL inhibitors was undertaken by screening an arylthioamide series. The evaluation of arylthioamides derivatives activity as MGL inhibitors measured by the hydrolysis of [^{3}H]-2-oleoylglycerol by human purified MGL led to the identification of (2-chloro-phenyl)-morpholin-4-yl-methanethione (**2**) and (3-nitro-phenyl) morpholin-4-yl-methanethione (**12**), which moreover exhibit good selectivity compared with human fatty acid amide hydrolase inhibition.

Keywords Monoglyceride lipase · 2-Arachidonoylglycerol · Arylthioamides derivatives · Monoglyceride lipase inhibitors

Introduction

The endocannabinoid system is involved in many physiopathological processes, in particular the regulations of pain, cognition, appetite, and cellular proliferation (Lambert and Fowler, 2005). Among the endogenous ligands, 2-arachidonoylglycerol (2-AG) and arachidonoylethanolamide (AEA) are considered the most important ligands of the CB_1 cannabinoid receptors (Devane *et al.*, 1992; Mechoulam, 1995; Sugiura *et al.*, 1995), which are predominantly located on presynaptic terminals in the central nervous system, and CB_2 cannabinoid receptors expressed mainly but not

C. N. Kapanda · G. G. Muccioli · G. Labar · N. Draoui · D. M. Lambert · J. H. Poupaert (✉)
Drug Design and Discovery Center, Unité de Chimie Pharmaceutique et de Radiopharmacie, Département des Sciences Pharmaceutiques, Faculté de Médecine, Université Catholique de Louvain, Avenue E. Mounier 73-40, 1200 Bruxelles, Belgium
e-mail: jacques.poupaert@uclouvain.be; poupaert@cmfa.ucl.ac.be

exclusively in immune cells (Facci *et al.*, 1995; Galiegue *et al.*, 1995; Ishac *et al.*, 1996).

AEA, a so-called *trans*-endocannabinoid, behaves as a partial agonist at the cannabinoid receptors, and is able to activate other receptors such as transient receptor potential vanilloid 1 (TRPV1), peroxisome proliferator-activated receptor gamma (PPARgamma), and NMDA receptor. Its levels in the brain are comparable with those of other neurotransmitters such as dopamine or serotonin (Saario and Laitinen, 2007; Sagan *et al.*, 1999). On the other hand 2-AG, considered by several authors as a true endocannabinoid, is a full agonist at the cannabinoid receptors. Its activity on other receptors have not been reported so far (Wilson and Nicoll, 2001; Kishimoto *et al.*, 2004).

AEA is thought to be transported into the cell by a putative specific transporter and hydrolyzed by the serine hydrolase fatty acid amide hydrolase (FAAH) (Cravatt *et al.*, 1996). Similarly, 2-AG is thought to be removed from its sites of action by cellular uptake. Inside the cells, 2-AG is primarily hydrolyzed by monoglyceride lipase (MGL) (Di Marzo *et al.*, 1994; Hillard *et al.*, 1997; Moore *et al.*, 2005). Because endocannabinoids are rapidly inactivated by cellular reuptake followed by intracellular hydrolysis by specific enzymes (Hashimotodani *et al.*, 2007; Dinh *et al.*, 2002; Muccioli *et al.*, 2007), in vivo cannabimimetic effects of the endocannabinoids are rather weak and non-lasting.

An increase in endocannabinoids levels could lead to several beneficial therapeutic effects including treatment of pain, inflammation, and mood control (Saario *et al.*, 2006; Pacher *et al.*, 2006). Thus, inhibition of FAAH and MGL represents a convenient way to elevate endocannabinoid levels, thereby increasing CB_1/CB_2 cannabinoid receptors activity. As endocannabinoids are biosynthesized upon demand, by inhibiting FAAH or MGL their effect could be enhanced in a more physiological manner when compared with the administration of synthetic cannabinoids (Jayamanne *et al.*, 2006; Kathuria *et al.*, 2003). In fact, a number of potent FAAH inhibitors have been reported, including the nonselective methyl arachidonylfluorophosphonate (Deutsch *et al.*, 1997), hexadecylsulfonyl fluoride, the selective URB597, OL-53, and OL-135 (Labar and Michaux, 2007). With respect to MGL, only a few inhibitors have been reported (such as URB602 and NAM), and so far these inhibitors lack selectivity (Vandevoorde *et al.*, 2007). Note that only *N*-arachidonylmaleimide (NAM) displays some selectivity for MGL compared with FAAH (Saario *et al.*, 2005).

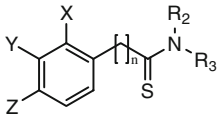
Herein we report the synthesis and screening of an arylthioamide series for potential MGL inhibitors. Some hits were identified and further characterized for their inhibition of MGL and FAAH by determining their pI_{50} values using human recombinant MGL and FAAH.

Results and discussion

Chemistry

The arylthioamide derivatives reported in this study (Table 1) were obtained using the synthetic pathways outlined below.

Table 1 Structures of arylthioamides synthesized



Compounds	X	Y	Z	*n*		Method
1	H	H	H	0	C_4H_9NO	A
2	Cl	H	H	0	C_4H_9NO	B
3	H	Cl	H	0	C_4H_9NO	A
4	H	H	Cl	0	C_4H_9NO	A
5	Cl	H	Cl	0	C_4H_9NO	B
6	H	Cl	Cl	0	C_4H_9NO	A
7	H	H	Br	0	C_4H_9NO	A
8	OH	H	H	0	C_4H_9NO	A
9	H	H	OH	0	C_4H_9NO	A
10	H	H	C_6H_{11}	0	C_4H_9NO	A
11	H	H	C_6H_5	0	C_4H_9NO	A
12	H	NO_2	H	0	C_4H_9NO	A
13	H	H	$N(CH_3)_2$	0	C_4H_9NO	A
14	H	H	$COOCH_3$	0	C_4H_9NO	A
15	H	H	H	1	C_4H_9NO	A
16	H	H	CH_3	1	C_4H_9NO	A
17	H	H	Cl	1	C_4H_9NO	A
18	H	OCH3	OCH_3	1	C_4H_9NO	A
19	H	H	C_6H_5	1	C_4H_9NO	A
20	Cl	H	H	1	C_4H_9NO	B
21	H	H	H	0	$C_5H_{11}N$	A
22	H	CH_3	H	0	$C_5H_{11}N$	A
23	H	H	H	0	$C_8H_{11}N$	A
24	H	OH	OH	0	$C_8H_{11}N$	A
25	H	H	$COOCH_3$	0	$C_8H_{11}N$	A
26	H	H	Br	0	$C_8H_{11}N$	A
27	H	H	Cl	0	$C_8H_{11}N$	A
28	Cl	H	H	0	$C_8H_{11}N$	B
29	Cl	H	Cl	0	$C_8H_{11}N$	B
30	H	Cl	Cl	0	$C_8H_{11}N$	A
31	H	H	F	0	$C_8H_{11}N$	A
32	H	H	C_6H_5	1	$C_8H_{11}N$	A

C_4H_9NO, $C_5H_{11}N$, $C_8H_{11}N$ refer, respectively, to morpholine, piperidine, and phenethylamine. Note that, for phenethylamine R_2 is an hydrogen atom

Willgerodt–Kindler reaction (method A): The Willgerodt–Kindler reaction was the main route used in the synthesis of these derivatives and involves the reaction between an aldone (aldehyde or ketone), sulfur, and a primary or secondary amine to yield a thioamide derivative (Fig. 1). Note that, when applied to an arylalkylketone, this reaction behaves as an autoredox system in which the carbonyl is reduced while the terminal methyl group of the alkyl side chain is oxidized. This reaction, overall, has good yield (60–70%) (Poupaert *et al.*, 2004; Rolfs and Liebscher, 1998).

Amide thionation (method B): When the Willgerodt–Kindler reaction (method A) was found to be unsatisfactory, for instance, when an aldehyde or ketone having a chlorine substituent at the *ortho* position of the aromatic ring were used (e.g., compounds **2**, **20**, and **28**), thionation of the amide to thioamide by using P_4S_{10}/Al_2O_3 in dioxane was used (Fig. 2), with yields ranging from 62% to 93% (Poupaert *et al.*, 2005).

With respect to the amines, we mainly used morpholine. We also synthesized some derivatives using the primary amine phenethylamine and the cyclic amine piperidine to allow for the establishment of preliminary structure–activity relationships. When considering the aromatic ring, we used phenyl ring substituted by different groups: alkyl, halogeno, nitro, phenyl, alkylamine, hydroxyl. Depending on the series, a spacer between the phenyl and the thioamide was introduced (i.e., $n = 1$ or 0). The compounds synthesized are summarized in Table 1.

To determine the putative relevance of the thioamide function, we synthesized some amides and amines of similar substitution pattern. The amides corresponding to the thioamides **2**, **12**, and **19** (i.e., **33**–**35**, Fig. 3 and Table 2) were obtained by action of substituted benzoyl chloride derivatives on amines. Similarly, to obtain the tertiary amines derivatives (**36**–**38**) the corresponding amides were reduced by action of $LiAlH_4$ in anhydrous diethyl ether (Fig. 3).

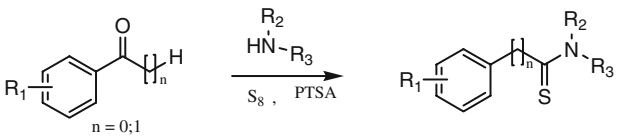


Fig. 1 Willgerodt–Kindler reaction. Reagents and conditions: aldehyde or ketone (0.2 mol), sulfur (0.2 mol), *p*-toluenesulfonic acid monohydrate (PTSA; 1 g), and amine (0.41 mmol). Reflux 4 h, filtered, and recrystallization from ethanol

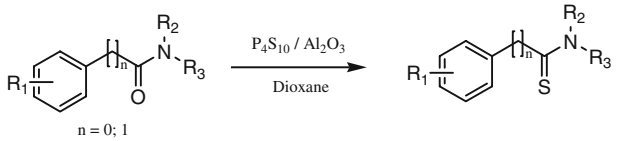


Fig. 2 Thionation of amides to thioamides. Reagents and conditions: amide (2.5 mmol), P_4S_{10}/Al_2O_3 (1 g), and anhydrous dioxane (20 mL). Reflux 3 h, filtered on ice, and precipitate recrystallized from ethanol to yield the corresponding thioamide

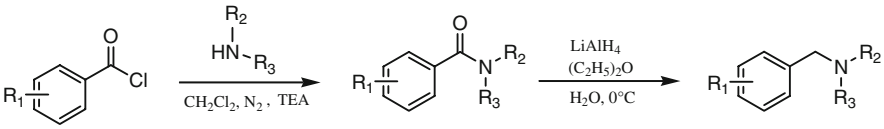
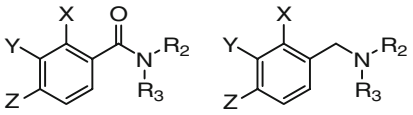


Fig. 3 Synthesis of amides and amines derivatives. Amides, reagents and conditions: substituted benzoyl chloride (0.05 mol), amine (0.1 mol), triethylamine (0.05 mol), methylene chloride (100 mL). Stirred for 12 h from 0°C to room temperature, under inert atmosphere. Crystallization of amide from ethanol. Amines, reagents and conditions: amide (11 mmol), LiAlH4 (50 mmol), anhydrous diethyl ether (200 mL) and ice. The organic phase is removed to afford the amine (oil)

Table 2 Structures of arylamides and arylamine synthesized



33-35 36-38

Compounds	X	Y	Z	R_2 HN R_3
33	Cl	H	H	C_4H_9NO
34	H	H	Cl	C_4H_9NO
35	H	H	C_6H_5	C_4H_9NO
36	Cl	H	H	C_4H_9NO
37	H	H	Cl	C_4H_9NO
38	H	H	C_6H_5	C_4H_9NO

Pharmacological evaluation

Hydrolysis of tritiated 2-oleoylglycerol by human monoglyceride lipase (Fig. 4) was used to evaluate the ability of our compounds to inhibit MGL esterase activity (Labar *et al.*, 2007).

The compounds were first screened at 10 μM and 100 μM. The compounds showing a good inhibition at 10 μM were further characterized by determining their pI_{50} values in inhibiting MGL activity (Table 2).

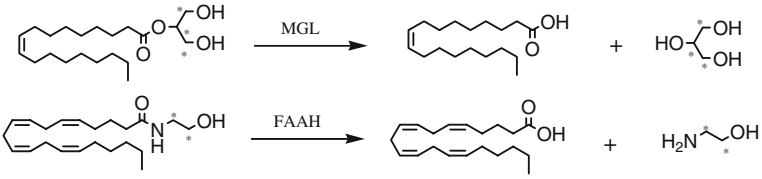


Fig. 4 [^{3}H]-2-OG hydrolysis by human MGL and [^{3}H]-AEA hydrolysis by human FAAH

In order to evaluate the selectivity of our compounds for MGL inhibition, compared to FAAH, we measured, according to the same principle, the hydrolysis of tritiated anandamide by human FAAH (Labar *et al.*, 2008). This is an important element because current MGL inhibitors usually lack selectivity over FAAH. Table 3 reports the pI_{50} values obtained for MGL and FAAH inhibition.

According to the results summarized in Table 3, the arylthioamide derivatives are monoglyceride lipase inhibitors. Note that compounds **2** and **11** have pI_{50} values close to the value we reported for the reference MGL inhibitor URB602 (Labar *et al.*, 2007). With respect to the amine moiety of the thioamide, morpholine-bearing compounds (e.g., **2**, **12**, and **19**) show higher activity compared with others amines. The substitution on the aryl moiety is crucial for the activity. Indeed halides and phenyl when used as substituents increase significantly monoglyceride lipase inhibition, whereas an unsubstituted aryl moiety (e.g., **1** and **21**) leads to inactive compounds. On the other hand, substitution of the aryl with an amine decreases the activity (**13**). Finally the substituent's position seems to be very important; in fact, derivatives with *ortho* and *para* substitution are potent inhibitors compared with *meta* substitution (e.g., compare **2** and **4** with the inactive **3**). The spacer arm has an

Table 3 pI_{50} values for MGL and FAAH inhibition

Compound	Inhibition (pI_{50})		Compound	Inhibition (pI_{50})	
	MGL	FAAH		MGL	FAAH
1	<3	<3	**20**	4.61 ± 0.06	<3
2	4.70 ± 0.03	<3	**21**	<3	<3
3	<3	<3	**22**	<3	4.21 ± 0.09
4	4.11 ± 0.07	3.52 ± 0.03	**23**	<3	<3
5	<3	<3	**24**	3.28 ± 0.09	3.14 ± 0.03
6	<3	<3	**25**	4.58 ± 0.03	< 3
7	4.50 ± 0.04	<3	**26**	4.01 ± 0.11	<3
8	4.22 ± 0.01	<3	**27**	3.36 ± 0.05	3.39 ± 0.04
9	3.69 ± 0.05	3.25 ± 0.03	**28**	3. 78 ± 0.09	< 3
10	<3	<3	**29**	4.10 ± 0.07	< 3
11	4.71 ± 0.07	4.51 ± 0.09	**30**	4.12 ± 0.04	4.27 ± 0.01
12	5.02 ± 0.08	3.61 ± 0.05	**31**	<3	<3
13	<3	<3	**32**	3.27 ± 0.02	3.14 ± 0.04
14	4.51 ± 0.04	<3	**33**	<3	<3
15	<3	<3	**34**	<3	<3
16	4.62 ± 0.15	4.31 ± 0.09	**35**	<3	<3
17	4.83 ± 0.20	4.11 ± 017	**36**	<3	<3
18	<3	<3	**37**	<3	<3
19	5.24 ± 0.20	4.51 ± 0.19	**38**	<3	<3

Values are the mean ± standard error on the mean (SEM) from three independent experiments performed in duplicate. Note that, the reference MGL inhibitor URB602 displays in our hands a pI_{50} value equal to 5.00 ± 0.11 (Labar *et al.*, 2007)

influence on MGL inhibition as evidenced, for example, by compounds **17** ($pI_{50} = 4.8$) and **4** ($pI_{50} = 4.1$). High activity was obtained with phenyl as substituent (e.g., **19**) whereas good selectivity, compared with FAAH inhibition, was obtained with halides substituents (e.g., **2**).

The synthesis and pharmacological evaluation of amides (**33–35**) and amines (**36–38**) corresponding to the active thioamides **2**, **4**, and **19** evidenced that the thioamide moiety is crucial to the inhibition of MGL by this series of compounds. Indeed, corresponding amide and amine derivatives were devoid of activity against both MGL and FAAH.

Interestingly, following thioamide synthesis, the crude crystallized products showed strong inhibition of MGL activity. However, this inhibition was found to be decreased after extensive purification using flash chromatography on silica gel column and further recrystallization from ethanol. For instance, compound **2** exhibited pI_{50} values before and after column purification of 6.20 and 4.70, respectively, suggesting the inhibitory activity of some contaminant.

To investigate this issue, elemental analysis was undertaken on the initial products and showed the presence of excess sulfur compared with the expected calculated value. This impurity was absent following flash chromatography and recrystallization of the compounds. In this regard, of interest is the report by Piomeli's group of a similar problem. Indeed, the activity on MGL initially attributed to URB754 (Makara *et al.*, 2005) was actually due to the presence of a bis (methylthio) mercurane impurity in their initial batch of URB754 (Tarzia *et al.*, 2007). These two examples highlight once again the risk of evaluating chemical libraries of compounds of insufficient purity during the drug discovery process. Careful resynthesis and purification of the initially discovered hits have been needed to definitely confirm the MGL inhibition potential of these compounds. In this regard, note that the pI_{50} values reported in Table 3 were obtained for compounds that were purified by column chromatography and subsequently crystallized.

Conclusions

We have shown that arylthioamides derivatives are monoglyceride lipase inhibitors. The compounds reported in this study will constitute useful templates for designing new monoglyceride lipase inhibitors. Indeed, their structure is characterized by a relatively low molecular weight and a log P value around 3, which will allow further possibilities of pharmacomodulations.

Experimental section

General procedures

All reagents were purchased from Acros organics or Sigma-Aldrich. Nuclear magnetic resonance (NMR) spectra were taken by using a Bruker 400

Ultrashield^{TM}. Chemical shifts (δ) are reported relative to the tetramethylsilane peak set at 0 ppm. In the case of multiplets the signals are reported as intervals. Signals are abbreviated as s: singlet; t: triplet; m: multiplet. Melting points were determined in open capillaries using the Electrothermal type IA 9000, apparatus and are reported uncorrected. Mass spectra were recorded by using a Finnigan MAT 44S, with an ionization voltage.

Willgerodt–Kindler reaction

A 100-mL round-bottomed flask was charged with 0.2 mol aldone, 1 g *p*-toluenesulfonic acid monohydrate (PTSA), 0.41 mol amine, and 0.2 mol sulfur. The flask was equipped with a reflux condenser and was heated at reflux for 4 h. The resulting reddish-brown solution was filtered and poured into 100 mL stirred hot methanol (55–60°C). The wall of the beaker was scratched with a glass rod for seeding. The beaker was sealed with aluminum foil and put into a refrigerator for 6 h. The resulting crystalline product was filtered and washed twice with ice-cold methanol and recrystallized from ethanol.

Synthesis of amides

A 250-mL round-bottomed flask was charged with amine (1.5 mol) in methylene chloride, triethylamine (1.5 mol); under cooling, a specific acyl chloride (1 mol) in methylene chloride was added dropwise. The mixture was stirred overnight. The solution was washed with water to remove triethylamine salt, and evaporated by using a rotary evaporator. The residue was crystallized from ethanol.

Thionation of amides

One gram of P_4S_{10}/Al_2O_3 reagent (0.85 mmol) was suspended in a solution of the amide (2.5 mmol) in 10–25 ml dry dioxane. The reaction was stirred and refluxed for 1 h, and filtered. The filtrate was poured on to ice (150 g) and the resulting mixture was stirred for 0.5 h. The precipitate was filtered and recrystallized from ethanol. The Al_2O_3-supported P_4S_{10} reagent was prepared by grinding together in a mortar 6.0 g of tetra phosphorus decasulfide with 10.0 g basic alumina until a homogeneous powder was obtained. The reagent was kept in a desiccator in a closed vessel before use.

Reduction of amides to amines

A 500-mL round-bottomed flask was charged with amide (11 mmol) in anhydrous diethyl ether (200 mL). Lithium aluminium hydrid was added. The mixture was stirred and reflux for 3 h. Ice was added, the organic phase extracted, and the solvent removed under reduced pressure.

The structures for all synthesized compounds, after purification by column chromatography and recrystallization, were consistent with their ^{1}H NMR, ^{13}C NMR, infrared (IR), and mass spectra.

Characterization of original compounds

*(4-Cyclohexyl-phenyl)-morpholin-4-yl-methanethione (**10**)*

^{1}H-NMR (400MHZ): (CDCl3) δ ppm: 1.21, 1.47 (m, 5H), 1.65, 1.85 (m, 5H), 2.50 (s,1H), 3.68 (2H, s), 3.86 (s, 2H), 4.40 (4H, s), 7.26 (m, 4H). ^{13}C-NMR: (CDCl3) δ ppm: 26.07, 26.79, 34.26, 44.38, 49.70, 52.60, 66.55, 66.81, 126.07, 139.93, 149.16, 201.48. MS: m/z = 289. m.p.: 143-144°C.

*2-Biphenyl-4-yl-1-morpholin-4-yl-ethanethion (**19**)*

^{1}H-NMR (400 MHZ): (CDCl3) δ ppm: 3.17 (t, 2H), 3.45 (t, 2H), 3.55 (t, 2H), 3.73 (t, 2H), 4.35 (s, 2H), 7.32–7.52 (m, 9H). ^{13}C-NMR: (CDCl3) δ ppm: 50.18, 50.27, 50.85, 66.19, 66.38, 127.01, 127.46, 127.65,128.24, 128.82, 134.90, 140.14, 198.52. MS: m/z = 297. m.p.: 73-74°C.

*4-Phenethylthiocarbamoyl-benzoic acid methyl ester (**25**)*

^{1}H-NMR (400 MHZ): (CDCl3) δ ppm: 3.09 (t, 2H), 3.24 (t, 2H), 3.89 (s, 3H), 4.04 (s, 1H), 7.29–7.96 (m, 9H). ^{13}C-NMR: (CDCl3) δ ppm: 33.43, 47.24, 52.09, 126.27, 126.70, 128.47, 128.66, 129.47,131.73, 137.81, 145.23, 166.01, 197.85. MS: m/z = 299. m.p.: 138–139°C.

*2-Chloro-N-phenethyl-thiobenzamide (**28**)*

^{1}H-NMR (400 MHZ): (DMSO-d6) δ ppm: 3.06 (t, 2H), 3.91 (t, 2H,), 4.40 (s, 1H), 7.26–7.57 (m, 9H) ^{13}C-NMR: (DMSO-d6) δ ppm: 32.66, 46.48, 126.26, 1226.83, 128.29, 128.55, 129.25, 129.64, 138.72, 142.43, 195.33. MS: m/z = 275. m.p.: 93–94°C.

*2,4-Dichloro-N-phenethyl-thiobenzamide (**29**)*

^{1}H-NMR (400 MHZ): (CDCl3) δ ppm: 3.06(t, 2H), 3.91(t, 2H), 4.40(s, 1H), 6.92–7.54 (m, 8H) ^{13}C-NMR: (CDCl3) δ ppm: 33.61, 47.24, 127.37, 128.74, 128.89, 129.05, 129.23, 129.63, 131.10, 135.70, 137.85, 140.30, 196.03. MS: m/z = 309. m.p.: 121–122°C.

*3,4–Dichloro-N-phenethyl-thiobenzamide (**30**)*

^{1}H-NMR (400 MHZ): (CDCl3) δ ppm: 3.70 (t, 2H), 4.04 (t, 2H), 4.17 (s, 1H), 7.13–7.75 (m, 8H). ^{13}C-NMR: (CDCl3) δ ppm: 33.68, 47.56, 125.56, 127.06, 128.63, 128.74, 128.99, 130.39, 132.82, 135.36, 137.99, 141.33, 196.21. MS: m/z = 309. m.p.: 118–119°C.

*2-Biphenyl-4-yl-N-phenethyl-thioacetamide (**32**)*

^{1}H-NMR (400 MHZ): (CDCl3) δ ppm: 2.01 (t, 2H), 2.88 (t, 2H), 3.91 (s, 2H), 4.05 (s, 1H), 6.97–7.54 (m, 14H) ^{13}C-NMR: (CDCl3) δ ppm: 32.89, 46.13, 52.10, 125.99, 126.31, 126.91, 127.19, 127.87, 128.09, 128.22, 129.33, 132.70, 137.17, 139.65, 140.04, 201.12. MS: m/z = 331. m.p.:148–149°C.

Pharmacological evaluation

The production of human recombinant MGL and pharmacological assay were previously reported. (Labar *et al.*, 2007). Briefly, [^{3}H]-2-oleoylglycerol (10 μM, 50 000 dpm, American Radiolabeled Chemicals) and human MGL (5 ng in Tris buffer, pH 8.0; 200 μL total volume assay) were incubated at 37°C for 10 min in the presence of inhibitors or dimethyl sulfoxide (DMSO) (10 μl, vehicle). The incubation was stopped by adding an ice-cold methanol–chloroform mixture (1:1, 400 μL), and the radioactivity in the upper aqueous phase was measured by liquid scintillation. Results are reported as pI_{50} value ($pI_{50} = -\log IC_{50}$). Graph Pad prism was used to treat the data.

For human FAAH evaluation (Labar *et al.*, 2008), radiolabeled [^{3}H]-anandamide was incubated for 10 min at 37°C in the presence of inhibitors or DMSO (10 μl, vehicle). The incubation was stopped by adding an ice-cold methanol–chloroform mixture (1:1, 400 μL), and the radioactivity in the upper aqueous phase was measured by liquid scintillation. Results are reported as pI_{50} value ($pI_{50} = -\log IC_{50}$). Graph Pad prism was used to treat data.

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