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Development and characterization of endocannabinoid hydrolases FAAH and MAGL inhibitors bearing a benzotriazol-1-yl carboxamide scaffold

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ABSTRACT

A series of (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-benzylpiperazin-1-yl)methanones and of (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-phenylpiperazin-1-yl)methanones has been prepared and tested on human fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). In the benzylpiperazinyl series, compound **29** (ML30) exhibited an IC_{50} value of 0.54 nM on MAGL, combined with a 1000-fold selectivity versus FAAH, while compounds **11** and **16** acted as potent dual FAAH-MAGL inhibitors (IC_{50} <10 nM). In the phenylpiperazinyl series, compounds **37**, **38**, **42**, and **43** displayed IC_{50} values against MAGL in the nanomolar range, whilst being between one and two orders of magnitude less potent on the FAAH, while compounds **31** and **32** were potent FAAH inhibitors (IC_{50} <20 nM) and over 12-fold selective versus MAGL. The key structural determinants driving the structure–activity relationships were explored by the minimization of the inhibitors inside the active site of both enzymes.

1. Introduction

The quest for enzyme inhibitors intended to increase endocannabinoid levels, in particular *N*-arachidonoylethanolamine (ananda-mide, AEA) and 2-arachidonoylglycerol (2-AG), is well underway. Since the early nineties and the identification of these endogenous lipids able to bind to CB_1 and CB_2 cannabinoid receptors, thus producing a wide range of physiological effects, the unraveling of the enzymes controlling this type of signaling as well as the design of compounds counteracting their action have constituted—and still do—major challenges.[1–7]

Fatty acid amide hydrolase (FAAH), which terminates anandamide signaling, is undoubtedly the best known representative of these enzymes. Its gene was cloned in 1996 and since then, numerous increasingly potent and selective inhibitors have been reported.[1–5] These efforts have culminated with compound PF04457845 from Pfizer, which completed phase II clinical trials (NCT00981357) for relief of pain in patients with osteoarthritis of the knee, after a phase I study demonstrating a good tolerability as well as a complete FAAH inhibition and an increase in AEA and three other fatty acid amides levels.[8]

Beside FAAH—and other enzymes whose existence was discovered more recently—monoacylglycerol lipase (MAGL) constitutes the second foremost enzyme in the endocannabinoid system. Since 1997 and the cloning of its gene,[9] much work has been devoted to the characterization of MAGL. In mammals, this enzyme is ubiquitous and appears exclusively dedicated to the hydrolysis of monoacylglycerols. In the last years, several teams have contributed, using various approaches, to unravel the leading role played by MAGL in the degradation of 2-AG, the most abundant endocannabinoid in the brain,[10–13] generating an intense interest for the design of MAGL inhibitors. The role of MAGL in promoting the malignancy of several aggressive cancer cell lines has recently further underscored the need for such compounds.[14–16] Moreover, the possibility of taking advantage of an inhibition of the enzyme in other pathological conditions, including neuroinflammation, pain and colitis, has also been demonstrated.[11,17,18]

However, apart from the *O*-phenyl carbamate-based compound JZL184, the *N*,*N*-diphenyl tetrazole-carboxamide **1** (Fig. 1)[19] and the *O*-hexafluoroisopropyl (HFIP) carbamates, very recently reported by Cravatt and co-workers,[20] MAGL inhibitors exhibiting both selectivity and potency have been lacking for a long time, and we became therefore interested into a project aimed at the development of such compounds. Thus, besides the determination of the tridimensional structure of the human MAGL,[21] we have reported in the last four years that a number of carbamoyl tetrazoles (e.g. **2**) turned out to constitute potent, although non selective, MAGL inhibitors[22] and that one analogue of the mammalian lipase inhibitor tetrahydrolipstatin was a potent and relatively selective MAGL-inhibitor (OMDM169).[23] Additional structural classes of MAGL inhibitors described recently by us and other groups are triazole-carboxamides,[24] bis(dialkylaminethiocarbonyl)disulfides,[25]

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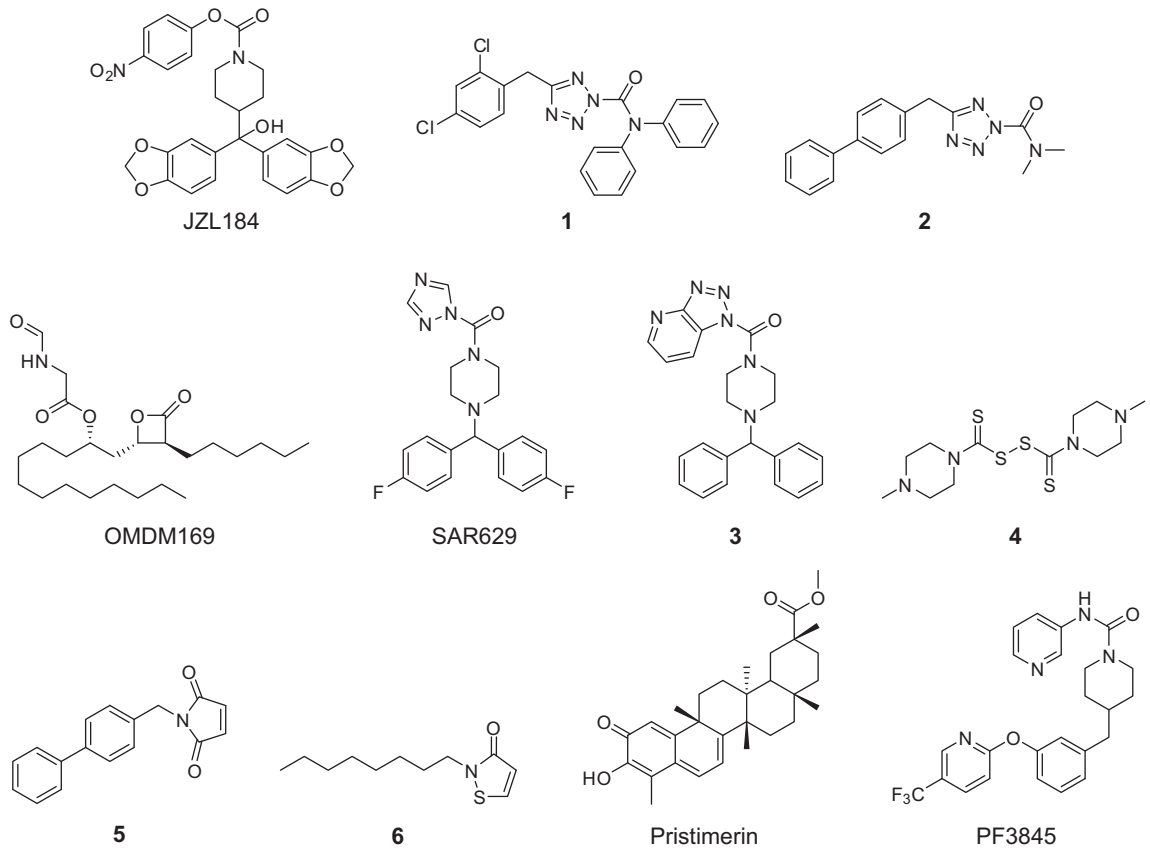


Figure 1. Structures of some MAGL inhibitors and of the FAAH inhibitor PF3845.

maleimides,[26] isothiazolinones,[27] and pentacyclic triterpenoids,[28] exemplified by compounds SAR629 and **3**, **4**, **5**, **6** and pristimerin, respectively, and most of them target MAGL cystein residues.

At the time, during a preliminary screening of our chemical library, we identified compound **7** (Fig. 2), an urea based on a benzotriazole-1-carboxamide template, as a MAGL inhibitor. This compound was of limited value (IC_{50}^{hMAGL} = 10 μM), and we therefore envisaged a few simple pharmacomodulations, after which we could identify compound **8** and the phenylpiperazinyl moiety as promising candidates for further medicinal chemistry efforts. This compound showed a more than respectable potency for the pure MAGL, with an IC_{50} value of 21 nM (Table 1).

We first explored with compounds **9**–**11** some linkers joining the phenyl ring to the piperazinyl moiety and the replacement of the latter by a piperidinyl ring. Comparison of compounds **8** and **9** revealed that the introduction of a methylene linker conferred additional potency towards MAGL, while the contemporaneous replacement of the core piperazine of compound **9** with a piperidine resulted in a seven times increase of the potency (compound **11**). Besides this, as one of the aims of this work was the design of selective MAGL inhibitors, the affinity for the human FAAH was also measured, using a recombinant enzyme fused to the maltose-binding protein. Compounds **8**–**11** turned out to constitute potent inhibitors in this assay as well. In particular, compound **11**, which exhibited a very high inhibitory activity for both FAAH and MAGL, has the potential to serve as a useful pharmacological probe for the evaluation of behavioral consequences of simultaneous elevations in AEA and 2-AG.[29] Although the issue of selectivity of inhibition of FAAH versus MAGL (or vice versa) is certainly the most important one,[20,30] the identification of dual inhibitors with nanomolar potencies on both targets appears to be of interest in view of their potential therapeutic exploitation. In this respect, Makriyannis and co-workers reported this year that the dual FAAH-MAGL inhibitor **2** is more protective against seizure pathology that its 1,5-regioisomer which is more selective for FAAH than MAGL.[31] In order to achieve selectivity for MAGL over FAAH, we next synthesized compounds **12**–**43**, starting from inhibitors **8** and **9**, which were chosen as lead compounds, on the basis of their good affinity for the principal target, the opportunities that they offered for pharmacomodulations and the gain in solubility that could be achieved compared to compounds bearing the piperidinyl moiety (compound **11**).

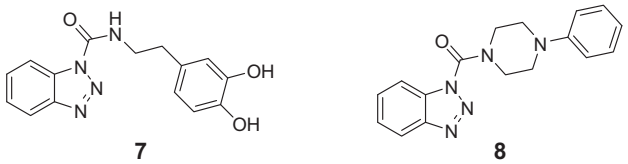
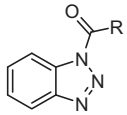


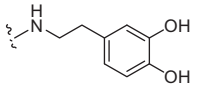
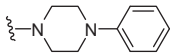
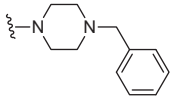
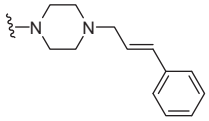
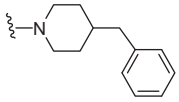
Figure 2. Structures of compounds **7** and **8**.

2. Chemistry

Compounds **8**–**43** were synthesized as shown in Scheme 1. Compounds **8**–**14**, **24**, and **29**–**43** were synthesized by the reaction of 1*H*-benzotriazole-1-carbonyl chloride, generated by treatment of benzotriazole with triphosgene, with the appropriate substituted piperazine/piperidine. Compounds **15**–**18** and **25**–**28** were prepared by reaction of appropriate benzyl halides with (1*H*-benzotriazol-1-yl)(piperazin-1-yl)methanone trifluoroacetate (**45**), in turn obtained from 1*H*-benzotriazole-1-carbonyl chloride and *N*-Boc-piperazine and deprotection with trifluoroacetic acid in

Table 1
In vitro activity of benzotriazol-1-yl carboxamides **7**–**11** and JZL184 on human MAGL and FAAH recombinant enzyme[a]



Compds	R=	IC$_{50}$, nM		Selectivity[b]
		[h]MAGL	[h]FAAH	
7		10471	ND[c]	–
8		21	11	0.5
9		8	19	2.4
10		33	28	0.8
11		3	2	0.7
JZL184	–	37	36308	981

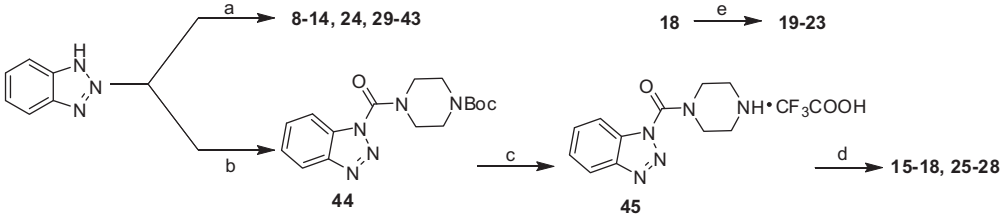
[a] Values are the means of three independent experiments performed in duplicate. Standard errors are not shown for the sake of clarity and were never higher than 10% of the means.
[b] Selectivity is expressed as IC$_{50}^{\text{hFAAH}}$/IC$_{50}^{\text{hMAGL}}$.
[c] ND, not determined.

chloroform of the Boc-derivative **44**. Benzamides **19**–**23** were prepared by condensing the carboxylic group of compound **18** with appropriate amines, using 1-hydroxybenzotriazole (HOBt)/*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as the carboxylate activator.

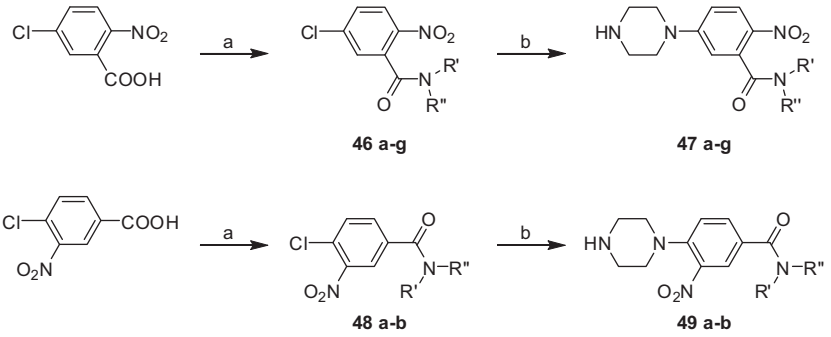
Non commercially available phenylpiperazines **47a**–**g** and **49a**–**b** were prepared by reaction of the appropriate chloro nitro benzoic acid with thionyl chloride, followed by addition of the appropriate amine and heating in DMF with an excess of piperazine of the resulting benzamides **46a**–**g** and **48a**–**b** (Scheme 2).

3. Results and discussion

We started our optimization efforts by exploring first the benzylpiperazinyl series. For this series, substitution in the *para* or *meta* position of the phenyl ring of the benzyl moiety with halogens or a nitro group generally maintained the activity towards both MAGL and FAAH (Table 2, compounds **12**–**17**) to afford some promising potent dual FAAH-MAGL inhibitors, in particular compound **16**, with a slight increase of the selectivity in the case of compounds **15** and **17**. The introduction of a carboxylic group in the *meta* position (compound **18**) led, on the contrary, to a dramatic 80 and 1500 times decrease of the activity for MAGL and FAAH, respectively, although resulting in a twenty times improvement of the selectivity. Therefore, we synthesized a limited series of benzylpiperazines bearing various carboxamide groups in the *meta* position of the benzyl moiety (compounds **19**–**23**), with the purpose of recovering the activity for MAGL, whilst maintaining low FAAH activities. However, this strategy turned out to be inefficient, as we were not able to dissociate the improvement of the activity for MAGL from that for FAAH. The addition of a second phenyl ring to various positions of the benzyl moiety (compounds **24**–**26**) or using a 1- or 2-naphtylmethyl moiety (compounds **27**–**28**) also led us to the same result.



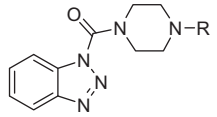
Scheme 1. Synthesis of compounds **8**–**43**. Reagents and conditions: (a) $(Cl_3CO)_2CO$, TEA, CH_2Cl_2, rt, 1 h, then appropriate piperazine or piperidine, TEA, rt, 16 h; (b) $(Cl_3CO)_2CO$, TEA, CH_2Cl_2, rt, 1 h, then *N*-Boc-piperazine, TEA, rt, 16 h; (c) CF_3CO_2H, $CHCl_3$, rt, 2 h; (d) $ArCH_2X$, K_2CO_3, DMF, rt, 16 h; (e) EDC, HOBt, DMF, 0 °C, 15 min, rt, 45 min, then RNH_2, rt, 16 h.

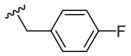
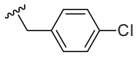
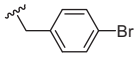
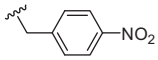
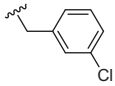
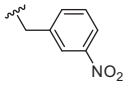
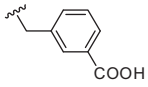
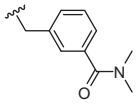
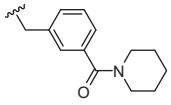
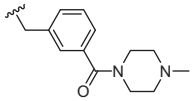
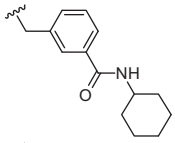
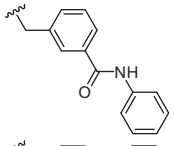
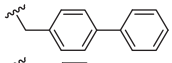
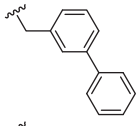
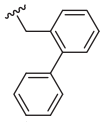
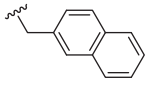
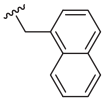
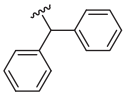


a R' = H R'' = $CH(CH_3)_2$ **b** R', R'' = $(CH_2CH_2)_2O$ **c** R', R'' = CH_3 **d** R' = H R'' = C_6H_5
e R' = H R'' = C_6H_{11} **f** R', R'' = $(CH_2CH_2)_2CH_2$ **g** R', R'' = $(CH_2CH_2)_2NCH_3$

Scheme 2. Synthesis of phenylpiperazines **47a**–**g** and **49a**–**b**. Reagents and conditions: (a) $SOCl_2$, 80 °C, 2 h, then R′R″NH, CH_2Cl_2, rt, 16 h; (b) piperazine, DMF, 130 °C, 1 h.

Table 2
In vitro activity of 4-benzyl piperazinylbenzotriazole carboxamides on human MAGL and FAAH recombinant enzymes[a]



Compds	R=	IC_{50}, nM		Selectivity[b]
		^{h}MAGL	^{h}FAAH	
12		10	15	1.5
13		11	17	1.5
14		9	22	2.5
15		12	89	7.6
16		2	9	4.5
17		8	43	5.4
18		676	27542	41
19		102	275	2.7
20		36	58	1.6
21		324	1380	4.3
22		13	44	3.4
23		7	35	4.8
24		62	18	0.3
25		23	9	0.4
26		33	32	1.0
27		9	12	1.3
28		3	13	4
29 (ML30)		0.54	562	1040

[a] Values are the means of three independent experiments performed in duplicate. Standard errors are not shown for the sake of clarity and were never higher than 10% of the means.
[b] Selectivity is expressed as $IC_{50}^{hFAAH}/IC_{50}^{hMAGL}$.

Long *et al.* reported compound JZL184 (Fig. 1), the first MAGL inhibitor displaying high efficacy and selectivity against MAGL (IC_{50}^{MAGL} = 10 nM; IC_{50}^{FAAH} = 4690 nM,[11] although in other studies JZL184 exhibited lower inhibition activity on MAGL,[32,33]) with the selectivity in part due to the inability of the bulky lipophilic bisarylcarbinol moiety to fit into the relatively narrow FAAH acyl chain binding pocket. On the basis of these results, we introduced a benzhydryl group on the 4-piperazine position. As expected, a spectacular increase in both potency and selectivity for MAGL was obtained when adding steric hindrance with the bulkier diphenylmethyl substituent (compound **29**, ML30). Compound **29** allowed us to gain potency for the MAGL by a factor of ten (IC_{50} = 0.54 nM) whilst substantially decreasing FAAH inhibitory activity (IC_{50} = 562 nM), thus resulting in a three order of magnitude selectivity, and, being 70 times more potent, compares favorably with JZL184 in the same assay conditions (Table 1).

Other close analogues of ML30 are a couple of triazolo-carboxamides described by Sanofi-Aventis, SAR629 and **3** (Fig. 1). The triazolopyridine carboxamide-based compound **3** has been shown to inhibit MAGL with an IC_{50} value of 4 nM,[24] but the inhibitory potency against FAAH has not been determined, while no details of the inhibitory potency against MAGL nor of its capability of inhibiting MAGL selectively over FAAH have been provided in the case of SAR629.[34]

Based on structure and reactivity similarities, the mechanism of MAGL inhibition by ML30 and its mode of recognition should be similar to those of JZL184 and Sanofi-Aventis triazolo-carboxamides (Fig. 3). To investigate the reversibility of the inhibition of MAGL by ML30, we first used the rapid dilution assay (Fig. 4a). Concentrations were carefully chosen according to the IC_{50} curve to achieve an almost complete inactivation of the enzyme at the initial concentration, whilst the final concentration—that is, after a 300-fold dilution—should not lead to inhibition. After dilution however, the enzyme did not recover activity, indicating the irreversible character of the inhibition. We next recorded the kinetics of MAGL inhibition over time in the presence of ML30. MAGL enzymatic velocity decreased over time in the presence of the inhibitor (Fig. 4b), giving us an additional evidence of the irreversible character of the inhibition. The k_{inact} and K_i inactivation constants, which are often used in the case of an irreversible inhibition, were also calculated using k_{obs}, the first-order constant for enzyme inactivation (Fig. 4c).

The tridimensional structure of FAAH has been known since 2002,[35] and we as well as other groups have recently reported that of human MAGL as the apoenzyme or as the complex with several inhibitors.[21,34] Therefore, in order to gain a better understanding of

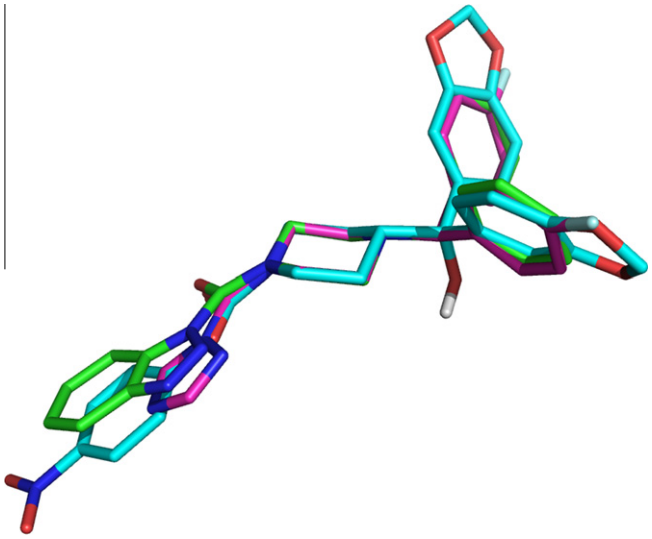


Figure 3. Overlay of the minimized structures of compound **29**, JZL184 and SAR629 (carbon atoms in green, blue and magenta, respectively).

the structure–activity relationships obtained within benzylpiperazinyl series, we then studied the binding mode of several inhibitors inside the MAGL and FAAH active sites, as covalent adducts by carbamoylation of a nucleophilic serine of the two enzymes. We first minimized compound **9** inside the FAAH active site, bound to the nucleophilic Ser241 (Fig. 5a). To this end, we used the crystal coordinates of the rat humanized FAAH in complex with the inhibitor PF3845 (Fig. 1).[36] Noteworthy, we considered for these studies both the open and closed conformations of the Phe432, which constitutes a lock controlling the access to the active site and the shape of the FAAH membrane access channel and acyl-binding pocket.[37] As expected, the benzylic ring of compound **9** closely superimposes with the proximal phenyl ring of PF3845 and makes a CH–π interaction with Phe192. This minimization also shows us that the *meta* position of the phenyl ring is well oriented to project toward the distal pocket which, in the experimental structure, is filled by the 4-trifluoromethyl-2-pyridyl group of the inhibitor PF3845. The lipophilicity of this distal pocket, lined with Met495, Val491 and Phe432 side chains, likely accounts for the dramatic loss of potency observed for compound **18**, due to the negative charge of the carboxylate. Similarly, it explains the progressive recovery of activity when filling the pocket with *N,N*-dimethylcarboxamide, piperidin-1-ylmethanone, *N*-cyclohexylcarboxamide or *N*-phenylcarboxamide groups (compounds **19**, **20**, and **22**, **23**). Due to the positively charged *N*-methylpiperazinyl moiety, compound **21** is also twenty times less potent than **20** on the FAAH.

In the biphenyl series, the *meta*-linked biphenyl compound **25** was the most potent FAAH inhibitor. This position allows to reach the distal pocket of the acyl-binding pocket of FAAH, in which compound **25** superimposes nicely with PF3845 (Fig. 5b). In order to fit into the FAAH active site, the benzhydryl group of **29** adopts an energetically unfavorable conformation (Fig. 5b). Moreover, the only possibility for the second phenyl group is to protrude toward a pocket lined, among others, by the hydroxyl groups of Ser213 and Thr488 and by the main chain carbonyl of Phe192, Leu401, Cys400, Pro484, and Gly485. This pocket, which constitutes the bottom of the channel leading from the membrane access port to the active site, has thus a less lipophilic character which, together with the torsion which is imposed to fit into the active site of the FAAH, likely account for the loss of potency of compound **29** compared to **9** or **25**.

In order to better understand the high selectivity of compound **29** for the MAGL, we next studied its binding mode inside the

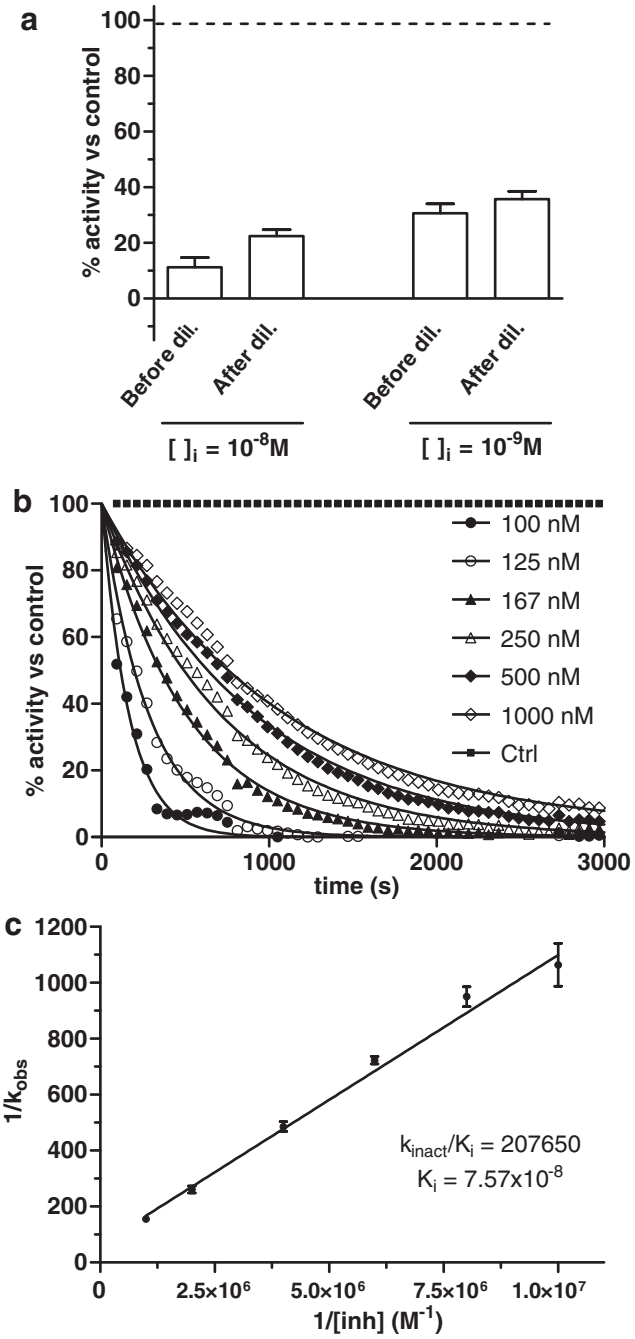


Figure 4. (a) Rapid dilution assay. After a 30 min incubation of MAGL with ML30 (10^{-8} or 10^{-9} M) followed by a 300-fold dilution, the 2-OG hydrolase activity was measured to investigate the reversibility of MAGL inhibition. The percentage of residual activity observed using 10^{-8} or 10^{-9} M of the inhibitor (without dilution) is also indicated. The experiment was performed two times in triplicate. (b) MAGL residual activity in the presence of ML30 was measured over time using *p*-nitrophenylpropionate as a substrate. The experiment was done four times in quadruplicate. (c) k_{obs} (first order constant for enzyme inactivation) values at each inhibitor concentration were used to calculate k_{inact} and K_i values for MAGL inhibition by ML30.

MAGL active site. Several tridimensional MAGL structures have been reported to date.[21,34,38] A wide hydrophobic site able to accommodate the acyl chain of 2- AG is present in each, with the major difference being observed in the organization of the cap, which occludes or not part of the active site entry. We used, for our study, the coordinates of the human enzyme in complex with the inhibitor SAR629. As expected, the diphenylmethyl group of **29**

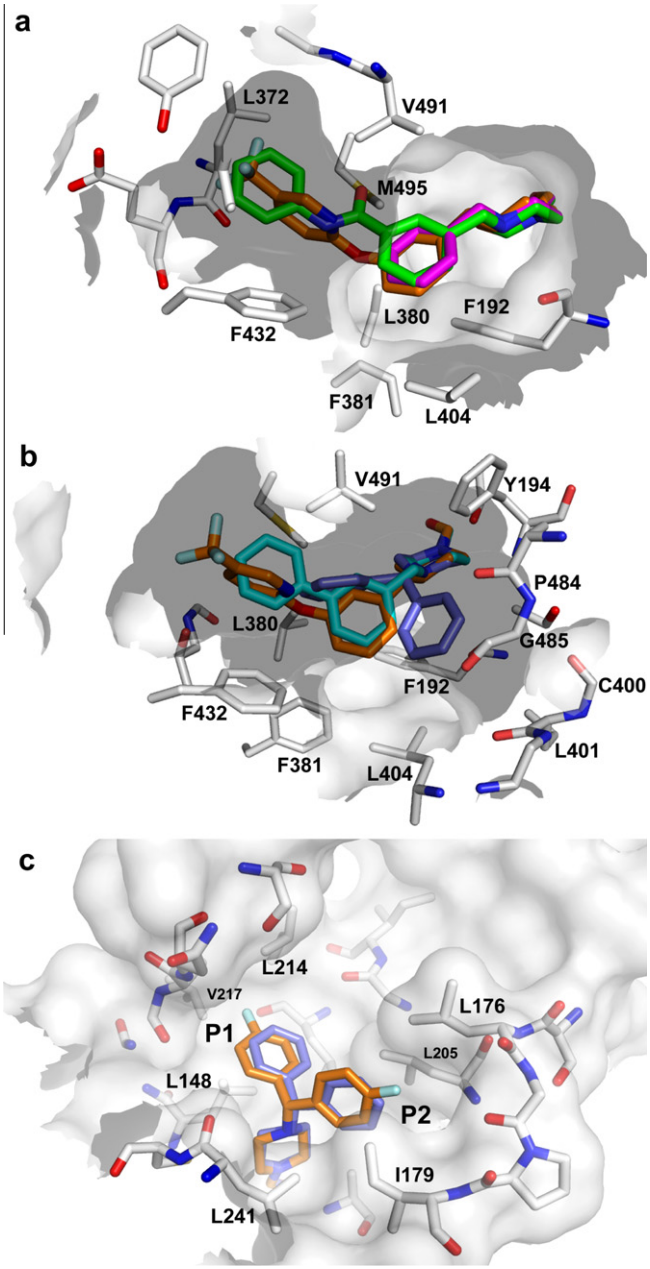
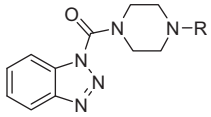


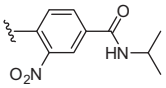
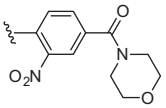
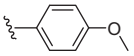
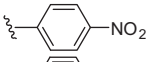
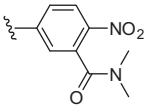
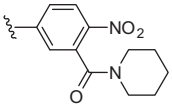
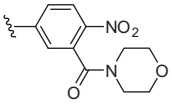
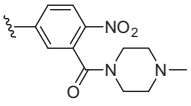
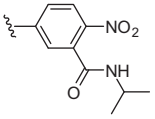
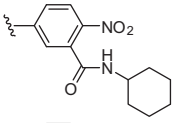
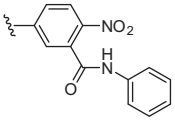
Figure 5. (a) Minimization of compounds **9** (magenta) and **23** (green) in the active site of the rat humanized FAAH. (b) Minimization of compounds **25** (cyan) and **29** (blue) in the active site of the rat humanized FAAH. (c) Minimization of compounds **29** (blue) in the active site of the human MAGL. The FAAH inhibitor PF3845 (Fig. 5a and b) or the MAGL inhibitor SAR629 (Fig. 5c) are also represented (orange).

closely superimposes with the corresponding substituent of SAR629, making CH–π interactions either with Leu205 and Leu241 alkyl chains or with Leu148 (Fig. 5c). Unlike in the case of FAAH, compound **29** fits in the MAGL active site in a energetically favorable conformation and makes Van der Waals interactions with both P1 and P2 pockets.

We next explored the phenylpiperazinyl series (Table 3). Substitution in *ortho* with a fluoro, methyl or nitro group gradually decreased the activity for MAGL (compounds **30**–**32** compared to **8**) while maintaining excellent activity for FAAH. In particular, compounds **31** and **32** were potent FAAH inhibitors (IC_{50} <20 nM) and over 12-fold selective versus MAGL. The association of a nitro

Table 3
In vitro activity of 4-aryl piperazinylbenzotriazole carboxamides on human MAGL and FAAH recombinant enzymes[a]



Compds	R=	IC_{50}, nM		Selectivity[b]
		^{h}MAGL	^{h}FAAH	
30		30	13	0.44
31		71	3	0.04
32		224	19	0.08
33		372	158	0.43
34		955	1950	2.0
35		32	15	0.47
36		20	34	1.7
37		63	5012	79
38		38	2291	60
39		162	40	% inh (10^{-5} M)>100
40		141	40	% inh (10^{-5}M)>100
41		234	2089	8.9
42		41	490	12
43		19	363	19

[a] Values are the means of three independent experiments performed in duplicate. Standard errors are not shown for the sake of clarity and were never higher than 10% of the means.
[b] Selectivity is expressed as $IC_{50}^{hFAAH}/IC_{50}^{hMAGL}$.

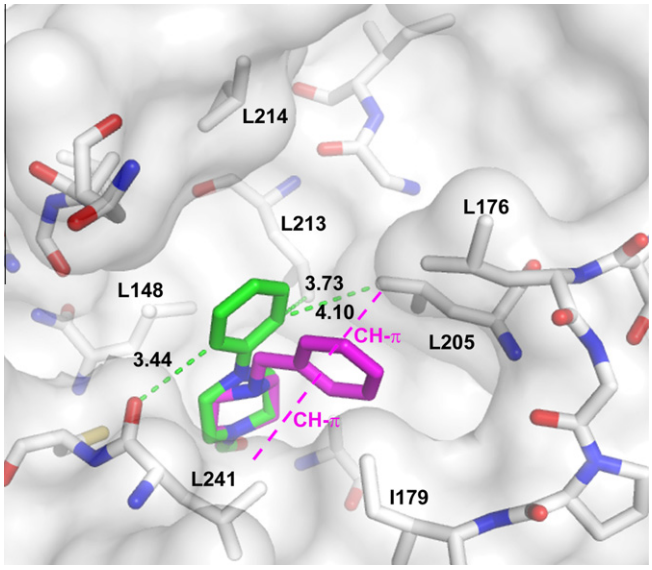


Figure 6. Minimization of compounds **8** (green) and **9** (magenta) in the active site of the human MAGL.

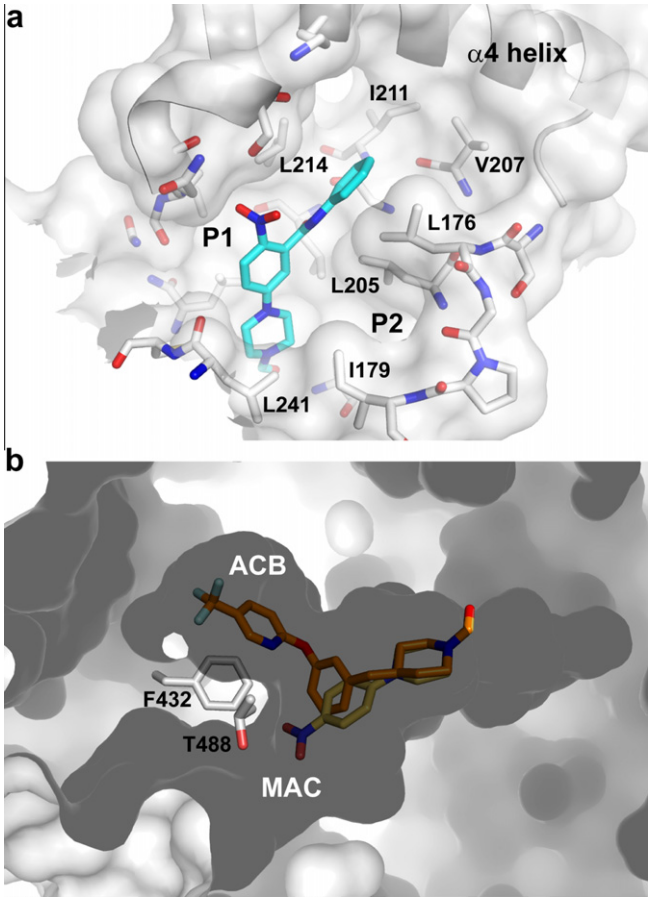


Figure 7. (a) Minimization of compound **43** in the active site of the human MAGL. (b) Minimization of compounds **36** (yellow) in the active site of the FAAH. The FAAH inhibitor PF3845 is represented in orange. The position of the acyl-binding pocket (ACB) and the membrane access channel (MAC) is also indicated.

group in *ortho* and of a carboxamide in the *para* position led to a general loss of potency (compounds **33**–**34**). However, when the nitro group was placed in *para*, the potency against MAGL was slightly increased, while the activity for FAAH was reduced by a factor of three (compound **36**). In the hope to increase the MAGL selectivity, we planned therefore to synthesize a series of 1-(4-nitrophenyl)piperazines bearing various carboxamides in the *meta* position of the aromatic ring (compounds **37**–**43**). In the case of tertiary amides, a substantial decrease of the potency at FAAH was observed, ranging from a ~70 times diminution for compound **38** compared to compound **36** to an almost complete loss of activity (compounds **39**–**40**). This effect was less spectacular with secondary amides, although the presence of a *meta* *N*-isopropylcarboxamide group led to a compound (**41**) 60 times less potent than the *meta* unsubstituted analogue **36** on FAAH.

Although the majority of the inhibitors bearing a carboxamide group showed also a decreased potency on MAGL, the diminution was less dramatic than that on FAAH. Indeed, only a slight decrease was observed for compounds **38** and **42**, and compound **43** was equipotent to compound **8**. These differences in structure–activity relationships of the two enzymes resulted in an improved MAGL selectivity. In fact, when compared to the parent inhibitor **8**, compound **43** gained more than one order of magnitude in selectivity and compounds **37**, **38**, and **39**, although two to eight times less potent than compound **8**, were 120–200 times more selective.

As for the benzylpiperazine series, we then tried, based on the tridimensional structure of both enzymes, to gain further understanding of the structure–activity relationships obtained in the phenylpiperazinyl series. We first minimized **8** in the cavity of the MAGL, covalently linked to the Ser122 (Fig. 6). Due to the absence of a methylene bridge between the phenyl ring and the piperazinyl moiety, the aromatic ring fails to project toward the P1 or P2 pocket. Moreover, despite partly filling the hydrophobic acyl-binding channel, Leu148 and Val217 are not well oriented or not close enough to be engaged in stabilizing CH–π interactions with the inhibitor. Therefore, at first glance, it could be rather surprising to see that **8** was almost equipotent to its benzylpiperazine analogue, compound **9**. However, at the pH of the enzymatic assay, unlike compound **8**, compound **9** bears a positively charged nitrogen, and, in MAGL, this charge is enclosed in an apolar environment, directly facing the CD1 and CD2 atoms of Leu148 and Leu241 alkyl chains. It is thus likely that, regarding compound **8**, the disappearance of this unfavourable condition counterbalances the lack of CH–π interaction involving its phenyl ring. Besides this, the *ortho* substitution on the phenyl ring leads to less active MAGL inhibitors (**30**–**32**). This can be explained by the very close proximity of the phenyl *ortho* position with the Leu205 CD2, Leu213 CD1, and Leu241 main chain oxygen (Fig. 6). This situation creates, in the near environment of the phenyl ring, a steric hindrance, leading to a steric clash when a substituent is introduced in the *ortho* position. On the contrary, compound **36**, with a nitro group in the *para* position, fits well in the active site, even if the nitro is not optimal for the hydrophobic cavity, which is lined by Ala151, Leu214, Leu205 and Leu176 side chains.

A minimization of compound **43** in the MAGL showed that the inhibitor can project the phenylcarboxamide group toward the top of the active site, just below the hydrophobic α4 helix that constitutes the lid that controls access to the enzyme (Fig. 7a).

On the other hand, although compound **36** fits in the FAAH cavity, the 4-nitro group comes in close contact with Thr488 and Phe432 side chains, two residues that form the junction between the acyl binding pocket and the membrane access channel (Fig. 7b). Along this line, the geometric constrains imposed by the combination of nitro and carboxamide groups in the *para* and *meta* positions, respectively, seem to explain the poor inhibitory potency of compounds **37**–**43** on FAAH.

4. Conclusion

A series of (1*H*-benzo[*d*][1,2,3]triazol-1-yl)(4-benzylpiperazin-1-yl)methanones (compounds **9**, **12**–**29**) and of (1*H*-benzo[*d*] [1,2,3]triazol-1-yl)(4-phenylpiperazin-1-yl)methanones (compounds **8**, **30**–**43**) has been synthesized and its inhibitory activity measured against human FAAH and MAGL recombinant enzymes. Starting from compounds **8** and **9**, we were able to identify some MAGL- and FAAH-selective or dual FAAH-MAGL inhibitors. In particular, compound **29** (ML30) exhibited an IC_{50} value of 0.54 nM on MAGL, combined with a 1000-fold selectivity versus FAAH, while compounds **11** and **16** acted as potent dual FAAH-MAGL inhibitors (IC_{50} <10 nM). Compounds **37**, **38**, **42**, and **43** displayed IC_{50} values against MAGL in the nanomolar range, whilst being between one and two orders of magnitude less potent on the FAAH, while compounds **31** and **32** were potent FAAH inhibitors (IC_{50} <20 nM) and over 12-fold selective versus MAGL. The structural features driving the recognition profile on FAAH versus MAGL and vice versa were also studied by a comparison of the binding mode of the inhibitors inside the enzyme active sites.

Taken together, these data suggest that these inhibitors could serve as promising leads for the development of therapeutics based on the inhibition of AEA and/or 2-AG degradation.

5. Experimental section

5.1. Chemistry

5.1.1. General

All chemical reagents were commercially available unless otherwise indicated and were used without further purification. Solvents were of analytical grade. Column chromatographies were carried out using Merck silica gel 60 (230–400 mesh). Melting points were determined on a Kofler hot-stage apparatus or in open capillaries using the Electro thermal 9100 apparatus and are reported uncorrected. IR spectra were recorded on a Perkin-Elmer 1000 FT-IR spectrophotometer as $CHCl_3$ solutions unless otherwise indicated. For compounds **20**, **21** and **23**, IR spectra were run on a SpectrumOne FT-ATR spectrophotometer (Perkin Elmer). Band position and absorption ranges are given in cm^{-1}. ^1H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 spectrometer using $CDCl_3$ as solvent unless otherwise indicated and TMS as internal standard. Satisfactory elemental analyses were obtained for the newly synthesized compounds (C, H, N ± 0.4%).

5.1.2. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-phenylpiperazin-1-yl) methanone (8)

Bis(trichloromethyl) carbonate (1.000 g, 3.42 mmol) was added to a solution of benzotriazole (1.230 g, 10.3 mmol) and triethylamine (TEA) (1.5 mL) in CH_2Cl_2 (15 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 1 h. Then, a solution of 1-phenylpiperazine (1.330 g, 8.2 mmol) and TEA (1.0 mL) in CH_2Cl_2 (15 mL) was added dropwise, and the reaction mixture was stirred at room temperature overnight. The reaction was quenched by adding a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and evaporated in vacuo to give a residue of 770 mg, which was chromatographed on silica gel (98:2 CH_2Cl_2/*n*-hexane as eluent) to give 600 mg (57%) of pure **8** as solid. Mp = 152–153 °C; IR (KBr) 2829, 1695, 1441, 1233, 1011, 749; ^1H NMR δ 3.38 (4H, m), 4.10 (4H, m), 6.92–6.99 (3H, m), 7.31 (2H, t, J = 7.8 Hz), 7.47 (1H, t, J = 7.6 Hz), 7.62 (1H, t, J = 7.6 Hz), 8.02 (1H, d, J = 8.4 Hz), 8.11 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 49.60, 113.65, 116.77, 119.92, 120.80, 125.34, 129.32, 129.50, 133.22, 145.38, 149.42, 150.75. Anal. Calcd for $C_{17}H_{17}N_5O$: C, 66.43; H, 5.58; N, 22.79. Found: C, 66.58; H, 5.57; N, 22.75.

5.1.3. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-benzylpiperazin-1-yl) methanone (9)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-benzylpiperazine instead of 1-phenylpiperazine. Chromatography on silica gel (8:2 CH_2Cl_2/AcOEt as eluent) afforded pure **9** as solid. Yield = 29%. Mp = 91–92 °C; IR 2815, 2771, 1712, 1450, 1246, 1011, 749; ^1H NMR δ 2.64 (4H, m), 3.60 (2H, s), 3.93 (4H, m), 7.27–7.35 (5H, m), 7.45 (1H, t, J = 7.6 Hz), 7.60 (1H, t, J = 7.6 Hz), 7.98 (1H, d, J = 8.2 Hz), 8.09 (1H, d, J = 8.2 Hz); ^{13}C NMR δ 52.82, 62.82, 113.57, 119.86, 125.20, 127.38, 128.40, 129.15, 129.34, 133.21, 137.45, 145.34, 149.36. Anal. Calcd for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79. Found: C, 67.48; H, 5.97; N, 21.73.

5.1.4. (*E*)-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-cinnamylpiperazin-1-yl)methanone (10)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-cinnamylpiperazine instead of 1-phenylpiperazine. Chromatography on silica gel (5:5 *n*-hexane/AcOEt as eluent) afforded pure **10** as solid. Yield = 46%. Mp = 111 °C; IR 2936, 2813, 1711, 1417, 1229, 999, 747; ^1H NMR δ 2.70 (4H, t, J = 5.1 Hz), 3.25 (2H, d, J = 6.7 Hz), 3.96 (4H, m), 6.28 (1H, dt, J = 15.8, 6.7 Hz), 6.57 (1H, d, J = 15.8 Hz), 7.21–7.28 (1H, m), 7.28–7.36 (2H, m), 7.40 (2H, d, J = 7.5 Hz), 7.45 (1H, t, J = 7.6 Hz), 7.60 (1H, t, J = 7.6 Hz), 7.99 (1H, d, J = 8.3 Hz), 8.10 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 52.92, 53.42, 60.84, 113.57, 119.87, 125.23, 125.66, 126.36, 127.71, 128.61, 129.38, 133.19, 133.73, 136.62, 145.34, 149.36. Anal. Calcd for $C_{20}H_{21}N_5O$: C, 69.14; H, 6.09; N, 20.16. Found: C, 68.98; H, 6.10; N, 20.17.

5.1.5. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-benzylpiperidin-1-yl) methanone (11)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 4-benzylpiperidine instead of 1-phenylpiperazine. Chromatography on silica gel (8:2 *n*-hexane/AcOEt as eluent) afforded pure **11** as solid. Yield = 95%. Mp = 80–81 °C; IR 2948, 2929, 1705, 1430, 1250, 1051, 749; ^1H NMR δ 1.49 (2H, m), 1.74–1.98 (3H, m), 2.63 (2H, d, J = 6.9 Hz), 3.00–3.13 (2H, m), 4.51 (2H, d, J = 12.6 Hz), 7.14–7.35 (5H, m), 7.44 (1H, t, J = 7.6 Hz), 7.59 (1H, t, J = 7.6 Hz), 7.96 (1H, d, J = 8.4 Hz), 8.09 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 32.10, 38.19, 42.89, 53.45, 113.47, 119.82, 125.11, 126.17, 128.38, 129.12, 129.24, 133.20, 139.78, 145.37, 149.39. Anal. Calcd for $C_{19}H_{20}N_4O$: C, 71.23; H, 6.29; N, 17.49. Found: C, 71.38; H, 6.28; N, 17.47.

5.1.6. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(4-fluorobenzyl) piperazin-1-yl)methanone (12)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(4-fluorobenzyl)piperazine instead of 1-phenylpiperazine. Chromatography on silica gel (99:1 CH_2Cl_2/MeOH as eluent) afforded pure **12** as white solid. Yield = 62%. Mp = 113 °C; IR (KBr) 3417, 2811, 1716, 1510, 1428, 1209, 1024, 750; ^1H NMR δ 2.63 (4H, m), 3.56 (2H, s), 3.93 (4H, m), 7.03 (2H, m), 7.32 (2H, m), 7.46 (1H, t, J = 7.6 Hz), 7.60 (1H, t, J = 7.6 Hz), 7.99 (1H, d, J = 8.4 Hz), 8.09 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 44.96, 52.76, 61.99, 113.59, 115.26 (d, J_{C-F} = 21.1 Hz), 119.88, 125.23, 129.37, 130.0 (d, J_{C-F} = 7.9 Hz), 130.64, 133.19, 133.22, 145.36, 149.37, 162.17 (d, J_{C-F} = 244.9 Hz). Anal. Calcd for $C_{18}H_{18}FN_5O$: C, 63.70; H, 5.35; N, 20.64. Found: C, 63.87; H, 5.36; N, 20.60.

5.1.7. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(4-chlorobenzyl) piperazin-1-yl)methanone (13)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(4-chlorobenzyl)piperazine instead of 1-phenylpiperazine. Chromatography on silica gel (8:2 CH_2Cl_2/AcOEt as eluent) afforded pure **13** as white solid. Yield = 70%. Mp = 121–122 °C; IR (KBr) 3417, 2812, 1703, 1433, 1229, 990, 760; ^{1}H NMR δ 2.62 (4H, m), 3.56 (2H, s), 3.92 (4H, m), 7.30 (4H, m), 7.46 (1H, t, J = 7.6 Hz), 7.60 (1H, t, J = 7.6 Hz), 7.99 (1H, d, J = 8.4 Hz), 8.09 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 52.79, 62.01, 63.82, 113.56, 119.87, 125.25, 128.56, 129.39, 130.37, 133.11, 133.18, 136.03, 145.33, 149.35. Anal. Calcd for $C_{18}H_{18}ClN_5O$: C, 60.76; H, 5.10; N, 19.68. Found: C, 60.93; H, 5.10; N, 19.63.

5.1.8. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(4-bromobenzyl) piperazin-1-yl)methanone (14)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(4-bromobenzyl)piperazine instead of 1-phenylpiperazine. Chromatography on silica gel (98:2 CH_2Cl_2/MeOH as eluent) afforded pure **14** as white solid. Yield = 66%. Mp = 131 °C; IR (KBr) 3418, 2807, 1692, 1433, 1228, 991, 760; ^{1}H NMR δ 2.62 (4H, t, J = 4.4 Hz), 3.54 (2H, s), 3.92 (4H, m), 7.23 (2H, d, J = 8.0 Hz), 7.46 (3H, m), 7.60 (1H, t, J = 7.6 Hz), 7.98 (1H, d, J = 8.0 Hz), 8.09 (1H, d, J = 8.0 Hz); ^{13}C NMR δ 47.51, 52.77, 62.05, 113.56, 119.87, 121.21, 125.25, 129.40, 130.74, 131.53, 133.19, 136.57, 145.33, 149.36. Anal. Calcd for $C_{18}H_{18}BrN_5O$: C, 54.01; H, 4.53; N, 17.50. Found: C, 54.17; H, 4.54; N, 17.49.

5.1.9. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(4-nitrobenzyl) piperazin-1-yl)methanone (15)

A solution of 4-nitrobenzylbromide (65 mg, 0.3 mmol), **45** (104 mg, 0.3 mmol), and potassium carbonate (250 mg, 1.8 mmol) in DMF (1 mL) was stirred overnight at room temperature. The reaction was quenched by adding a saturated $NaHCO_3$ solution and extracted with AcOEt. The organic phase was dried over Na_2SO_4 and evaporated in vacuo to give a residue of 109 mg, which was chromatographed on silica gel (55:45 *n*-hexane/AcOEt as eluent) to give 74 mg (67%) of pure **15** as yellow solid. Mp = 170–172 °C; IR (KBr) 2813, 1715, 1605, 1513, 1429, 1345, 1231, 1025, 1001, 754; ^{1}H NMR δ 2.67 (4H, m), 3.70 (2H, s), 3.96 (4H, m), 7.46 (1H, t, J = 7.7 Hz), 7.56 (2H, d, J = 8.6 Hz), 7.61 (1H, t, J = 7.7 Hz), 7.99 (1H, d, J = 8.3 Hz), 8.09 (1H, d, J = 8.3 Hz), 8.21 (2H, d, J = 8.6 Hz); ^{13}C NMR δ 46.52, 52.93, 61.86, 113.58, 119.89, 123.70, 125.31, 129.46, 129.54, 133.20, 145.35, 147.42, 149.37. Anal. Calcd for $C_{18}H_{18}N_6O_3$: C, 59.01; H, 4.95; N, 22.94. Found: C, 59.07; H, 4.95; N, 22.99.

5.1.10. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(3-chlorobenzyl) piperazin-1-yl)methanone (16)

The title compound was prepared from 1-(bromomethyl)-3-chlorobenzene following the same procedure used for the synthesis of **15**. Chromatography on silica gel (6:4 *n*-hexane/AcOEt as eluent) afforded pure **16** as white solid. Yield = 91%. Mp = 69–70 °C; IR (KBr) 2925, 2800, 1698, 1439, 1235, 1020, 752; ^{1}H NMR δ 2.62 (4H, m), 3.55 (2H, s), 3.93 (4H, m), 7.21–7.28 (3H, m), 7.36 (1H, br s), 7.44 (1H, t, J = 7.7 Hz), 7.59 (1H, t, J = 7.7 Hz), 7.98 (1H, d, J = 8.3 Hz) 8.08 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 45.20, 47.64, 52.75, 62.07, 113.55, 119.80, 125.18, 127.10, 127.51, 128.96, 129.32, 129.62, 133.15, 134.28, 139.74, 145.28, 149.28. Anal. Calcd for $C_{18}H_{18}ClN_5O$: C, 60.76; H, 5.10; N, 19.68. Found: C, 60.83; H, 5.11; N, 19.65.

5.1.11. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(3-nitrobenzyl) piperazin-1-yl)methanone (17)

The title compound was prepared from 1-(chloromethyl)-3-nitrobenzene following the same procedure used for the synthesis of **15**. Chromatography on silica gel (9:1 CH_2Cl_2/AcOEt as eluent) afforded pure **17** as yellow solid. Yield = 66%. Mp = 164–165 °C; IR (KBr) 2799, 2780, 1698, 1533, 1438, 1342, 1018, 735; ^{1}H NMR δ 2.68 (4H, m), 3.69 (2H, s), 3.96 (4H, m), 7.45 (1H, t, J = 7.6 Hz), 7.52 (1H, t, J = 8.0 Hz), 7.60 (1H, t, J = 7.6 Hz), 7.71 (1H, d, J = 7.6 Hz), 7.99 (1H, d, J = 8.4 Hz) 8.09 (1H, d, J = 8.4 Hz), 8.14 (1H, d, J = 8.0 Hz), 8.26 (1H, s); ^{13}C NMR δ 46.83, 52.75, 61.66, 113.49, 119.76, 122.41, 123.61, 125.17, 129.27, 129.32, 133.10, 134.84, 139.95, 145.24, 148.41, 149.25. Anal. Calcd for $C_{18}H_{18}N_6O_3$: C, 59.01; H, 4.95; N, 22.94. Found: C, 59.12; H, 4.95; N, 22.99.

5.1.12. 3-((4-(1H-Benzo[d][1,2,3]triazole-1-carbonyl)piperazin-1-yl)methyl)benzoic acid (18)

A solution of 3-(chloromethyl)benzoic acid (512 mg, 3.0 mmol) in DMF (9 mL) was added dropwise to a stirring solution of **7** (1.04 g, 3.0 mmol) and DIPEA (2.5 mL, 15.0 mmol) in DMF (6 mL), and the mixture was stirred at room temperature overnight. The reaction was quenched by adding a 2 N HCl solution and extracted with Et_2O; the aqueous layer was basified with saturated $NaHCO_3$ and extracted with Et_2O. The aqueous phase was neutralized (pH ~6.5) and extracted with AcOEt. The organic phase was dried over Na_2SO_4, and evaporated in vacuo to give a residue of 603 mg, which was chromatographed on silica gel (9:1 AcOEt/MeOH as eluent) to give 476 mg (43%) of pure **18** as white solid. Mp = 197–199 °C; IR (KBr) 3649, 3447, 1708, 1434, 1285, 1235, 981, 740; ^{1}H NMR (DMSO-d_6) δ 2.57 (4H, m), 3.64 (2H, s), 3.78 (4H, t, J = 4.6 Hz), 7.48 (1H, t, J = 7.6 Hz), 7.54 (1H, t, J = 7.6 Hz), 7.59 (1H, d, J = 7.6 Hz), 7.70 (1H, t, J = 7.6 Hz), 7.86 (1H, d, J = 7.6 Hz), 7.93 (2H, m), 8.18 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 52.15, 61.14, 113.10, 119.50, 125.29, 128.04, 128.42, 129.37, 129.67, 130.89, 132.43, 133.27, 138.19, 144.59, 148.61, 167.27. Anal. Calcd for $C_{19}H_{19}N_5O_3$: C, 62.46; H, 5.24; N, 19.17. Found: C, 62.61; H, 5.22; N, 19.23.

5.1.13. 3-((4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)methyl)-*N*,*N*-dimethylbenzamide (19)

To a stirring solution of **18** (82 mg, 0.22 mmol) in DMF (2 mL) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (45 mg, 0.23 mmol) and hydroxybenzotriazole (HOBt) (40 mg, 0.23 mmol) were added at 0 °C and the mixture was stirred at 0 °C for 15 min, then at room temperature for 45 min. Dimethylamine (33% in ethanol) (30 μl, 0.22 mmol) was added dropwise and the reaction mixture was stirred at room temperature overnight. The reaction was quenched by adding a saturated $NaHCO_3$ solution and extracted with AcOEt. The organic phase was dried over Na_2SO_4, and evaporated in vacuo to give a residue of 87 mg, which was chromatographed on silica gel (8:2 AcOEt/*n*-hexane as eluent) to afford 65 mg (75%) of pure **19** as oil. IR 3011, 2932, 1701, 1626, 1449, 1433, 999, 787, 697; ^{1}H NMR δ 2.67 (4H, m), 2.99 (3H, s), 3.12 (3H, s), 3.63 (2H, s), 3.94 (4H, m), 7.32–7.47 (5H, m), 7.60 (1H, t, J = 7.7 Hz), 7.98 (1H, d, J = 8.3 Hz), 8.09 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 35.40, 39.62, 42.52, 52.78, 62.34, 113.57, 119.85, 125.25, 126.17, 127.71, 128.48, 129.39, 130.25, 133.19, 136.49, 137.62, 145.33, 149.33, 171.55. Anal. Calcd for $C_{21}H_{24}N_6O_2$: C, 64.27; H, 6.16; N, 21.41. Found: C, 64.36; H, 6.17; N, 21.37.

5.1.14. (3-((4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)methyl)phenyl)(piperidin-1-yl)methanone (20)

The title compound was prepared following the same procedure used for the synthesis of **19**, using piperidine as amine. Chromatography

on silica gel (8:2 AcOEt/*n*-hexane as eluent) afforded pure **20** as oil. Yield = 81%. IR (neat) 2934, 1698, 1626, 1424, 1281, 1228, 996, 747; ^{1}H NMR δ 1.53 (2H, br s), 1.69 (4H, m), 2.65 (4H, m), 3.35 (2H, br s), 3.62 (2H, s), 3.72 (2H, br s), 3.93 (4H, m), 7.30 (1H, m), 7.39 (3H, m), 7.45 (1H, t, J = 7.7 Hz), 7.60 (1H, t, J = 7.7 Hz), 7.99 (1H, d, J = 8.3 Hz), 8.09 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 24.61, 25.61, 26.54, 43.17, 48.86, 52.83, 62.41, 113.59, 119.87, 125.24, 125.88, 127.39, 128.53, 129.38, 130.01, 133.22, 136.75, 137.86, 145.36, 149.37, 170.18. Anal. Calcd for $C_{24}H_{28}N_6O_2$: C, 66.65; H, 6.53; N, 19.43. Found: C, 66.83; H, 6.54; N, 19.48.

5.1.15. (3-((4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)methyl)phenyl)(4-methylpiperazin-1-yl)methanone (21)

The title compound was prepared following the same procedure used for the synthesis of **19**, using 1-methylpiperazine as amine. Chromatography on silica gel (95:5 CH_2Cl_2/MeOH as eluent) afforded pure **21** as oil. Yield = 77%. IR (neat) 2924, 2794, 1698, 1629, 1424, 1288, 997, 747; ^{1}H NMR δ 2.34 (3H, s), 2.38 (2H, br s), 2.51 (2H, br s), 2.64 (4H, m), 3.47 (2H, br s), 3.62 (2H, s), 3.82 (2H, br s), 3.93 (4H, m), 7.31 (1H, m), 7.42 (4H, m), 7.60 (1H, t, J = 7.7 Hz), 7.98 (1H, d, J = 8.3 Hz), 8.08 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 41.97, 45.94, 47.55, 52.80, 55.14, 62.34, 113.55, 119.82, 125.21, 126.01, 127.57, 128.54, 129.35, 130.27, 133.17, 135.95, 138.18, 145.30, 149.31, 170.19. Anal. Calcd for $C_{24}H_{29}N_7O_2$: C, 64.41; H, 6.53; N, 21.91. Found: C, 64.58; H, 6.51; N, 21.87.

5.1.16. 3-((4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)methyl)-*N*-cyclohexylbenzamide (22)

The title compound was prepared following the same procedure used for the synthesis of **19**, using cyclohexylamine as amine. Chromatography on silica gel (7:3 AcOEt/*n*-hexane as eluent) afforded pure **22** as white solid. Yield = 92%. Mp = 158–159 °C; IR (KBr) 3321, 2936, 1722, 1631, 1525, 1435, 1281, 754; ^{1}H NMR δ 1.13–1.27 (4H, m), 1.37 (2H, m), 1.62 (1H, m), 1.72 (2H, m), 2.00 (2H, m), 2.59 (4H, m), 3.57 (2H, s), 3.88 (4H, m), 6.22 (1H, d, J = 7.7 Hz), 7.34 (1H, t, J = 7.6 Hz), 7.43 (2H, m), 7.56 (1H, t, J = 7.6 Hz), 7.63 (1H, d, J = 7.7 Hz), 7.74 (1H, br s), 7.94 (1H, d, J = 8.3 Hz), 8.04 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 24.99, 25.57, 33.19, 48.80, 52.80, 62.40, 113.56, 119.82, 125.23, 125.86, 127.75, 128.54, 129.37, 131.88, 133.18, 135.37, 138.00, 145.31, 149.31, 166.53. Anal. Calcd for $C_{25}H_{30}N_6O_2$: C, 67.24; H, 6.77; N, 18.82. Found: C, 67.33; H, 6.75; N, 18.77.

5.1.17. 3-((4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)methyl)-*N*-phenylbenzamide (23)

The title compound was prepared following the same procedure used for the synthesis of **19**, using aniline as amine. Chromatography on silica gel (6:4 AcOEt/*n*-hexane as eluent) afforded pure **23** as white solid. Yield = 93%. Mp = 65–68 °C; IR (neat) 3308, 2919, 2814, 1696, 1529, 1429, 1320, 1228, 997, 749; ^{1}H NMR δ 2.66 (4H, m), 3.65 (2H, s), 3.93 (4H, m), 7.15 (1H, t, J = 7.4 Hz), 7.36 (2H, t, J = 8.0 Hz), 7.44 (2H, m), 7.52 (1H, d, J = 7.6 Hz), 7.59 (1H, t, J = 7.7 Hz), 7.68 (2H, d, J = 7.8 Hz), 7.79 (1H, d, J = 7.7 Hz), 7.92 (1H, br s), 7.98 (1H, d, J = 8.3 Hz), 8.07 (1H, d, J = 8.3 Hz), 8.12 (1H, br s); ^{13}C NMR δ 52.82, 60.49, 62.38, 113.59, 119.87, 120.29, 124.60, 125.30, 126.21, 127.93, 128.88, 129.08, 129.44, 132.48, 133.21, 135.36, 138.02, 145.35, 149.34, 165.67. Anal. Calcd for $C_{25}H_{24}N_6O_2$: C, 68.17; H, 5.49; N, 19.08. Found: C, 68.25; H, 5.48; N, 19.13.

5.1.18. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(biphenyl-4-ylmethyl) piperazin-1-yl)methanone (24)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(biphenyl-4-ylmethyl)piperazine instead of 1-phenylpiperazine. Chromatography on silica gel (99:1 CH_2Cl_2/MeOH as eluent) afforded pure **24** as white solid. Yield = 72%. Mp = 100–101 °C; IR (KBr) 3427, 2818, 1707, 1430, 1230, 998, 759; ^{1}H NMR δ 2.68 (4H, m), 3.64 (2H, s), 3.95 (4H, m), 7.34 (1H, t, J = 7.2 Hz), 7.43 (5H, m), 7.59 (5H, m), 7.99 (1H, d, J = 8.4 Hz), 8.09 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 52.84, 53.42, 62.49, 113.57, 119.86, 125.22, 127.06, 127.14, 127.27, 128.77, 129.36, 129.57, 133.20, 136.51, 140.35, 140.82, 145.34, 149.36.3. Anal. Calcd for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.67; H, 5.84; N, 17.63.

5.1.19. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(biphenyl-3-ylmethyl) piperazin-1-yl)methanone (25)

The title compound was prepared from 3-(bromomethyl)biphenyl following the same procedure used for the synthesis of **15**. Chromatography on silica gel (8:2 CH_2Cl_2/AcOEt as eluent) afforded pure **25** as oil. Yield = 81%. IR 3009, 2812, 1700, 1449, 1433, 1018, 999, 675; ^{1}H NMR δ 2.63 (4H, m), 3.61 (2H, s), 3.91 (4H, m), 7.29–7.43 (6H, m), 7.48–7.60 (5H, m), 7.97 (1H, d, J = 8.3 Hz), 8.05 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 45.48, 47.55, 52.73, 62.71, 113.52, 119.75, 125.11, 126.11, 127.08, 127.29, 127.79, 127.99, 128.71, 128.76, 129.23, 133.14, 137.95, 140.88, 141.25, 145.24, 149.23. Anal. Calcd for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.77; H, 5.84; N, 17.58.

5.1.20. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(biphenyl-2-ylmethyl) piperazin-1-yl)methanone (26)

The title compound was prepared from 2-(bromomethyl)biphenyl following the same procedure used for the synthesis of **15**. Chromatography on silica gel (75:25 *n*-hexane/AcOEt as eluent) afforded pure **26** as oil. Yield = 84%. IR 3025, 3011, 2816, 1699, 1449, 1433, 1214, 775, 717; ^{1}H NMR δ 2.51 (4H, m), 3.50 (2H, s), 4.10 (4H, m), 7.25–7.42 (9H, m), 7.54 (2H, m), 7.95 (1H, d, J = 8.3 Hz), 8.05 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 47.93, 52.39, 59.52, 113.49, 119.75, 125.11, 126.91, 127.09, 127.20, 127.88, 129.23, 129.32, 129.87, 130.17, 133.12, 134.93, 141.29, 142.79, 145.24, 149.21. Anal. Calcd for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.35; H, 5.82; N, 17.63.

5.1.21. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(naphthalen-2-ylmethyl) piperazin-1-yl)methanone (27)

The title compound was prepared from 2-(bromomethyl)naphthalene following the same procedure used for the synthesis of **15**. Chromatography on silica gel (8:2 CH_2Cl_2/AcOEt as eluent) afforded pure **27** as oil. Yield = 70%. IR 3009, 2817, 1699, 1449, 1433, 1018, 999, 677; ^{1}H NMR δ 2.65 (4H, m), 3.70 (2H, s), 3.92 (4H, m), 7.41–7.51 (4H, m), 7.56 (1H, t, J = 7.7 Hz), 7.73 (1H, s), 7.81 (3H, m), 7.99 (1H, d, J = 8.4 Hz), 8.07 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 45.76, 52.84, 62.84, 113.53, 119.78, 125.13, 125.75, 126.06, 127.17, 127.63, 127.66, 127.73, 128.06, 129.27, 132.83, 133.16, 133.25, 134.99, 145.28, 149.27. Anal. Calcd for $C_{22}H_{21}N_5O$: C, 71.14; H, 5.70; N, 18.85. Found: C, 71.36; H, 5.69; N, 18.82.

5.1.22. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(naphthalen-1-ylmethyl) piperazin-1-yl)methanone (28)

The title compound was prepared from 1-(chloromethyl)naphthalene following the same procedure used for the synthesis of **15**. Chromatography on silica gel (95:5 CH_2Cl_2/AcOEt as eluent) afforded pure **28** as oil. Yield = 27%. IR 3009, 2817, 1700, 1448, 1433, 1231, 997, 677; ^{1}H NMR δ 2.71 (4H, m), 3.90 (4H, m), 4.00 (2H, s), 7.40–7.61 (6H, m), 7.81 (1H, d, J = 8.1 Hz), 7.87 (1H, d, J = 8.3 Hz), 7.98 (1H, d, J = 8.3 Hz), 8.08 (1H, d, J = 8.3 Hz), 8.31 (1H, d, J = 8.1 Hz); ^{13}C NMR δ 42.73, 53.02, 61.01, 113.58, 119.86, 124.66, 125.13, 125.20, 125.77, 125.92, 127.73, 128.39, 128.51, 129.34, 132.50, 133.23, 133.94, 145.36, 149.36. Anal. Calcd for $C_{22}H_{21}N_5O$: C, 71.14; H, 5.70; N, 18.85. Found: C, 70.95; H, 5.70; N, 18.89.

5.1.23. (4-Benzhydrylpiperazin-1-yl)(1*H*-benzo[*d*][1,2,3]triazol-1-yl)methanone (29)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-benzhydrylpiperazine instead of 1-phenylpiperazine. Chromatography on silica gel (9:1 CH_2Cl_2/AcOEt as eluent) afforded pure **29** as white solid. Yield = 65%. Mp = 164–165 °C; IR (KBr) 3418, 2814, 1692, 1426, 1231, 992, 754; ^{1}H NMR δ 2.59 (4H, m), 3.93 (4H, m), 4.33 (1H, s), 7.21 (2H, m), 7.30 (4H, m), 7.43 (5H, m), 7.59 (1H, t, J = 7.6 Hz), 7.97 (1H, d, J = 8.4 Hz), 8.07 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 51.72, 58.93, 75.92, 113.55, 119.85, 125.17, 127.27, 127.87, 128.68, 129.31, 133.17, 141.93, 145.30, 149.30. Anal. Calcd for $C_{24}H_{23}N_5O$: C, 72.52; H, 5.83; N, 17.62. Found: C, 72.38; H, 5.83; N, 17.66.

5.1.24. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(2-fluorophenyl) piperazin-1-yl)methanone (30)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(2-fluorophenyl)piperazine instead of 1-phenylpiperazine. Chromatography on silica gel (98:2 CH_2Cl_2/AcOEt as eluent) afforded pure **30** as solid. Yield = 91%. Mp = 115–116 °C; IR (KBr) 2841, 1698, 1500, 1426, 1239, 1012, 749; ^{1}H NMR δ 3.29 (4H, t, J = 5.0 Hz), 4.11 (4H, m), 6.98–7.12 (4H, m), 7.47 (1H, t, J = 7.6 Hz), 7.62 (1H, t, J = 7.6 Hz), 8.02 (1H, d, J = 8.2 Hz), 8.11 (1H, d, J = 8.2 Hz); ^{13}C NMR δ 50.68, 53.45, 113.63, 116.35 (d, J_{C-F} = 20.5 Hz), 119.37 (d, J_{C-F} = 2.6 Hz), 119.93, 123.42 (d, J_{C-F} = 7.9 Hz), 124.64 (d, J_{C-F} = 3.6 Hz), 125.35, 129.49, 133.24, 139.35 (d, J_{C-F} = 8.6 Hz), 145.40, 149.45, 155.83 (d, J_{C-F} = 244.6 Hz). Anal. Calcd for $C_{17}H_{16}FN_5O$: C, 62.76; H, 4.96; N, 21.53. Found: C, 62.83; H, 4.95; N, 21.47.

5.1.25. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(*o*-tolyl)piperazin-1-yl)methanone (31)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-*o*-tolylpiperazine dihydrochloride instead of 1-phenylpiperazine and TEA (3.6 equiv). Chromatography on silica gel (98:2 CH_2Cl_2/AcOEt as eluent) afforded pure **31** as solid. Yield = 61%. Mp = 136 °C; IR (KBr) 2823, 1695, 1490, 1414, 1225, 1009, 758; ^{1}H NMR δ 2.38 (3H, s), 3.10 (4H, t, J = 4.8 Hz), 4.08 (4H, m), 7.04 (2H, m), 7.21 (3H, m), 7.47 (1H, t, J = 7.4 Hz), 7.62 (1H, t, J = 7.4 Hz), 8.02 (1H, d, J = 8.4 Hz), 8.11 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 17.84, 47.97, 51.85, 113.60, 119.39, 119.91, 124.02, 125.28, 126.78, 129.43, 131.25, 132.80, 133.26, 145.39, 149.55, 150.60. Anal. Calcd for $C_{18}H_{19}N_5O$: C, 67.27; H, 5.96; N, 21.79. Found: C, 67.32; H, 5.96; N, 21.77.

5.1.26. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(2-nitrophenyl) piperazin-1-yl)methanone (32)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(2-nitrophenyl)piperazine[39] instead of 1-phenylpiperazine. Chromatography on silica gel (75:25 *n*-hexane/AcOEt as eluent) afforded pure **32** as yellow solid. Yield = 87%. Mp = 136–138 °C; IR (KBr) 3037, 2852, 2833, 1706, 1526, 1446, 1430, 1364, 1348, 1283, 1227, 1011, 783, 748; ^{1}H NMR δ 3.27 (4H, t, J = 4.7 Hz), 4.08 (4H, m), 7.15 (1H, t, J = 7.9 Hz), 7.23 (1H, d, J = 7.9 Hz), 7.45 (1H, t, J = 7.9 Hz), 7.53 (1H, t, J = 7.9 Hz), 7.61 (1H, t, J = 7.9 Hz), 7.81 (1H, d, J = 7.9 Hz), 8.00 (1H, d, J = 8.3 Hz), 8.09 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 46.20, 51.98, 113.59, 119.86, 121.81, 123.31, 125.32, 125.80, 129.47, 133.18, 133.66, 144.33, 145.33, 145.40, 149.46. Anal. Calcd for $C_{17}H_{16}N_6O_3$: C, 57.95; H, 4.58; N, 23.85. Found: C, 57.75; H, 4.60; N, 23.77.

5.1.27. 4-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-*N*-isopropyl-3-nitrobenzamide (33)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **49a** instead of 1-phenylpiperazine. Chromatography on silica gel (8:2 AcOEt/*n*-hexane as eluent) afforded pure **33** as yellow solid. Yield = 46%. Mp = 209–210 °C; IR (KBr) 3417, 3299, 2969, 1721, 1698, 1626, 1521, 1434, 1220, 1009, 749; ^{1}H NMR δ 1.28 (6H, d, J = 6.4 Hz), 3.36 (4H, t, J = 5.2 Hz), 4.12 (4H, m), 4.30 (1H, m), 5.99 (1H, d, J = 7.6 Hz), 7.20 (1H, d, J = 8.8 Hz), 7.48 (1H, t, J = 7.6 Hz), 7.63 (1H, t, J = 7.6 Hz), 7.97 - 8.03 (2H, m), 8.11 (1H, d, J = 8.4 Hz), 8.23 (1H, d, J = 2.0 Hz); ^{13}C NMR δ 22.82, 42.29, 51.21, 53.44, 113.61, 119.97, 120.71, 124.99, 125.48, 128.38, 129.66, 132.63, 133.15, 141.59, 145.36, 147.42, 149.51, 163.95. Anal. Calcd for $C_{21}H_{23}N_7O_4$: C, 57.66; H, 5.30; N, 22.41. Found: C, 57.76; H, 5.31; N, 22.47.

5.1.28. (4-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-3-nitrophenyl)(morpholino) methanone (34)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **49b** instead of 1-phenylpiperazine. Chromatography on silica gel (9:1 AcOEt/*n*-hexane as eluent) afforded pure **34** as yellow solid. Yield = 86%. Mp = 167–168 °C; IR (KBr) 3416, 2853, 1698, 1631, 1426, 1249, 1113, 1007, 751; ^{1}H NMR δ 3.25–3.44 (6H, m), 3.73 (6H, m), 4.12 (4H, m), 7.23 (1H, d, J = 8.2 Hz), 7.49 (1H, t, J = 7.6 Hz), 7.64 (2H, m), 7.96 (1H, s), 8.02 (1H, d, J = 8.3 Hz), 8.12 (1H, d, J = 8.3 Hz); ^{13}C NMR δ 30.95, 51.38, 53.46, 66.78, 113.62, 119.97, 121.14, 125.48, 125.87, 128.96, 129.66, 132.95, 133.16, 142.17, 145.37, 146.52, 149.50, 167.77. Anal. Calcd for $C_{22}H_{23}N_7O_5$: C, 56.77; H, 4.98; N, 21.06. Found: C, 56.88; H, 4.96; N, 21.03.

5.1.29. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(4-methoxyphenyl) piperazin-1-yl)methanone (35)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(4-methoxyphenyl)piperazine instead of 1-phenylpiperazine. Chromatography on silica gel (7:3 *n*-hexane/AcOEt as eluent) afforded pure **35** as white solid. Yield = 71%. Mp = 131 °C; IR (KBr) 3414, 2928, 2829, 1715, 1513, 1437, 1232, 1013, 755; ^{1}H NMR δ 3.26 (4H, m), 3.78 (3H, s), 4.09 (4H, m), 6.87 (2H, d, J = 9.2 Hz), 6.96 (2H, d, J = 9.2 Hz), 7.47 (1H, t, J = 7.6 Hz), 7.62 (1H, t, J = 7.6 Hz), 8.02 (1H, d, J = 8.4 Hz), 8.11 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 46.39, 51.11, 55.54, 113.62, 114.56, 119.08, 119.91, 125.31, 129.47, 133.21, 145.04, 145.37, 149.39, 154.56. Anal. Calcd for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76. Found: C, 64.28; H, 5.67; N, 20.82.

5.1.30. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(4-(4-nitrophenyl)piperazin-1-yl)methanone (36)

The title compound was prepared following the same procedure used for the synthesis of **8**, using 1-(4-nitrophenyl)piperazine instead of 1-phenylpiperazine. Chromatography on silica gel (98:2 CH_2Cl_2/AcOEt as eluent) afforded pure **36** as yellow solid. Yield = 57%. Mp = 220 °C dec; IR (KBr) 2865, 1682, 1596, 1473, 1301, 1148, 1004, 753; ^{1}H NMR δ 3.67 (4H, t, J = 4.8 Hz), 4.15 (4H, m), 6.89 (2H, d, J = 9.4 Hz), 7.50 (1H, t, J = 7.6 Hz), 7.65 (1H, t, J = 7.6 Hz), 8.04 (1H, d, J = 8.2 Hz), 8.13 (1H, d, J = 8.2 Hz), 8.18 (2H, d, J = 9.4 Hz); ^{13}C NMR δ 46.93, 66.42, 113.13, 113.69, 120.01, 125.57, 125.99, 129.76, 133.13, 139.34, 145.36, 149.49, 154.31. Anal. Calcd for $C_{17}H_{16}N_6O_3$: C, 57.95; H, 4.58; N, 23.85. Found: C, 58.09; H, 4.59; N, 23.80.

5.1.31. 5-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-*N*,*N*-dimethyl-2-nitrobenzamide (37)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **47c** instead of 1-phenylpiperazine. Chromatography on silica gel (1:1 CH_2Cl_2/AcOEt as eluent) afforded pure **37** as yellow solid. Yield = 62%. Mp = 208–210 °C; IR (KBr) 3020, 2932, 1704, 1651, 1574, 1433, 1318, 1241, 1005, 767; ^{1}H NMR δ 2.83 (3H, s), 3.16 (3H, s), 3.69 (4H, t, J = 5.0 Hz),

4.13 (4H, m), 6.70 (1H, d, J = 2.7 Hz), 6.87 (1H, dd, J = 2.7, 9.4 Hz), 7.49 (1H, t, J = 7.7 Hz), 7.64 (1H, t, J = 7.7 Hz), 8.04 (1H, d, J = 8.3 Hz), 8.12 (1H, d, J = 8.3 Hz), 8.17 (1H, d, J = 9.4 Hz); ^{13}C NMR δ 34.87, 38.05, 46.60, 111.33, 113.03, 113.73, 120.02, 125.61, 127.34, 129.79, 133.14, 135.34, 135.93, 145.39, 149.50, 153.95, 168.58. Anal. Calcd for $C_{20}H_{21}N_7O_4$: C, 56.73; H, 5.00; N, 23.16. Found: C, 56.83; H, 5.01; N, 23.14.

5.1.32. (5-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-2-nitrophenyl)(piperidin-1-yl)methanone (38)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **47f** instead of 1-phenylpiperazine. Chromatography on silica gel (7:3 AcOEt/*n*-hexane as eluent) afforded pure **38** as yellow solid. Yield = 73%. Mp = 174–177 °C; IR (KBr) 2917, 2850, 1701, 1637, 1447, 1323, 1245, 1232, 1007, 759; ^{1}H NMR δ 1.45 (1H, m), 1.52 (1H, m), 1.66 (3H, m), 1.78 (1H, m), 3.16 (2H, t, J = 5.5 Hz), 3.67 (4H, m), 3.75 (2H, m), 4.13 (4H, m), 6.67 (1H, d, J = 2.7 Hz), 6.86 (1H, dd, J = 2.7, 9.4 Hz), 7.49 (1H, t, J = 7.5 Hz), 7.64 (1H, t, J = 7.5 Hz), 8.03 (1H, d, J = 8.3 Hz), 8.11 (1H, d, J = 8.3 Hz), 8.15 (1H, d, J = 9.4 Hz); ^{13}C NMR δ 24.50, 25.05, 25.82, 42.68, 46.65, 47.75, 111.25, 113.00, 113.73, 120.01, 125.59, 127.33, 129.77, 133.17, 135.45, 136.02, 145.40, 149.51, 153.92, 166.88. Anal. Calcd for $C_{23}H_{25}N_7O_4$: C, 59.60; H, 5.44; N, 21.15. Found: C, 59.73; H, 5.43; N, 21.19.

5.1.33. (5-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-2-nitrophenyl)(morpholino) methanone (39)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **47b** instead of 1-phenylpiperazine. Chromatography on silica gel (9:1 AcOEt/*n*-hexane as eluent) afforded pure **39** as yellow solid. Yield = 70%. Mp = 213–215 °C; IR (KBr) 2922, 2858, 1703, 1641, 1427, 1259, 1005, 755; ^{1}H NMR δ 3.23 (2H, br s), 3.57–3.83 (8H, m), 3.86–3.97 (2H, m), 4.09–4.15 (4H, m), 6.70 (1H, d, J = 2.7 Hz), 6.89 (1H, dd, J = 2.7, 9.4 Hz), 7.50 (1H, t, J = 7.7 Hz), 7.65 (1H, t, J = 7.7 Hz), 8.04 (1H, d, J = 8.3 Hz), 8.13 (1H, d, J = 8.3 Hz), 8.19 (1H, d, J = 9.4 Hz); ^{13}C NMR δ 42.20, 46.57, 47.00, 66.39, 111.21, 113.18, 113.71, 120.04, 125.65, 127.51, 129.85, 133.13, 134.98, 135.36, 145.43, 149.45, 153.96, 167.29. Anal. Calcd for $C_{22}H_{23}N_7O_5$: C, 56.77; H, 4.98; N, 21.06. Found: C, 56.66; H, 4.99; N, 21.11.

5.1.34. (5-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-2-nitrophenyl)(4-methylpiperazin-1-yl)methanone (40)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **47g** instead of 1-phenylpiperazine. Chromatography on silica gel (95:5 CH_2Cl_2/MeOH as eluent) afforded pure **40** as yellow solid. Yield = 40%. Mp = 99–103 °C; IR (KBr) 3482, 2937, 2797, 1702, 1639, 1598, 1578, 1446, 1319, 1228, 1002, 754; ^{1}H NMR δ 2.31 (5H, m), 2.48 (1H, m), 2.58 (1H, m), 3.23 (2H, br s), 3.70 (5H, m), 3.91 (1H, m), 4.13 (4H, m), 6.67 (1H, d, J = 2.7 Hz), 6.86 (1H, dd, J = 2.7, 9.4 Hz), 7.48 (1H, t, J = 7.7 Hz), 7.63 (1H, t, J = 7.7 Hz), 8.02 (1H, d, J = 8.3 Hz), 8.10 (2H, m); ^{13}C NMR δ 41.64, 45.98, 46.46, 46.58, 54.08, 54.49, 111.10, 113.02, 113.70, 119.91, 125.54, 127.32, 129.70, 133.12, 135.23, 135.39, 145.32, 149.44, 153.88, 166.95. Anal. Calcd for $C_{23}H_{26}N_8O_4$: C, 57.73; H, 5.48; N, 23.42. Found: C, 57.87; H, 5.48; N, 23.47.

5.1.35. 5-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-*N*-isopropyl-2-nitrobenzamide (41)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **47a** instead of 1-phenylpiperazine. Chromatography on silica gel (7:3 AcOEt/*n*-hexane as eluent) afforded pure **41** as yellow solid. Yield = 94%. Mp = 129 °C; IR (KBr) 3333, 2972, 1687, 1651, 1578, 1317, 1271, 1006, 755; ^{1}H NMR δ 1.28 (6H, d, J = 6.4 Hz), 3.67 (4H, m), 4.13 (4H, m), 4.30 (1H, m), 5.64 (1H, d, J = 8.0 Hz), 6.79 (1H, d, J = 2.4 Hz), 6.85 (1H, dd, J = 2.4, 9.2 Hz), 7.50 (1H, t, J = 7.6 Hz), 7.65 (1H, t, J = 7.6 Hz), 8.02–8.13 (3H, m); ^{13}C NMR δ 22.40, 42.31, 46.68, 60.40, 112.70, 113.27, 113.70, 120.01, 125.60, 127.29, 129.79, 133.11, 135.77, 136.09, 145.35, 149.47, 153.51, 166.65. Anal. Calcd for $C_{21}H_{23}N_7O_4$: C, 57.66; H, 5.30; N, 22.41. Found: C, 57.75; H, 5.31; N, 22.39.

5.1.36. 5-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-*N*-cyclohexyl-2-nitrobenzamide (42)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **47e** instead of 1-phenylpiperazine. Chromatography on silica gel (8:2 CH_2Cl_2/AcOEt as eluent) afforded pure **42** as yellow solid. Yield = 43%. Mp = 218–219 °C; IR (KBr) 3269, 2930, 2853, 1702, 1644, 1600, 1579, 1504, 1428, 1323, 1241, 1229, 1005, 752; ^{1}H NMR (DMSO-d_6) δ 0.64 (5H, m), 0.95 (1H, m), 1.09 (2H, m), 1.26 (2H, m), 2.56 (4H, m), 3.08 (4H, m), 3.38 (1H, m), 6.22 (1H, d, J = 2.7 Hz), 6.40 (1H, dd, J = 2.7, 9.4 Hz), 6.91 (1H, t, J = 7.7 Hz), 7.08 (1H, t, J = 7.7 Hz), 7.35 (1H, d, J = 8.3 Hz), 7.39 (1H, d, J = 9.4 Hz), 7.55 (1H, d, J = 8.3 Hz), 7.61 (1H, d, J = 7.9 Hz); ^{13}C NMR δ 24.38, 25.05, 31.75, 45.62, 48.26, 78.27, 78.60, 78.93, 112.00, 112.33, 113.23, 119.33, 125.22, 126.42, 129.26, 132.38, 134.82, 136.37, 144.54, 148.78, 153.03, 165.71. Anal. Calcd for $C_{24}H_{27}N_7O_4$: C, 60.37; H, 5.70; N, 20.53. Found: C, 60.58; H, 5.71; N, 20.59.

5.1.37. 5-(4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl)piperazin-1-yl)-2-nitro-*N*-phenylbenzamide (43)

The title compound was prepared following the same procedure used for the synthesis of **8**, using **47d** instead of 1-phenylpiperazine. Chromatography on silica gel (7:3 $CHCl_3$/AcOEt as eluent) afforded pure **43** as yellow solid. Yield = 56%. Mp = 136–138 °C; IR (KBr) 3278, 2917, 1681, 1598, 1578, 1443, 1318, 1222, 1004, 752; ^{1}H NMR δ 3.48 (4H, m), 3.96 (4H, m), 6.51 (1H, dd, J = 1.9, 9.3 Hz), 6.62 (1H, d, J = 1.9 Hz), 7.04 (1H, t, J = 7.5 Hz), 7.21 (2H, t, J = 7.8 Hz), 7.40 (1H, t, J = 7.5 Hz), 7.54 (3H, m), 7.67 (1H, d, J = 9.3 Hz), 7.91 (1H, d, J = 8.3 Hz), 7.99 (1H, d, J = 8.3 Hz), 9.10 (1H, br s); ^{13}C NMR δ 45.97, 60.40, 111.79, 112.49, 113.62, 119.72, 120.29, 124.58, 125.54, 127.02, 128.92, 129.67, 132.96, 134.50, 135.19, 138.00, 145.11, 149.35, 153.07, 165.94. Anal. Calcd for $C_{24}H_{21}N_7O_4$: C, 61.14; H, 4.49; N, 20.80. Found: C, 61.33; H, 4.50; N, 20.83.

5.1.38. *tert*-Butyl 4-(1*H*-Benzo[*d*][1,2,3]triazole-1-carbonyl) piperazine-1-carboxylate (44)

The title compound was prepared following the same procedure used for the synthesis of **8**, using *tert*-butylpiperazine-1-carboxylate instead of 1-phenylpiperazine. Chromatography on silica gel (8:2 *n*-hexane/AcOEt as eluent) afforded pure **44** as white solid. Yield = 91%. Mp = 105–106 °C; IR (KBr) 2971, 1722, 1697, 1420, 1247, 1169, 998, 748; ^{1}H NMR δ 1.50 (9H, s), 3.65 (4H, t, J = 5.2 Hz), 3.90 (4H, m), 7.47 (1H, t, J = 7.7 Hz), 7.62 (1H, t, J = 7.7 Hz), 8.00 (1H, d, J = 8.4 Hz), 8.10 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 28.40, 80.57, 113.63, 119.96, 125.39, 129.56, 133.20, 145.40, 149.58, 154.54. Anal. Calcd for $C_{16}H_{21}N_5O_3$: C, 57.99; H, 6.39; N, 21.13. Found: C, 57.10; H, 6.39; N, 21.17.

5.1.39. (1*H*-Benzo[*d*][1,2,3]triazol-1-yl)(piperazin-1-yl)methanone 2,2,2-trifluoroacetate (45)

To a solution of **44** (4.50 g, 13.6 mmol) in $CHCl_3$ (5 mL) a solution of 2,2,2-trifluoroacetic acid (10 mL) in $CHCl_3$ (10 mL) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was then evaporated in vacuo and the residue was triturated with Et_2O and filtered to give 4.59 g of pure **45** (98%) as white solid. Mp = 205–207 °C; IR (KBr) 2983, 2480, 1723, 1675, 1440, 1202, 1122, 1002; ^{1}H NMR (CD_3OD) δ 3.47 (4H, t, J = 5.3 Hz),

4.17 (4H, m), 7.55 (1H, t, J = 7.4 Hz), 7.70 (1H, t, J = 7.4 Hz), 8.02 (1H, d, J = 8.4 Hz), 8.11 (1H, d, J = 8.4 Hz); ^{13}C NMR δ 41.70, 44.47, 114.89, 120.66, 127.00, 131.03, 134.40, 146.59, 150.70. Anal. Calcd for $C_{13}H_{14}F_3N_5O_3$: C, 45.22; H, 4.09; N, 20.28. Found: C, 45.16; H, 4.10; N, 20.25.

5.1.39.1. 5-Chloro-*N*-isopropyl-2-nitrobenzamide (46a). A solution of 5-chloro-2-nitrobenzoic acid (1.209 g, 6 mmol) in thionyl chloride (3.9 mL, 54 mmol) was stirred at 80 °C for 2 h. The reaction mixture was then evaporated in vacuo and to the residue, dissolved in CH_2Cl_2 (5 mL), isopropylamine (567 μl, 13.2 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight, and then quenched by adding a 1 N NaOH solution and extracted with AcOEt. The organic phase was washed successively with brine, 2 N HCl, and brine until neutral, dried over Na_2SO_4, and evaporated in vacuo to give a residue of 1.3 g (89%) of **46a** as white solid. Mp = 172 °C; IR (KBr) 3270, 3098, 2978, 1644, 1554, 1526, 1364, 1150, 906, 835; ^{1}H NMR δ 1.26 (6H, d, J = 6.4 Hz), 4.23 (1H, m), 7.44 (1H, d, J = 2.0 Hz), 7.50 (1H, dd, J = 2.0, 8.8 Hz), 8.01 (1H, d, J = 8.8 Hz); ^{13}C NMR δ 22.28, 42.51, 125.99, 128.94, 130.19, 134.76, 140.35, 144.35, 164.29. Anal. Calcd for $C_{10}H_{11}ClN_2O_3$: C, 49.50; H, 4.57; N, 11.54. Found: C, 49.63; H, 4.57; N, 11.52.

5.1.39.2. (5-Chloro-2-nitrophenyl)(morpholino)methanone (46b). The title compound was prepared following the same procedure used for the synthesis of **46a**, using morpholine as amine. The extraction afforded **46b** as white solid. Yield = 92%. Mp = 156–157 °C; IR (KBr) 2860, 1644, 1528, 1342, 1115, 1023, 844; ^{1}H NMR δ 3.24 (2H, m), 3.64 (2H, m), 3.84 (4H, m), 7.39 (1H, d, J = 2.2 Hz), 7.55 (1H, dd, J = 2.2, 8.8 Hz), 8.17 (1H, d, J = 8.8 Hz); ^{13}C NMR δ 42.33, 47.19, 66.23, 66.36, 126.37, 128.15, 130.12, 134.01, 141.40, 143.62, 165.07. Anal. Calcd for $C_{11}H_{11}ClN_2O_4$: C, 48.81; H, 4.10; N, 10.35. Found: C, 48.87; H, 4.11; N, 10.36.

5.1.39.3. 5-Chloro-*N*,*N*-dimethyl-2-nitrobenzamide (46c)[40]. The title compound was prepared following the same procedure used for the synthesis of **46a**, using dimethylamine (33% in ethanol) as amine. Chromatography on silica gel (6:4 *n*-hexane/AcOEt as eluent) afforded pure **46c** as white solid. Yield = 80%. Mp = 96–102 °C; IR (KBr) 3093, 2928, 1640, 1567, 1529, 1402, 1343, 1099, 859; ^{1}H NMR δ 2.86 (3H, s), 3.16 (3H, s), 7.39 (1H, d, J = 2.2 Hz), 7.52 (1H, dd, J = 2.2, 8.8 Hz), 8.16 (1H, d, J = 8.8 Hz); ^{13}C NMR δ 34.94, 38.21, 126.22, 128.27, 129.80, 134.94, 141.27, 143.43, 166.40.

5.1.39.4. 5-Chloro-2-nitro-*N*-phenylbenzamide (46d). The title compound was prepared following the same procedure used for the synthesis of **46a**, using aniline as amine. The extraction afforded **46d** as white solid. Yield = 91%. Mp = 166–168 °C; IR (KBr) 3259, 3058, 1658, 1531, 1443, 1354, 1321, 750. ^{1}H NMR 7.20 (1H, t, J = 7.6 Hz), 7.38 (2H, t, J = 7.6 Hz), 7.55–7.61 (4H, m), 8.09 (1H, d, J = 8.7 Hz); ^{13}C NMR δ 120.57, 125.49, 126.26, 128.87, 129.26, 130.76, 134.44, 137.02, 140.64, 144.56, 166.98. Anal. Calcd for $C_{13}H_9ClN_2O_3$: C, 56.43; H, 3.28; N, 10.13. Found: C, 56.33; H, 3.28; N, 10.15.

5.1.39.5. 5-Chloro-*N*-cyclohexyl-2-nitrobenzamide (46e). The title compound was prepared following the same procedure used for the synthesis of **46a**, using cyclohexylamine as amine. Chromatography on silica gel (AcOEt as eluent) afforded pure **46e** as white solid. Yield = 65%. Mp = 198–200 °C; IR (KBr) 3266, 2937, 2852, 1638, 1562, 1524, 1346, 1085, 839, 749; ^{1}H NMR (DMSO-d_6) δ 1.10–1.35 (5H, m), 1.57 (1H, m), 1.71 (2H, m), 1.85 (2H, m), 3.68 (1H, m), 7.66 (1H, d, J = 2.2 Hz), 7.76 (1H, dd, J = 2.2, 8.7 Hz), 8.08 (1H, d, J = 8.7 Hz), 8.56 (1H, d, J = 7.9 Hz); ^{13}C NMR δ 24.33, 25.07, 31.76, 48.15, 125.98, 128.81, 130.13, 134.58, 138.09, 145.29, 162.99. Anal. Calcd for $C_{13}H_{15}ClN_2O_3$: C, 55.23; H, 5.35; N, 9.91. Found: C, 55.15; H, 5.36; N, 9.89.

5.1.39.6. (5-Chloro-2-nitrophenyl)(piperidin-1-yl)methanone (46f). The title compound was prepared following the same procedure used for the synthesis of **46a**, using piperidine as amine. Chromatography on silica gel (7:3 *n*-hexane/AcOEt as eluent) afforded pure **46f** as white solid. Yield = 71%. Mp = 109–111 °C; IR (KBr) 2935, 2864, 1638, 1527, 1443, 1342, 1273, 1005, 830; ^{1}H NMR δ 1.53 (2H, m), 1.69 (4H, m), 3.20 (2H, t, J = 5.6 Hz), 3.74 (2H, m), 7.38 (1H, d, J = 2.4 Hz), 7.54 (1H, dd, J = 2.4, 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz); ^{13}C NMR δ 24.31, 25.00, 25.70, 42.71, 47.90, 126.26, 127.92, 129.69, 134.90, 140.99, 143.44, 164.62. Anal. Calcd for $C_{12}H_{13}ClN_2O_3$: C, 53.64; H, 4.88; N, 10.43. Found: C, 53.66; H, 4.88; N, 10.40.

5.1.39.7. (5-Chloro-2-nitrophenyl)(4-methylpiperazin-1-yl) methanone (46g). The title compound was prepared following the same procedure used for the synthesis of **46a**, using 1-methylpiperazine as amine. Chromatography on silica gel (7:3 AcOEt/CH_2Cl_2 as eluent) afforded pure **46g** as white solid. Yield = 87%. Mp = 146–151 °C; IR (KBr) 2980, 2806, 1636, 1528, 1346, 1294, 839, 762; ^{1}H NMR δ 2.32 (3H, s), 2.36 (2H, m), 2.54 (2H, m), 3.26 (2H, m), 3.81 (2H, m), 7.39 (1H, d, J = 2.0 Hz), 7.55 (1H, dd, J = 2.0, 8.8 Hz), 8.15 (1H, d, J = 8.8 Hz); ^{13}C NMR δ 41.77, 45.92, 46.72, 54.03, 54.39, 126.28, 128.05, 129.91, 134.35, 141.05, 143.60, 164.73. Anal. Calcd for $C_{12}H_{14}ClN_3O_3$: C, 50.80; H, 4.97; N, 14.81. Found: C, 50.98; H, 4.95; N, 14.77.

5.1.40. *N*-Isopropyl-2-nitro-5-(piperazin-1-yl)benzamide (47a)

A solution of **46a** (500 mg, 2 mmol) and piperazine (4.437 g, 51.5 mmol) in DMF (7 mL) was stirred at 130 °C for 1 h. After cooling to room temperature, the reaction was quenched by adding a 1 N NaOH solution and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and evaporated in vacuo. The residue was chromatographed on silica gel (9:1 CH_2Cl_2/MeOH as eluent) to give 600 mg (99%) of pure **47a** as yellow solid. Mp = 186–189 °C; IR (KBr) 3253, 2967, 1652, 1579, 1327, 1246, 1080, 854; ^{1}H NMR δ 1.26 (6H, d, J = 6.4 Hz), 3.01 (4H, t, J = 5.3 Hz), 3.40 (4H, t, J = 5.3 Hz), 4.28 (1H, m), 6.74 (1H, d, J = 2.8 Hz), 6.79 (1H, dd, J = 2.8, 9.2 Hz), 8.05 (1H, d, J = 9.2 Hz); ^{13}C NMR δ 22.40, 42.24, 45.64, 47.89, 112.23, 112.85, 127.28, 134.76, 136.08, 154.34, 167.06. Anal. Calcd for $C_{14}H_{20}N_4O_3$: C, 57.52; H, 6.90; N, 19.17. Found: C, 57.42; H, 6.92; N, 19.22.

5.1.41. Morpholino(2-nitro-5-(piperazin-1-yl)phenyl)methanone (47b)

The title compound was prepared from **46b** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 CH_2Cl_2/MeOH as eluent) afforded pure **47b** as yellow solid. Yield = 74%. Mp = 160–162 °C; IR (KBr) 3208, 2850, 1634, 1495, 1327, 1248, 1110, 1024; ^{1}H NMR δ 3.00 (4H, m), 3.22 (2H, m), 3.40 (4H, m), 3.60 (2H, m), 3.76 (2H, m), 3.87 (2H, m), 6.62 (1H, d, J = 2.2 Hz), 6.83 (1H, dd, J = 2.2, 9.6 Hz), 8.10 (1H, d, J = 9.6 Hz); ^{13}C NMR δ 42.09, 45.52, 46.94, 47.71, 50.21, 53.57, 66.22, 66.26, 110.53, 112.69, 127.37, 134.08, 134.90, 154.70, 167.66. Anal. Calcd for $C_{15}H_{20}N_4O_4$: C, 56.24; H, 6.29; N, 17.49. Found: C, 56.35; H, 6.31; N, 17.47.

5.1.42. *N*,*N*-Dimethyl-2-nitro-5-(piperazin-1-yl)benzamide (47c)

The title compound was prepared from **46c** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 CH_2Cl_2/MeOH as eluent) afforded pure **47c** as yellow solid. Yield = 96%. Mp = 63–70 °C; IR (KBr) 3405, 2924, 1653, 1612, 1599, 1492, 1329, 1257, 1116; ^{1}H NMR δ 2.82 (3H, s), 3.01 (4H, t, J = 5.3 Hz), 3.15 (3H, s), 3.42 (4H, t, J = 5.3 Hz), 6.64 (1H, d,

J = 2.8 Hz), 6.81 (1H, dd, *J* = 2.8, 9.5 Hz), 8.13 (1H, d, *J* = 9.5 Hz); ^{13}C NMR δ 34.86, 38.03, 45.64, 47.88, 110.92, 112.64, 127.28, 135.86, 154.78, 160.33, 168.99. Anal. Calcd for $C_{13}H_{18}N_4O_3$: C, 56.10; H, 6.52; N, 20.13. Found: C, 56.27; H, 6.54; N, 20.17.

5.1.43. 2-Nitro-*N*-phenyl-5-(piperazin-1-yl)benzamide (47d)

The title compound was prepared from **46d** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 acetone/MeOH as eluent) afforded pure **47d** as yellow solid. Yield = 94%. Mp = 120–123 °C; IR (KBr) 3272, 2952, 2849, 1660, 1597, 1444, 1317, 1251, 1036, 752; ^{1}H NMR (DMSO-d_6) δ 2.80 (4H, t, *J* = 4.9 Hz), 3.42 (4H, t, *J* = 4.9 Hz), 4.56 (1H, br s), 7.00–7.11 (3H, m), 7.34 (2H, m), 7.67 (2H, d, *J* = 7.7 Hz), 8.05 (1H, d, *J* = 9.4 Hz), 10.42 (1H, s); ^{13}C NMR δ 55.73, 68.41, 111.72, 112.41, 119.40, 123.42, 126.81, 128.60, 133.49, 136.13, 139.14, 154.04, 165.12. Anal. Calcd for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found: C, 62.45; H, 5.56; N, 17.22.

5.1.44. *N*-Cyclohexyl-2-nitro-5-(piperazin-1-yl)benzamide (47e)

The title compound was prepared from **46e** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 CH_2Cl_2/MeOH as eluent) afforded pure **47e** as yellow solid. Yield = 99%. Mp = 170–173 °C; IR (KBr) 3271, 2934, 2853, 1635, 1578, 1501, 1327, 1243, 1038, 828; ^{1}H NMR δ 1.18 (3H, m), 1.39 (2H, m), 1.63 (1H, m), 1.73 (2H, m),1.98 (1H, s), 2.08 (2H, m), 3.00 (4H, t, *J* = 5.2 Hz), 3.39 (4H, t, *J* = 5.2 Hz), 3.95 (1H, m), 5.76 (1H, d, *J* = 8.4 Hz), 6.72 (1H, d, *J* = 2.8 Hz), 6.78 (1H, dd, *J* = 2.8, 9.2 Hz), 8.02 (1H, d, *J* = 9.2 Hz); ^{13}C NMR δ 24.86, 25.54, 32.69, 45.59, 47.85, 48.92, 112.27, 112.78, 127.22, 134.78, 136.14, 154.34, 167.04. Anal. Calcd for $C_{17}H_{24}N_4O_3$: C, 61.43; H, 7.28; N, 16.86. Found: C, 61.55; H, 7.27; N, 16.88.

5.1.45. *N*-(2-Nitro-5-(piperazin-1-yl)benzoyl)piperidine (47f)

The title compound was prepared from **46f** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 $CHCl_3$/MeOH as eluent) afforded pure **47f** as yellow solid. Yield = 92%. Mp = 67–69 °C; IR (KBr) 3446, 2937, 2852, 1634, 1597, 1577, 1492, 1446, 1322, 1244, 1040, 780; ^{1}H NMR δ 1.48 (2H, m), 1.65 (3H, m), 1.75 (1H, m), 2.18 (1H, br s), 2.98 (4H, t, *J* = 4.9 Hz), 3.16 (2H, m), 3.40 (4H, t, *J* = 4.9 Hz), 3.74 (2H, m), 6.61 (1H, d, *J* = 2.6 Hz), 6.84 (1H, dd, *J* = 2.6, 9.5 Hz), 8.09 (1H, d, *J* = 9.5 Hz); ^{13}C NMR δ 24.41, 24.99, 25.65, 42.44, 45.50, 47.65, 47.72, 110.37, 112.44, 127.20, 133.89, 135.85, 154.64, 167.17. Anal. Calcd for $C_{16}H_{22}N_4O_3$: C, 60.36; H, 6.97; N, 17.60. Found: C, 60.57; H, 6.95; N, 17.58.

5.1.46. 1-(2-Nitro-5-(piperazin-1-yl)benzoyl)-4-methylpiperazine (47g)

The title compound was prepared from **46g** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 CH_2Cl_2/MeOH as eluent) afforded pure **47g** as oil. Yield = 82%. IR 3690, 3011, 2947, 1634, 1581, 1445, 1328, 1246, 1237, 772; ^{1}H NMR δ 2.32 (5H, m), 2.50 (1H, m), 2.58 (1H, m), 3.01 (4H, t, *J* = 5.1 Hz), 3.23 (2H, t, *J* = 5.1 Hz), 3.40 (4H, t, *J* = 5.1 Hz), 3.70 (1H, m), 3.97 (1H, m), 6.61 (1H, d, *J* = 2.8 Hz), 6.81 (1H, dd, *J* = 2.8, 9.4 Hz), 8.12 (1H, d, *J* = 9.4 Hz); ^{13}C NMR δ 41.67, 45.63, 46.02, 46.62, 47.91, 54.13, 54.54, 110.89, 112.72, 127.34, 134.51, 135.38, 154.74, 167.36. Anal. Calcd for $C_{16}H_{23}N_5O_3$: C, 57.64; H, 6.95; N, 21.01. Found: C, 57.83; H, 6.96; N, 20.99.

5.1.47. 4-Chloro-*N*-isopropyl-3-nitrobenzamide (48a)

The title compound was prepared following the same procedure used for the synthesis of **46a**, using 4-chloro-3-nitrobenzoic acid as starting material. The extraction afforded **48a** as white solid. Yield = 89%. Mp = 115–116 °C; IR (KBr) 3286, 2978, 1638, 1531, 1340, 1051, 851; ^{1}H NMR δ 1.28 (6H, d, *J* = 6.8 Hz), 4.25 (1H, m), 7.59 (1H, d, *J* = 8.0 Hz), 7.96 (1H, d, *J* = 8.0 Hz), 8.28 (1H, br s); ^{13}C NMR δ 22.52, 42.58, 124.15, 129.75, 131.72, 132.11, 134.70, 147.49, 163.51. Anal. Calcd for $C_{10}H_{11}ClN_2O_3$: C, 49.50; H, 4.57; N, 11.54. Found: C, 49.67; H, 4.58; N, 11.56.

5.1.48. (4-Chloro-3-nitrophenyl)(morpholino)methanone (48b)

The title compound was prepared following the same procedure used for the synthesis of **46a**, using 4-chloro-3-nitrobenzoic acid as starting material and morpholine as amine. The extraction afforded **48b** as white solid. Yield = 55%. Mp = 145–146 °C; IR (KBr) 3071, 2863, 1634, 1524, 1436, 1258, 1108, 1023, 835; ^{1}H NMR δ 3.47 (2H, br s), 3.75 (6H, m), 7.58 (1H, dd, *J* = 2.0, 8.4 Hz), 7.64 (1H, d, *J* = 8.4 Hz), 7.95 (1H, d, *J* = 2.0 Hz); ^{13}C NMR δ 42.86, 66.69, 124.63, 128.75, 131.78, 132.35, 135.01, 147.83, 166.73. Anal. Calcd for $C_{11}H_{11}ClN_2O_4$: C, 48.81; H, 4.10; N, 10.35. Found: C, 48.69; H, 4.11; N, 10.33.

5.1.49. *N*-Isopropyl-3-nitro-4-(piperazin-1-yl)benzamide (49a)

The title compound was prepared from **48a** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 CH_2Cl_2/MeOH as eluent) afforded pure **49a** as yellow solid. Yield = 96%. Mp = 60 °C; IR (KBr) 3287, 2973, 1615, 1522, 1235, 1128, 823; ^{1}H NMR δ 1.27 (6H, d, *J* = 6.8 Hz), 3.02 (4H, m), 3.12 (4H, m), 4.27 (1H, m), 7.11 (1H, d, *J* = 8.8 Hz), 7.92 (1H, dd, *J* = 2.0, 8.8 Hz), 8.16 (1H, d, *J* = 2.0 Hz); ^{13}C NMR δ 22.82, 42.14, 45.73, 52.19, 120.05, 125.02, 126.52, 132.46, 140.52, 148.13, 164.22. Anal. Calcd for $C_{14}H_{20}N_4O_3$: C, 57.52; H, 6.90; N, 19.17. Found: C, 57.37; H, 6.92; N, 19.12.

5.1.50. Morpholino(3-nitro-4-(piperazin-1-yl)phenyl)methanone (49b)

The title compound was prepared from **48b** following the same procedure used for the synthesis of **47a**. Chromatography on silica gel (9:1 CH_2Cl_2/MeOH as eluent) afforded pure **49b** as yellow solid. Yield = 73%. Mp = 196-198 °C; IR (KBr) 3337, 2838, 1619, 1528, 1437, 1330, 1113, 1026, 831; ^{1}H NMR δ 3.09 (4H, m), 3.16 (4H, m), 3.65 (4H, m), 3.71 (4H, m), 7.15 (1H, d, *J* = 8.8 Hz), 7.57 (1H, dd, *J* = 1.6, 8.8 Hz), 7.89 (1H, d, *J* = 1.6 Hz); ^{13}C NMR δ 45.44, 51.79, 66.80, 120.64, 125.96, 127.58, 132.82, 141.39, 147.10, 168.02. Anal. Calcd for $C_{15}H_{20}N_4O_4$: C, 56.24; H, 6.29; N, 17.49. Found: C, 56.38; H, 6.31; N, 17.48.

5.2. Biological evaluation

5.2.1. MAGL esterase activity assay

MAGL activity was measured by following [^{3}H]-2-oleoyl glycerol ([^{3}H]-2-OG) hydrolysis, as previously described.[41] Briefly, 6 ng of pure human MAGL[42] was preincubated during 30 min at 22 °C in the presence of the inhibitor at various concentrations (Tris buffer 50 mM, BSA 0.15% w/v, pH 8.0, inhibitor dissolved in 10 μl DMSO). 2-OG (10 μM final concentration; [^{3}H]-2-OG 50,000 dpm, American Radiolabeled Chemicals; 200 μl total volume assay) was then added and the tubes were placed at 37 °C for 10 min. The reaction was stopped by adding 400 μl of a cold methanol-chloroform (1-1) mixture and, after 5 min centrifugation at 700 g, the radioactivity was measured in the upper aqueous phase by liquid scintillation. The dpm value obtained for a blank (containing no enzyme) was systematically subtracted. Results were then expressed as percent of control activity (without inhibitor), after which Graph Pad prism software was used to treat the data and to analyze the dose-response curves. Inhibitors potency was expressed as IC_{50} values. JZL184 was purchased from Cayman Chemical.

5.2.2. MAGL rapid dilution assay

The reversibility of MAGL inhibition by compound **29** was investigated using the rapid dilution assay. The enzyme (800 ng in 38 μl of a buffer containing Tris 50 mM, BSA 0.15%, pH 8.0) was incubated during 30 min at room temperature with 2 μL of compound **29** (concentration of 10^{-8}M and 10^{-9}M in the mixture) dissolved in DMSO. The enzyme-inhibitor mixture was then diluted 300-fold with the buffer. After 15 min of incubation, the enzyme activity was measured on a 150 μl aliquot (corresponding to 10 ng of enzyme) according to the above-described standard procedure.

5.2.3. Measurement of k_{inact} and K_i values

Inactivation constants for MAGL inactivation by compound **29** (**1**) were measured using *p*-nitrophenylpropionate as substrate. In a 96-well plate, MAGL (3 ng in 180 μl of a buffer containing Tris 50 mM, LDAO 0.1%, pH 8.0) was added to a mixture of *p*-nitrophenylpropionate (1.25 mM) and various concentrations of the inhibitor, both dissolved in 10 μl DMSO, after which the production of *p*-nitrophenol was followed by measuring the absorbance at 405 nM on a SpectraMax II^e spectrophotometer. Blank values (without enzyme) were subtracted to the optical density and the time course of MAGL velocity was calculated using the first derivative of the optical density *vs* time curve in the GraphPad Prism software. Then, the residual enzyme activity (expressed as percent versus a control without inhibitor) as a function of time was fitted to a first order decay equation to obtain the rate of inhibition (k_{obs}, which is the first-order constant for enzyme inactivation) at each inhibitor concentration. The inactivation rate (k_{inact}) and inhibitor dissociation constant (K_i) were then obtained by linear regression using $1/k_{obs}$ and 1/[I] values according to equations (**2**) and (**3**).

$$E + I \overset{K_i}{\leftrightarrow} EI \overset{k_{inact}}{\leftrightarrow} EI^* \quad (1)$$

$$1/k_{obs} = K_i/k^*_{inact}(1 + [S]/KM)^*1/[I] + 1/k_{inact} \quad (2)$$

$$k_{inact}/K_i = (1 + [S]/KM)/slope \quad (3)$$

5.2.4. FAAH anandamidase activity assay

FAAH activity was measured by following [^{3}H]-anandamide hydrolysis ([^{3}H]-AEA), as previously described.[43] Briefly, 4 μg of a preparation of human FAAH fused to the maltose-binding protein was preincubated during 30 min at 22 °C in the presence of the inhibitor at various concentrations (Tris buffer 10 mM, EDTA 1 mM, pH 8.0, inhibitor dissolved in 10 μl DMSO). The substrate (AEA, 2 μM final concentration; [^{3}H]-AEA 50,000 dpm, American radiolabeled Chemicals; 200 μl total volume assay) was then added and the tubes were placed at 37 °C for 10 min. The reaction was then stopped by adding 400 μl of a cold methanol/chloroform (1:1) mixture and, after 5 min centrifugation at 700 g, the radioactivity was measured in the upper aqueous phase by liquid scintillation. The dpm value obtained for a blank (containing no enzyme) was systematically subtracted. Results were then expressed as percent of control activity (without inhibitor), after which Graph Pad prism software was used to treat the data and to analyze the dose-response curves. Inhibitors potency was expressed as IC_{50} values. JZL184 was purchased from Cayman Chemical.

5.3. Molecular modeling

The binding mode of the inhibitors was investigated using Hyperchem. The compounds were covalently linked, as the result of a carbamoylation reaction, to the nucleophilic serine of rat humanized FAAH[37] (Ser241, pdb entry 3LJ6) and human MAGL[34] (Ser122, pdb entry 3JWE), after removing the coordinates of the originally bound inhibitor. Following the manual positioning of the piperazinyl moiety in order to match the conformation of PF3845 (for the FAAH) or SAR629 (for the MAGL), the inhibitors as well as key surrounding residues were minimized using the MM+ force field. For the study of FAAH-inhibitors complexes, both the open and closed rotamers that were described[37] for the Phe432 were considered. Figures were prepared using Pymol.

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