Bioorganic & Medicinal Chemistry Letters 21 (2011) 6782–6787

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The design, synthesis and biological evaluation of novel URB602 analogues as potential monoacylglycerol lipase inhibitors

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ARTICLE INFO

Article history:
Received 8 August 2011
Revised 9 September 2011
Accepted 12 September 2011
Available online 18 September 2011

Keywords:
Endocannabinoid system
2-Arachidonylglycerol
Monoacylglycerol lipase
URB602
Structure–activity relationship

ABSTRACT

We have synthesised an extensive series of URB602 analogues as inhibitors of monoacylglycerol lipase (MAGL), which is the major enzyme responsible for metabolising the endocannabinoid 2-arachidonylglycerol. The recently identified crystal structure of MAGL was used in the design strategy and revealed three possible binding sites for URB602 and the proposed analogues. A test series of carbamate analogues were docked into the identified sites to predict the most favourable binding location. The synthesised analogues of URB602 explored the biological effects of isosteric replacement, ring size and substitution, *para* substitution of the biphenyl moiety and the incorporation of a bicyclic element. The compounds were tested for their ability to inhibit human MAGL. The carbamate analogue **16** displayed the most significant inhibitory activity, reducing MAGL activity to 26% of controls at 100 μM compared to 73% for the parent compound URB602.

The endocannabinoid system (ECS) is a newly discovered physiological drug target composed of receptors, endogenous ligands, enzymes and accessory proteins. The ECS consists of two main receptors, namely the CB_1 receptor, which is predominately expressed on nerve terminals and modulates the inhibition of neurotransmitter release, and the CB_2 receptor, which is mainly associated with the modulation of cytokine release and migration of immune cells.[1]

The two main endogenous ligands of the ECS are 2-arachidonylglycerol (2-AG, **1**) and anandamide (AEA, **2**) (Fig. 1), which act as agonists at CB_1 and CB_2 receptors.[2] Unlike AEA, 2-AG is a full agonist at these receptors.[3] Monoacylglycerol lipase (MAGL) is the primary enzyme involved in the hydrolysis of 2-AG in the brain, whereas AEA is predominately hydrolysed by fatty acid amide hydrolase (FAAH).[4,5] Research into 2-AG signalling has revealed its role in inflammation, nociception and immune reactions.[1,6] Additionally, MAGL inhibitors have been found to increase 2-AG levels in the brain and spinal cord and also cause weakened mechanical and cold allodynia via CB_1 receptors, confirming their ability to reduce neuropathic pain.[7,8] Therefore MAGL inhibitors provide an opportunity to investigate the role of 2-AG in various cascades and complex pathways.

Selective inhibition of MAGL results in an increase of 2-AG concentration at ECS receptors. Thus, MAGL inhibition avoids the need for direct activation of CB_1 receptors, which is advantageous since it may result in less psychoactive (and other unwanted) side effects.[9] The catalytic triad of MAGL was identified by site-directed mutagenesis and is found in the active site (Ser122, Asp239 and His269).[10] Potent inhibitors of MAGL, such as JZL184[11] (IC_{50} = 8 nM; **3**) and Ly2183240[12] (IC_{50} = 20 nM; **4**) (Fig. 1), act via this catalytic triad to form a covalent bond with the serine residue. However, both these inhibitors lack selectivity.[13,14] Another inhibitor, SAR629, (**5**), (Fig. 1) has been co-crystallised with MAGL and appeared outside of the active site suggesting an alternative mechanism of action.[15]

The *N*-aryl carbamate URB602[16] (**15c**, Fig. 1) was discovered to be a selective inhibitor of MAGL[17] since it increases levels of 2-AG without altering levels of AEA in both in vitro and in vivo models. URB602 acts via a non-competitive mechanism, in which the activity can only partially be reversed; hence URB602 is also termed a partially reversible inhibitor.[18,19] Additionally, URB602 does not affect the levels of other lipid metabolising enzymes in the ECS.[18] The selectivity of URB602 makes it a suitable scaffold for designing MAGL inhibitors and an important pharmacological tool for understanding the effects of 2-AG.

In this Letter, we have explored a variety of structural modifications of URB602 with the aim of producing a more potent inhibitor. These structural modifications included isosteric replacement, ring size and substitution, *para* substitution of the biphenyl moiety and the incorporation of a bicyclic element (Fig. 2).

To aid in the design and synthesis of URB602 analogues, we docked the target carbamates and literature compounds into the crystal structure of MAGL[15] using the molecular modelling package Glide.[20,21] A total of 26 ligands, including literature compounds and multiple carbamate analogues were docked (Supplementary data).

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doi:10.1016/j.bmcl.2011.09.038

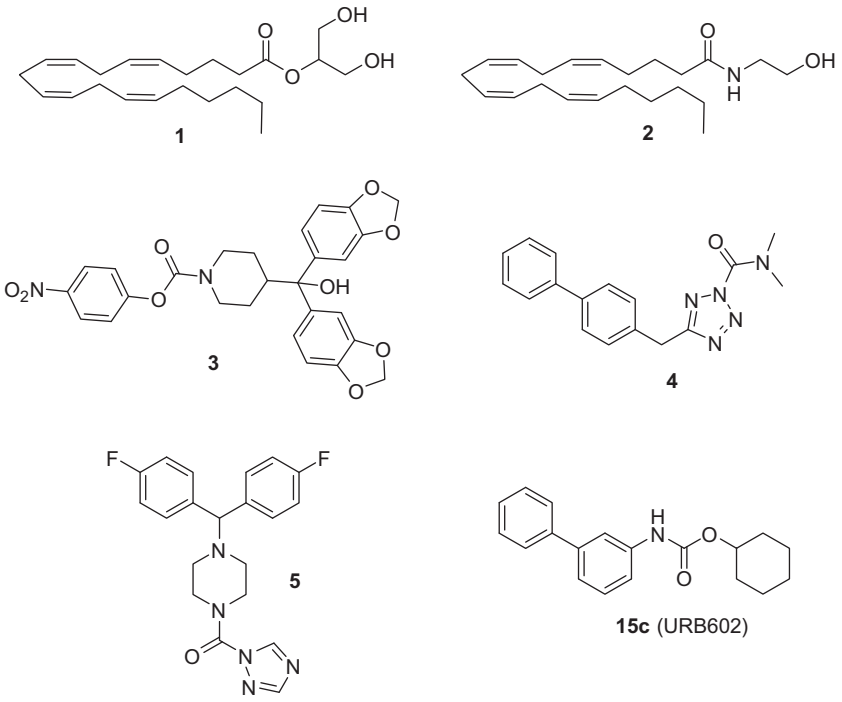


Figure 1. Chemical structures of the endocannabinoid ligands 2-AG (**1**), AEA (**2**), the potent covalent inhibitors of MAGL JZL184 (**3**), Ly2183240 (**4**), SAR629 (**5**) and the selective non-covalent inhibitor URB602 (**15c**).

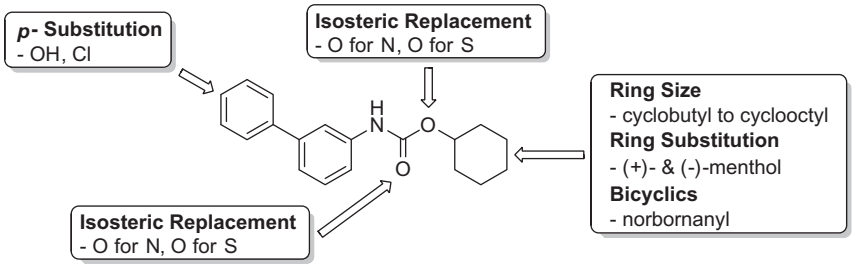


Figure 2. Structural modifications to URB602.

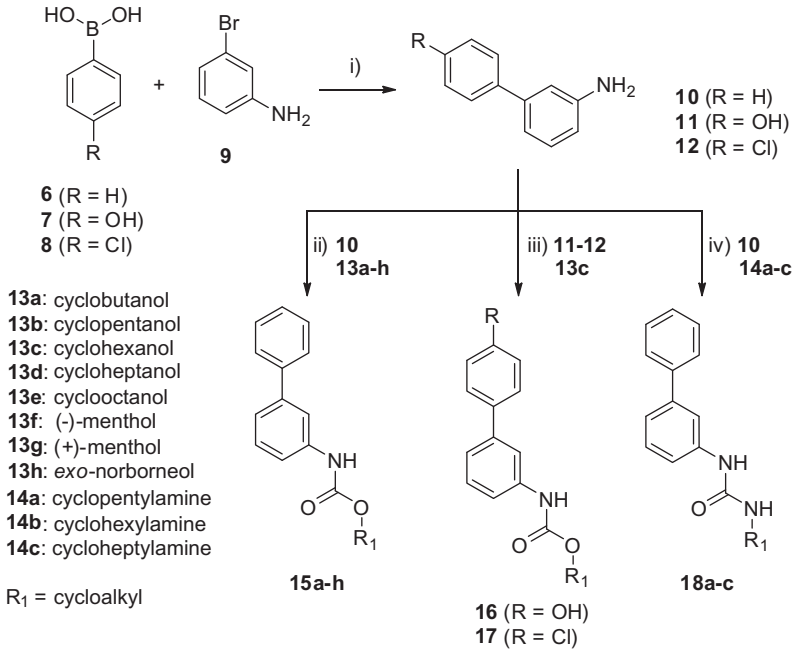
Several of the carbamate compounds docked were synthesised, as depicted in Scheme 1, whilst all other functional classes (urea, thiourea, thiocarbamate and guanidine) were chosen for synthesis for comparative purposes. The docking was performed to determine (i) how and where the compounds may be binding to MAGL and (ii) which compounds may be better inhibitors.

Prior to docking, SiteMap[22,23] was used to identify possible sites/cavities for docking. The top scoring site was a region in between the two monomers, termed the 'dimer site' (Fig. 3a). The second and third top scoring sites were the active site and the cavity in close proximity to the active site (termed 'upper active site') on each of the separate monomers (Fig. 3b). The residues Cys201 and Cys208, found in the upper active site, have been identified as important for MAGL function.[15] King et al. also recognised this hydrophobic pocket as a potential site for reversible inhibition of MAGL.[24] The site equivalent to the dimer site could not be identified by SiteMap when using a single monomer. This suggested that the site may only be operational when the enzyme is in its dimer form. Docking to this site revealed that only six ligands were able to be accommodated by this cavity, suggesting that 'breathing' between the two monomers may be necessary for binding to occur at this site.

Covalent and non-covalent docking strategies were explored, to each of the active site, upper active site and dimer site regions. Our results indicated that the most suitable binding site for URB602 carbamate analogues was the upper active site cavity (via a non-covalent mechanism). The results showed that the *p*-OH substituted carbamate **16** produced the best Glide GScore value. A Glide GScore of −6.643 was obtained for URB602, compared with −7.425 obtained for **16**.

In the crystal structure of MAGL in complex with URB602, a hydrogen bond exists between the amide hydrogen of Asn162 and the oxygen atom adjacent to the carbonyl group of URB602. The hydrogen bond with Asn162 was able to be replicated by the majority of ligands in the compound library, as indicated by molecular docking. The molecular docking experiments also suggested that the ligands were likely to form hydrogen bonds with Ser165. We expected that the interactions between the ligands in the compound library and the enzyme would predominantly be hydrophobic, since the ligands are moderately lipophilic and the upper active site cavity sits near the hydrophobic lid of MAGL. Residues Ala174, Gly220 and Leu186 were consistently contacted by the majority of compounds and are therefore likely to be the key residues involved in the non-covalent binding of compounds to this site.

Docking to the upper active site suggested that 5- to 7-membered rings were the most preferable (Supplementary data). Therefore, we decided to limit our synthetic efforts to only these ring



Scheme 1. Synthesis of carbamate and urea analogues of URB602. Reagents and conditions: (i) $Pd(OAc)_2$ or $Pd(PPh_3)_4$, Na_2CO_3, methanol, reflux; (ii) CDI, DMAP, reflux 14 h followed by cycloalkanol (**13a**–**h**), CH_3CN, reflux, 24 h; (iii) CDI, DMAP, reflux, 14 h followed by cyclohexanol (**13c**), CH_3CN, reflux, 24 h; (iv) CDI, TEA, reflux, 2 h, followed by cycloalkylamine (**14a**–**c**), CH_3CN, reflux, 4 h.

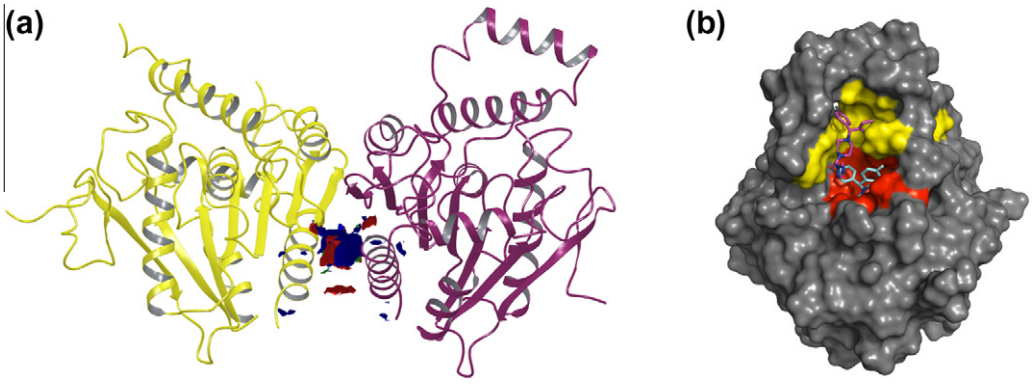
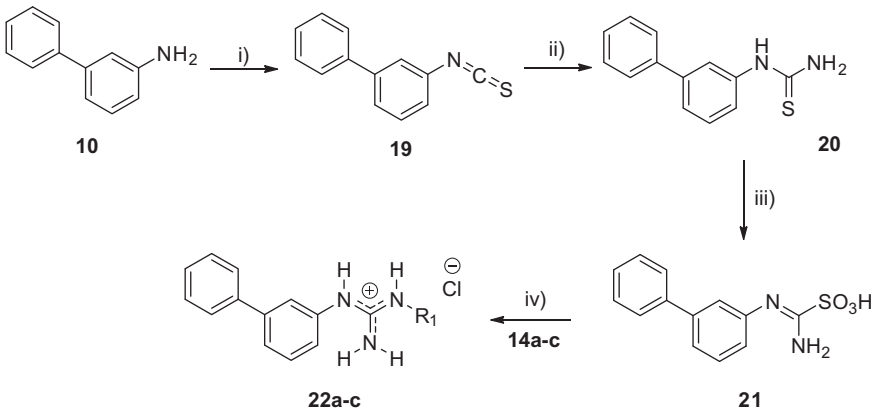


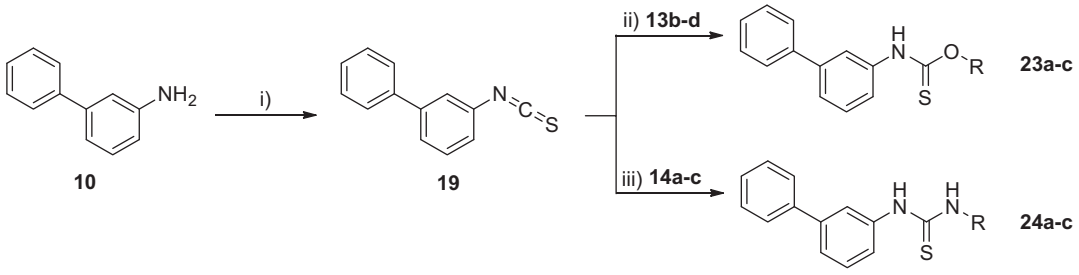
Figure 3. (a) Ribbon representation of MAGL. Dimer site indicated by Sitemap as the region between the two monomers (coloured red, blue and green). (b) Monomer B of MAGL, with the upper active site indicated in yellow, with SAR629 non-covalently bound and the active site indicated in red, with SAR629 covalently bound to the enzyme.

sizes in making the urea, guanidine, thiocarbamate and thiourea analogues of URB602. In addition to compound **16**, we also decided to synthesise the *p*-Cl substituted carbamate **17** for comparison of the docking results with pharmacological data, as both compounds docked in a very similar conformation and orientation (Fig. 1, Supplementary data). However, **17** gave a lower Glide GScore (−6.563) when compared with URB602 and **16**.

We envisaged the use of the Suzuki coupling reaction as integral to the synthesis of the target compounds via the key biphenylamine intermediates (**10**–**12**), involving phenylboronic acids **6**–**8**, 3-bromoaniline **9** and a suitable palladium catalyst (Scheme 1). These intermediates were isolated in good yield (50–76%). Compounds **10**–**12** were reacted with carbonyldiimidazole (CDI) under basic conditions to generate an activated carbamoyl imidazole intermediate, upon which treatment with the designated cycloalkanol (**13a**–**h**) afforded the target carbamates (**15a**–**h**, **16**, **17**). The urea analogues (**18a**–**c**) were synthesised by reacting **10** with CDI and TEA followed by the addition of the appropriate cycloalkylamine (**14a**–**c**). The synthesis of the guanidinum analogues proceeded as shown in Scheme 2 and used thiocarbonyldiimidazole (TCDI) to generate the thioisocyanate intermediate **19**. The formation of this intermediate was supported by ^{13}C NMR spectroscopy, with the presence of a carbon resonance at δ 135.7 ppm corresponding to the isothiocyanate functional group. Methanolic ammonia was then added to furnish the thiourea product **20**. Formation of the sulfonic acid derivative **21** was achieved by an oxidation reaction with hydrogen peroxide in the presence of a molybdenum salt catalyst. The product was confirmed by LC–MS exhibiting a peak at $m/z = 277$ and then reacted, without further purification, to generate the guanidine analogues. These compounds were converted to their corresponding salts to yield the target guanidinium analogues **22a**–**c**. The synthesis of the target thiocarbamates proceeded as described in Scheme 3, in which the initial step involved the isolation of the key isothiocyanate **19**. The desired cycloalkanols (**13b**–**d**) were added to give the corresponding thiocarbamates **23a**–**c**. Similarly, the thiourea analogues (**24a**–**c**) were furnished via the reaction of **19** with the appropriate cycloalkylamine (**14a**–**c**) in good yields (Scheme 3).



Scheme 2. Synthesis of guanidinium analogues of URB602. Reagents and conditions: (i) TCDI, DMAP, rt, 1 h, CH_2Cl_2; (ii) methanolic ammonia (iii) H_2O_2 (30% v/v), NaCl, $Na_2MoO_4{\cdot}2H_2O$, 0 °C → rt, H_2O; (iv) (a) cycloalkylamine (**14a**–**c**), rt, 24 h, CH_3CN; (b) 1 M HCl.



Scheme 3. Synthesis of thiocarbamate and thiourea analogues. Reagents and conditions: (i) TCDI, DMAP, rt, 1 h, DCM; (ii) cycloalkanol (**13b**–**d**), DMAP, 120 °C, 24 h, DMF; (iii) cycloalkylamine (**14a**–**c**), DMAP, rt, 2 h, CH_3CN.

Compounds were tested for their ability to inhibit human recombinant MAGL in serial dilutions (1:10) from 100 μM to 1 nM (six concentrations in triplicate) using methanol as the solvent. We have quoted the % MAGL activity (Table 1) at three concentrations (1 μM , 10 μM and 100 μM). The activity data for a handful of compounds at the highest concentration of 100 μM were not determined due to the emergence of solubility issues. A similar method was employed by Muccioli et al.[25] to describe the activity of URB602 and other carbamate inhibitors of MAGL. Importantly, the results for URB602 (e.g., 71 ± 4% MAGL activity at 10 μM) were comparable to that reported previously (Muccioli et al.[25] 84% MAGL activity 10 μM). As documented in the literature,[19] it is also significant to note that complete inhibition of MAGL was not observed; a feature possibly resulting from the target compounds sharing the scaffold of URB602, a partially reversible inhibitor. Not all synthesised compounds are shown in Table 1 due to un-obtainable or inconclusive results.

To evaluate the biological effects of the isosteric replacements, we compared the profiles of all the analogues containing the described functional groups and a cyclohexyl side chain with that of URB602. The guanidine analogue (**22b**) produced little to no inhibition. Furthermore this result suggests that MAGL does not accommodate a positively charged species at its binding site. Future analogues should presumably be designed to remain neutral at physiological pH to retain activity. The thiocarbamate (**23b**) and thiourea (**24b**) functional groups showed minimal inhibition at 1 and 10 μM (Table 1). The urea analogue (**18b**; previously synthesised by King et al.[19]) showed a comparable % inhibition of MAGL activity to URB602.

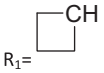
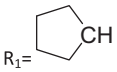
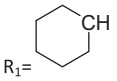
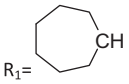
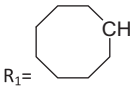
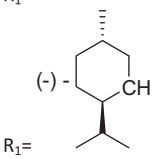
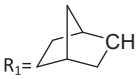
There was no clear relationship evident between ring sizes 5–7 and pharmacological activity. However, ring sizes greater than seven, such as compound **15e** (R_1 = cyclooctyl), had a much weaker inhibitory effect, as was the case with ring substituted analogues (**15f**), suggesting that bulkier groups may impede activity due to steric hindrance. The introduction of a bicyclic moiety (**15h**) also seemed to offer no beneficial effects towards decreasing MAGL activity.

From the library of compounds synthesised representing the disparate functional classes, the most effective inhibitor of MAGL was clearly the *p*-OH substituted carbamate **16** significantly reducing MAGL activity to 83%, 46% and 26% of control values at 1, 10 and 100 μM, respectively. These values were notably enhanced compared to the parent compound URB602 (93%, 71% and 73% of control values at 1, 10 and 100 μM, respectively). The cycloheptyl urea analogue **18c**, although not noteworthy at 1 and 10 μM, produced a signficant reduction of MAGL activity to 55% of controls at 100 μM. The *p*-Cl substituted carbamate **17** reduced MAGL activity to 85% and 81% of control values at 1 and 10 μM, respectively, which was consistent with molecular modelling predictions. Figure 4 illustrates the pronounced inhibitory activity of compounds **16** and **18c** against MAGL compared to the parent compound, URB602 (**15c**) across all concentrations.

Figure 5 gives a detailed view of the docked carbamate **16** interacting with key residues in the upper active site. Significantly, the modelling suggests that it makes a hydrogen bond between its *p*-hydroxyl group and Ser185. This compound was the only ligand to make this interaction, as neither URB602 nor SAR629 interacts with Ser185, which could explain its enhanced pharmacological activity. The design of 'next generation' ligands as MAGL inhibitors may possibly include the use of this hydrogen bond interaction as a starting point.

In summary, molecular modelling identified the upper active site as the most favourable binding site for carbamate analogues. Due to the close proximity of the active site and upper active site

Table 1
Selected synthesised compounds and their inhibitory effects against human recombinant MAGL

			MAGL activity (% of control ± SEM)		
			1 μM	10 μM	100 μM
15a		X = O, Z = O, R = H	97 ± 4	86 ± 4	85 ± 2
15b		X = O, Z = O, R = H	93 ± 5	71 ± 9	n.d.[a]
18a		X = O, Z = NH, R = H	80 ± 5	73 ± 4	n.d.
15c (URB602)		X = O, Z = O, R = H	93 ± 6	71 ± 4	73 ± 1
16		X = O, Z = O, R = OH	83 ± 1	46 ± 2	26 ± 2
17		X = O, Z = O, R = Cl	85 ± 2	81 ± 1	81 ± 4
18b		X = O, Z = NH, R = H	98 ± 6	79 ± 3	78 ± 8
23b		X = S, Z = O, R = H	73 ± 6	83 ± 3	n.d.
24b		X = S, Z = NH, R = H	93 ± 2	76 ± 3	n.d.
15d		X = O, Z = O, R = H	94 ± 2	71 ± 6	n.d.
18c		X = O, Z = NH, R = H	96 ± 1	80 ± 4	55 ± 5
23c		X = S, Z = O, R = H	79 ± 4	83 ± 5	n.d.
15e		X = O, Z = O, R = H	101 ± 1	92 ± 2	n.d.
15f		X = O, Z = O, R = H	106 ± 2	101 ± 3	n.d.
15h		X = O, Z = O, R = H	95 ± 3	79 ± 4	n.d.

[a] Not determined; due to solubility issues at the nominated concentration.

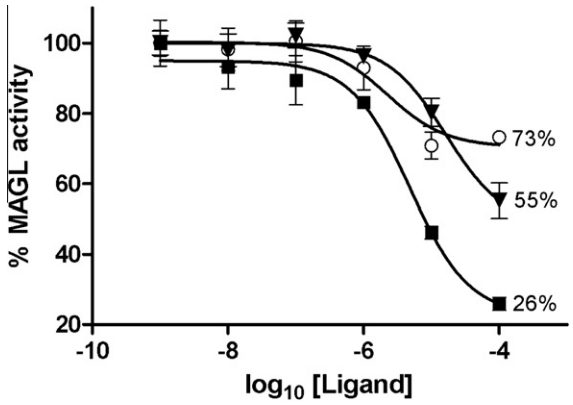


Figure 4. MAGL inhibition by URB602 (○, carbamate), analogue **18c** (▼, urea) and analogue **16** (■, carbamate) expressing the% of enzyme activity relative to controls at the highest ligand concentration of 100 μM.

regions, prospective work may extend towards the possibility of developing bitopic ligands as potential inhibitors of MAGL.[26] SAR studies of URB602 analogues found the carbamate **16** to be the most promising MAGL inhibitor reducing activity to 46% and 26% of controls at a concentration of 10 and 100 μM, respectively. Molecular modelling proposed that this may be due to the unique hydrogen bond with Ser185 resulting from the introduction of the *p*-OH group. Additionally, the urea analogue (**18c**) also proved to be a moderate inhibitor of MAGL thereby supporting carbamate and urea functionalities as promising scaffolds for the design of improved MAGL inhibitors.

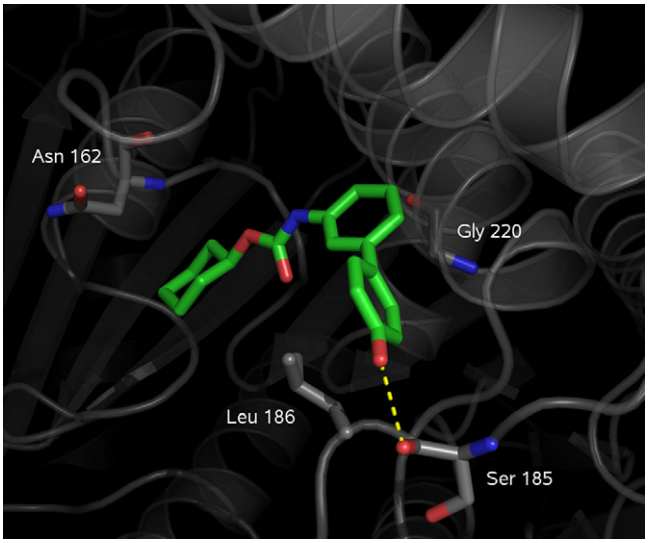


Figure 5. Intermolecular interactions of the *p*-OH substituted ligand **16** docked non-covalently into the upper active site cavity, showing key hydrogen bond (yellow) and hydrophobic interactions with highlighted residues.

Acknowledgments

M.S. and M.A. are recipients of the Australian Postgraduate Award (APA). M.A. is a recipient of the Monash University Postgraduate Publication Award (PPA). Thank you to Dr. Jason Dang for helping obtain NMR spectral data and HPLC chromatograms.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.038.

References and notes

1. Pertwee, R. G. *Aaps J.* **2005**, *7*, 625.
2. Pertwee, R. G. *Curr. Med. Chem.* **2010**, *17*, 1360.
3. Stella, N.; Schweitzer, P.; Plomelli, D. *Nature* **1997**, *388*, 773.
4. Dinh, T. P.; Carpenter, D.; Leslie, F. M.; Freund, T. F.; Katona, I.; Sensi, S. L.; Kathuria, S.; Piomelli, D. *PNAS* **2002**, *99*, 10819.
5. Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature* **1996**, *384*, 83.
6. Oka, S.; Yanagimoto, S.; Ikeda, S.; Gokoh, M.; Kishimoto, S.; Waku, K.; Ishima, Y.; Sugiura, T. *J. Biol. Chem.* **2005**, *280*, 18488.
7. Kinsey, S. G.; Long, J. Z.; Cravatt, B. F.; Lichtman, A. H. *J. Pain* **2010**, *11*, 1420.
8. Kinsey, S. G.; Long, J. Z.; O'Neal, S. T.; Abdullah, R. A.; Poklis, J. L.; Boger, D. L.; Cravatt, B. F.; Lichtman, A. H. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 902.
9. Hohmann, A. G. *Brit. J. Pharmacol.* **2007**, *150*, 673.
10. Karlsson, M.; Contreras, J. A.; Hellman, U.; Tornqvist, H.; Holm, C. *J. Biol. Chem.* **1997**, *272*, 27218.
11. Long, J. Z.; Li, W. W.; Booker, L.; Burston, J. J.; Kinsey, S. G.; Schlosburg, J. E.; Pavon, F. J.; Serrano, A. M.; Selley, D. E.; Parsons, L. H.; Lichtman, A. H.; Cravatt, B. F. *Nat. Chem. Biol.* **2009**, *5*, 37.
12. Ortar, G.; Cascio, M. G.; Moriello, A. S.; Camalli, M.; Morera, E.; Nalli, M.; Di Marzo, V. *Eur. J. Med. Chem.* **2008**, *43*, 62.
13. Alexander, J. P.; Cravatt, B. F. *J. Am. Chem. Soc.* **2006**, *128*, 9699.
14. Long, J. Z.; Nomura, D. K.; Cravatt, B. F. *Chem. Biol.* **2009**, *16*, 744.
15. Bertrand, T.; Augé, F.; Houtmann, J.; Rak, A.; Vallée, F.; Mikol, V.; Berne, P. F.; Michot, N.; Cheuret, D.; Hoornaert, C.; Mathieu, M. *J. Mol. Biol.* **2010**, *396*, 663.
16. Tarzia, G.; Duranti, A.; Tontini, A.; Piersanti, G.; Mor, M.; Rivara, S.; Plazzi, P. V.; Park, C.; Kathuria, S.; Piomelli, D. *J. Med. Chem.* **2003**, *46*, 2352.
17. Makara, J. K.; Mor, M.; Fegley, D.; Szabó, S. I.; Kathuria, S.; Astarita, G.; Duranti, A.; Tontini, A.; Tarzia, G.; Rivara, S.; Freund, T. F.; Piomelli, D. *Nat. Neurosci.* **2005**, *8*, 1139.
18. Hohmann, A. G.; Suplita, R. L.; Bolton, N. M.; Neely, M. H.; Fegley, D.; Mangieri, R.; Krey, J. F.; Walker, J. M.; Holmes, P. V.; Crystal, J. D.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; Piomelli, D. *Nature* **2005**, *435*, 1108.
19. King, A. R.; Duranti, A.; Tontini, A.; Rivara, S.; Rosengarth, A.; Clapper, J. R.; Astarita, G.; Geaga, J. A.; Luecke, H.; Mor, M.; Tarzia, G.; Piomelli, D. *Chem. Biol.* **2007**, *14*, 1357.
20. *Glide*, version 5.5; Schrödinger, LLC, New York, NY: 2009.
21. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739.
22. *SiteMap*, version 2.3; Schrödinger, LLC, New York, NY: 2009.
23. Halgren, T. A. *J. Chem. Inf. Model.* **2009**, *49*, 377.
24. King, A. R.; Dotsey, E. Y.; Lodola, A.; Jung, K. M.; Ghomian, A.; Qiu, Y.; Fu, J.; Mor, M.; Piomelli, D. *Chem. Biol.* **2009**, *16*, 1045.
25. Muccioli, G. G.; Labar, G.; Lambert, D. M. *ChemBioChem* **2008**, *9*, 2704.
26. Valant, C.; Sexton, P. M.; Christopoulos, A. *Mol. Interv.* **2009**, *9*, 125.