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Loratadine analogues as MAGL inhibitors

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Dedicated to Dr. Sameer Agarwal, principal scientist at Zydus Research Centre (ZRC, India)

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ABSTRACT

Compound **12a** (JZP-361) acted as a potent and reversible inhibitor of human recombinant MAGL (hMAGL, IC_{50} = 46 nM), and was found to have almost 150-fold higher selectivity over human recombinant fatty acid amide hydrolase (hFAAH, IC_{50} = 7.24 μM) and 35-fold higher selectivity over human α/β-hydrolase-6 (hABHD6, IC_{50} = 1.79 μM). Additionally, compound **12a** retained H_1 antagonistic affinity (pA_2 = 6.81) but did not show cannabinoid receptor activity, when tested at concentrations ⩽10 μM. Hence, compound **12a** represents a novel dual-acting pharmacological tool possessing both MAGL-inhibitory and antihistaminergic activities.

Monoacylglycerol lipase (MAGL) is a 33-kDa membrane-associated enzyme that belongs to the serine hydrolase superfamily. In the central nervous system (CNS), MAGL is the principal enzyme responsible for the in vivo degradation of 2-arachidonoyl glycerol (2-AG), an endogenous ligand of the cannabinoid receptors.[1–3] In addition to MAGL, two additional serine hydrolases, α/β-hydrolase domain-containing 6 (ABHD6) and α/β-hydrolase domain-containing 12 (ABHD12) participate in the degradation of 2-AG in the CNS.[4,5]

Several studies support the hypothesis that inhibition of MAGL may represent a useful and novel approach for the treatment of pain, inflammation, vomiting, and nausea.[6–9] Additionally, MAGL has been reported to be involved in the regulation of the fatty acid network that promotes cancer pathogenesis.[10] Hence, inhibition of MAGL may well be a promising target in drug development programs to treat different diseases.[11] The active site of MAGL contains a classical catalytic triad (Ser^{122}-Asp^{239}-His^{269}) and a lipase motif GxSxG both of which are typical for serine hydrolases.[12] At present, various MAGL inhibitors targeting the nucleophilic Ser^{122} residue have been reported.[13–18] Additionally, three cysteine residues (Cys^{201}, Cys^{208}, and Cys^{242}) located close to the catalytic site of MAGL have been postulated to be targeted by some inhibitors[19–23] (see review articles[24–27]). The majority of the current MAGL inhibitors, act in an irreversible manner and only a few have been reported to have a reversible mode of action,[21,28,29] although reversibly-acting MAGL inhibitors have been claimed to be safer alternatives than their irreversible counterparts.[29,30]

In 2008, Sanofi-Aventis published a patent of constrained piperazine bearing triazolopyridine ureas as MAGL inhibitors (e.g., **1**, Fig. 1).[31] Subsequently, several research groups including our own, have described the synthesis of piperidine/piperazine based carbamates/ureas, such as SAR629 (**2**),[14,32] JZL184 (**3**),[15] ML30 (**4**),[33] KML29 (**5**),[17] CK16 (**6**),[16] MJN110 (**7**),[18] JJKK-048 (**8a**)[32] and compound **8b**[34] and characterized these molecules as potent and selective MAGL inhibitors (Fig. 1). When assessing the structure–activity-relationships (SARs) of these compounds, it is evident that the two bulky hydrophobic aromatic rings, a piperidine/piperazine linker, and a good leaving group are required in order to achieve both high potency and selectivity toward MAGL over other possible off-targets such as fatty acid amide hydrolase (FAAH) and/or ABHD6.[15–17,24–27]

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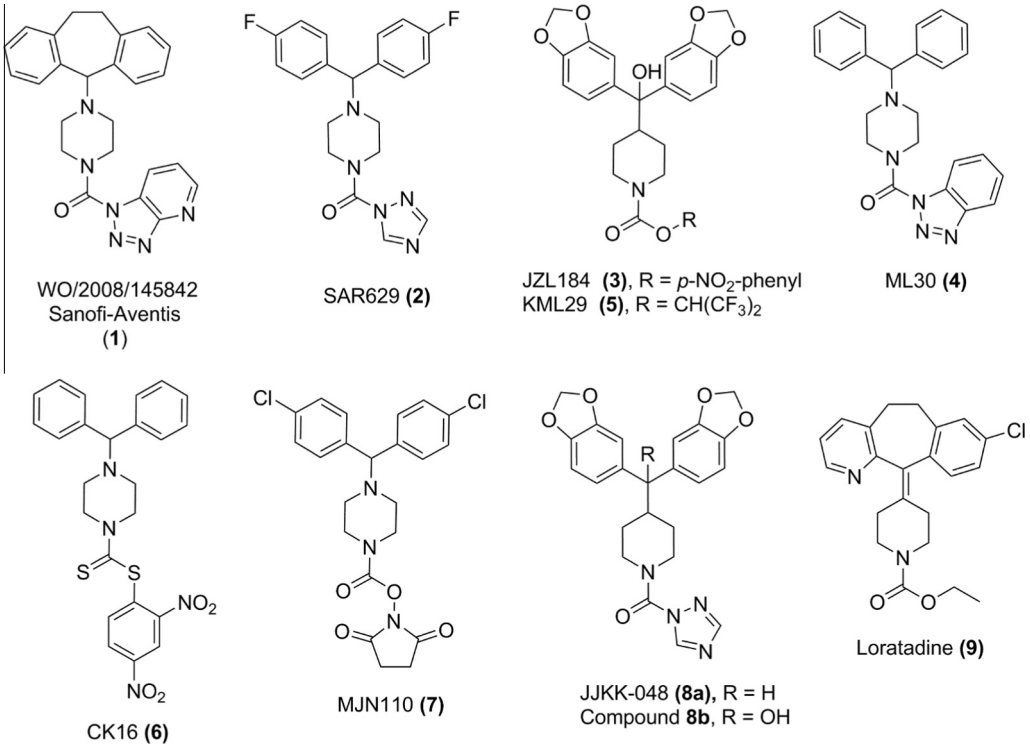


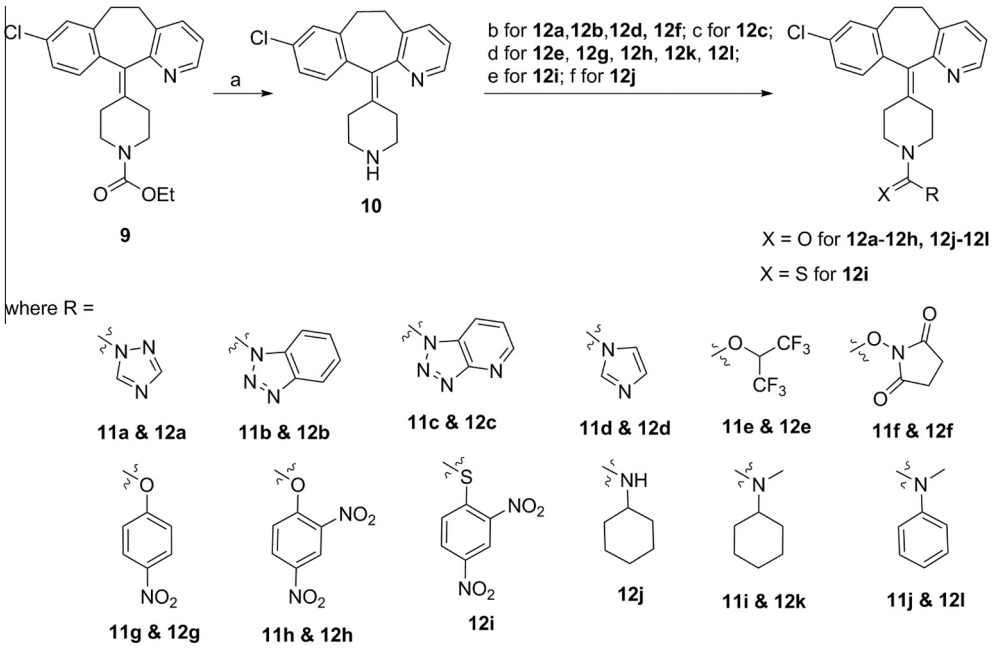
Figure 1. Representative structures of known MAGL inhibitors (**1**–**7**, **8a** and **8b**) and marketed drug loratadine (**9**).

Loratadine (**9**, see Fig. 1), a histamine H_1 receptor antagonist, is a marketed drug which has been used clinically to treat allergies. The structural features of this drug include two constrained aromatic rings, a piperidine moiety, and a carbamate functional group, features which are rather similar to those present in the previously reported active MAGL inhibitors. Since the carbamate functionality present in loratadine is not susceptible to MAGL attack, we decided to replace it with a more electrophilic carbamate/urea moiety. Therefore, we synthesized a small series of loratadine analogues and screened their activity against the human recombinant MAGL and FAAH (hMAGL and hFAAH, respectively). Selected compounds were tested further against other possible endocannabinoid targets and H_1 receptor antagonistic activity, and the obtained data were then used to explore the preliminary SAR. We also elucidated the mode of inhibition of the best compound in this series toward MAGL. Finally, a molecular modeling approach was employed to explore the binding interaction of the best compound with MAGL as well as with the histamine H_1 receptor.

A straightforward synthesis of loratadine analogues (**12a**–**12l**) was performed as per procedures described in the literature with some minor modifications (Scheme 1, see Supplementary information). Commercially available loratadine (**9**) was converted into desloratadine (**10**) with aqueous alkali.[35] The urea analogues (**12a**, **12b** and **12d**) were made by coupling of desloratadine with 1,1′-carbonyl-di-(1,2,4-triazole) (CDT), 1,1′-carbonylbis-benzotriazole, and, 1,1′-carbonyldiimidazole (CDI), respectively.[32] In the synthesis of the urea analogue **12c**, desloratadine was converted to the corresponding carbamoyl chloride via treatment with triphosgene and then it was coupled with 1*H*-1,2,3-triazolo [4,5-*b*]pyridine.[32] The carbamate analogue **12e** was synthesized by coupling of desloratadine with hexafluoroisopropanoyl chloride, produced in situ by the treatment of hexafluoroisopropanol with triphosgene.[17] The carbamate analogues **12f**–**12h** were made by coupling of desloratadine with *N*,*N*′-disuccinimidyl carbonate (DSC), 4-nitrophenyl chloroformate, and 2,4-dinitrophenyl chloroformate, respectively.[15,18] The latter intermediate was prepared via reaction of 2,4-dinitrophenol with triphosgene. The dithiocarbamate derivative **12i** was obtained by reaction of dithiocarbamic acid salt; prepared in situ by reaction of desloratadine with CS_2 in the presence of triethylamine, with 2,4-dinitrofluorobenzene.[16] The urea analogue **12j** was prepared by reaction of desloratadine with cyclohexyl isocyanate. The urea analogues **12k** and **12l** were prepared by coupling of desloratadine with *N*-cyclohexyl-*N*-methylcarbamoyl chloride and *N*-methyl-*N*-phenylcarbamoyl chloride, respectively. The former intermediate was prepared via the reaction of *N*-cyclohexyl-*N*-methylamine with triphosgene.

At first, the inhibitory activities of the synthesized compounds were tested at a 10 μM concentration against hFAAH and hMAGL using previously validated methodologies[36,37] (see Supplementary information) and the results are summarized in Table 1. In this study, loratadine (**9**) was found to be inactive towards both MAGL and FAAH when tested at 10 μM concentration. As the 1,2,4-triazole-based urea analogue JJKK-048 (**8a**) was recently found as the best MAGL inhibitor in terms of both potency and selectivity,[32] we replaced the ethoxy group present in loratadine with the 1,2,4-triazole group (compound **12a**). Consequently, compound **12a** displayed excellent inhibitory activity against MAGL in the low nanomolar range (IC_{50} = 46 nM) and importantly, it was found to possess ~150-fold higher selectivity for MAGL than towards hFAAH (IC_{50} = 7.24 μM).

Next, we tested loratadine analogues having various *N*-substitutions on the piperidine ring (compounds **12b**–**12l**, Table 1). Benzotriazole and triazolopyridine containing urea analogues (compounds **12b** and **12c**) were also found to be good MAGL inhibitors, albeit being less potent (2–4 fold) than compound **12a**. Surprisingly, the imidazole urea analogue **12d** was found to be inactive towards both MAGL and FAAH. Among the carbamate analogues, hexafluoroisopropyl (HFIP) and succinimide analogues (**12e** and **12f**) exhibited weak MAGL inhibition while the



Scheme 1. Synthesis of loratadine analogues **12a–12l**. Reagents and conditions: (a) aq KOH, EtOH, reflux, 16–18 h; (b) DIPEA, RCOR (R = **11a**, **11b**, **11d**, **11f**, respectively), DCM, 22–25 °C, 24–26 h; (c) (i) pyridine, DCM, triphosgene, 0–25 °C, 2–3 h, (ii) carbamoyl chloride from (i) followed by RH (R = **11c**), DIPEA, DMAP, DCM, 0–25 °C, 24–26 h; (d) DIPEA or pyridine, RCOCl (R = **11e**, **11g**, **11h**, **11i**, **11j**, respectively), DCM, 0–25 °C, 16–24 h; (e) (i) CS_2, Et_3N, 22–25 °C, 16 h; (ii) R-F (R = 2,4-dinitrophenyl), DMF, 22–25 °C, 24 h; (f) RNCO (R = cyclohexyl), THF, reflux, 4 h.

para-nitrophenyl analogue (**12g**) was found to be inactive. In order to cross-check the behaviour of compound **12g**, we synthesized the 2,4-dinitrophenyl analogue of compound **12g** (compound **12h**) as well as the corresponding dithiocarbamate analogue of compound **12h** (compound **12i**). However, these analogues also did not display any significant MAGL inhibitory activities. This could be because these compounds do not fit well into the MAGL binding pocket because of their bulky phenyl leaving group. As expected, analogues with less electrophilic/poor leaving groups (**12j–12l**) were found to be inactive towards MAGL. None of the compounds **12b–12l** showed any significant inhibition of FAAH. Overall, these results are consistent with our earlier findings demonstrating that high potency of MAGL inhibitors can be achieved if they have leaving-groups with a conjugate acid pK_a of 8–10.[32,34]

Then, we decided to study the effect of the 1,2,4-triazole leaving group on another marketed H_1-antihistamine known as cyproheptadine (**13**) which is also used to treat allergic reactions. Cyproheptadine (**13**) was converted into an ethyl ester analogue (**14**) via ethylchloroformate and subsequently treated with alkali to produce the amine (**15**) (Scheme 2, see Supplementary information). Finally, coupling of amine **15** with 1,1′-carbonyl-di-(1,2,4-triazole) (CDT) in DMSO produced the triazole urea analogue **16**. Compound **16** was found to be equally potent as compound **12a** as a MAGL inhibitor and had comparable selectivity over FAAH (see Table 1).

We further screened compounds **12a** and **16** at 10 μM against supplementary 2-AG hydrolases—hABHD6 and hABHD12 using the previously validated methodology[38] (see Supplementary information). Both compounds, **12a** and **16** were found to inhibit hABHD6 in the low micromolar range (IC_{50} 1.78 μM and 0.75 μM, respectively), however, no inhibition of hABHD12 was observed (Table 2). In short, compound **12a** shows 35-fold selectivity over hABHD6 while towards its main off-target FAAH, as described above, it has around 150-fold selectivity.

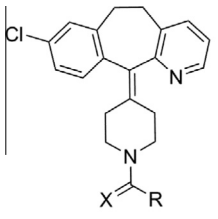
To rule out cross-activity with the other proteins of the endocannabinoid system, compound **12a** (JZP-361), which appeared to be the best MAGL inhibitor in this series in terms of both potency and selectivity, was evaluated for its ability to stimulate cannabinoid CB_1 and CB_2 receptor-mediated G protein activity using the [^{35}S]GTPγS binding assay, tested with previously validated methods.[39,40] In this assay, compound **12a** showed no agonist or antagonist activity when tested at 10 μM concentration (Table 3).

Since the above described analogues are derived from marketed H_1 antagonists, we tested the H_1 antagonist properties of compounds **12a** and **16** in the guinea pig ileum tissue preparation according to the method described by Arunlakshana et al.[41] (see Supplementary information). We found that both of these analogues retained H_1 antagonistic properties (Table 4) indicating that they behave as dual blockers of MAGL activity and H_1 receptors.

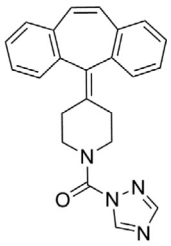
The reversibility of MAGL inhibition by compound **12a** was tested by using a dilution-method where the enzyme is first pretreated for 60 min with the inhibitor, followed by substrate addition and 40-fold dilution, essentially as previously described.[32] In these experiments, we found that within the time-frame studied, binding of **12a** (JZP-361) appeared to be slowly reversible, as the potency of this compound to inhibit MAGL was slightly but significantly lower after 90 min incubation in comparison to the early time point (10 min) (Fig. 2). A slowly reversible mode of inhibition has been previously described also for other serine hydrolases such as acetylcholinesterase[42] and acylprotein thioesterase.[43] The reference compounds, MAFP (methylarachidonyl fluorophosphonate, an established irreversible inhibitor) and ATM-114 (5-ethoxy-3-(3-phenoxyphenyl)-1,3,4-oxadiazol-2(3*H*)-one, a reversible inhibitor), behaved in this assay as previously reported[44] with the exception that we observed no statistically significant reduction in the potency of ATM-114 with a prolonged incubation time. Instead, the inhibitor efficacy, that is, maximal inhibition achieved at ATM-114 concentrations between 10^{-7} and 10^{-4} M, showed a time-dependent decline that was statistically significant at each time-point examined (Fig. 2), evidence for a rapid mode of reversible inhibition.

Molecular modeling studies were employed in order to gain molecular level insights into the binding of compound **12a** with human MAGL and human histamine H_1 receptor, (see Supplementary information). These studies suggested that compound **12a** (JZP-361) fitted well into the MAGL active site

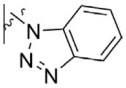
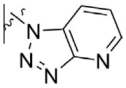
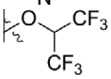
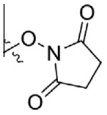
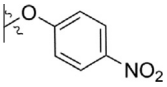
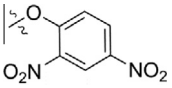
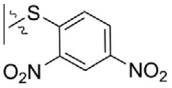
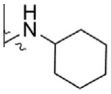
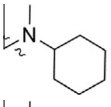
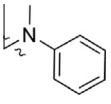
Table 1
Inhibitory activities of analogues **12a**–**12l** and **16** against MAGL and FAAH



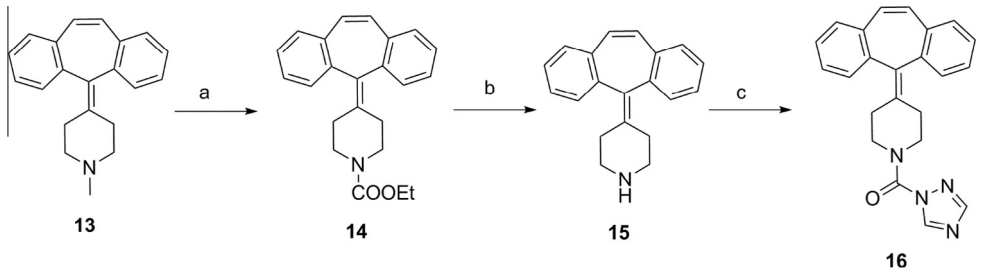
X = O for **12a-12h, 12j-12l**
X = S for **12i**



16

Compound	R	pI_{50} (range) [IC_{50}, μM][a] or % inhibition at 10 μM[b]	
		hMAGL (μM)	hFAAH (μM)
9	–	NI[c]	11%
12a (JZP-361)		7.34 (7.21–7.47) [0.046]	5.14 (5.12–5.16) [7.24]
12b		6.68 (6.66–6.69) [0.208]	22%
12c		7.01 (7.01–7.02) [0.098]	30%
12d		NI	7%
12e		6.19 (6.11–6.26) [0.645]	15%
12f		5.59 (5.58–5.59) [2.57]	12%
12g		27%	6%
12h		NI	14%
12i		14%	12%
12j		NI	9%
12k		NI	NI
12l		NI	11%
16	–	7.33 (7.32–7.34) [0.047]	5.16 (5.14–5.19) [6.91]
8a (JJKK-048)[d]	–	9.44 ± 0.05 [0.00036][e]	5.32 ± 0.05 [4.8][e]
8b[f]	–	9.16 (9.02–9.30) [0.0007]	5.79 (5.69–5.88) [1.62]

[a] pI_{50} values ($-\log_{10}$ [IC_{50}]) represent the mean (range) from two independent experiments performed in duplicate. IC_{50} values were calculated for those compounds having ⩾50% inhibition at 10 μM and were derived from the mean pI_{50} values as shown in brackets.
[b] The percentage (%) of inhibition is calculated as the mean from two independent experiments performed in duplicate.
[c] NI indicates no inhibition at 10 μM.
[d] JJKK-048: ({4-[bis-(benzo[*d*][1,3]dioxol-5-yl)methyl]-piperidin-1-yl}(1*H*-1,2,4-triazol-1-yl)methanone) used as MAGL reference standard (See Ref. 32).
[e] pI_{50} values ($-\log_{10}$ [IC_{50}]) represent the mean ± SEM from three independent experiments performed in duplicate.
[f] See Ref. 34.



Scheme 2. Synthesis of 1,2,4-triazole urea derivative of cyproheptadine **16**. Reagents and conditions: (a) toluene, ClCOOEt, reflux, 3 h; (b) aq KOH, EtOH, reflux, 16–18 h; (c) DIPEA, RCOR (R = **11a**), DMSO, 22–25 °C, 24 h.

Table 2
Inhibitory activities of analogues **12a** and **16** against ABHD6 and ABHD12

Compound	pI_{50} (range) [IC_{50}, μM][a] or % inhibition at 10 μM[b]	
	hABHD6	hABHD12
12a (JZP-361)	5.75 (5.66–5.84) [1.78]	NI[c]
16	6.12 (5.98–6.27) [0.75]	NI
THL[d]	7.32 ± 0.06 [0.048][e]	6.72 ± 0.07 [0.19][e]

[a–c,e] See footnotes for Table 1.
[d] THL, tetrahydrolipstatin (orlistat, See Ref. 38).

comprising the catalytic triad Ser^{122}-His^{169}-Asp^{239} and the oxyanion hole Met^{123}-Ala^{51} (Fig. 3), which is in good agreement with earlier findings with other MAGL inhibitors.[14,45–47] Compound **12a**, a slow reversible inhibitor of MAGL, was found to bind in a similar orientation as several other 1,2,4-triazole bearing irreversible MAGL inhibitors such as SAR629 (**2**) and JJKK-048 (**8a**) (image not shown). Thus the molecular modeling was able to explain, at least partly, how the Y-shaped ligands possess good shape complementarity with the MAGL active site. In the case of the histamine H_1 receptor, compound **12a** has a similar shape as doxepin, a ligand which has been complexed with the resolved H_1 receptor crystal structure.[48] Accordingly, our modeling studies indicated that compound **12a** docked in a similar orientation as doxepin, forming favourable interactions with the aromatic side chain residues $Trp158^{4.56}$, $Phe432^{6.52}$, $Phe435^{6.55}$ and $Trp428^{6.48}$ (Fig. 3). It is noteworthy that although the activity data were obtained using guinea pig preparation whereas the modeling study was based on human H_1 receptor structure, the two H_1 receptor orthologues share high sequence homology in their transmembrane domains and identical amino acid residues are present in the ligand binding cavity (data not shown).

In summary, our preliminary attempt to modify the carbamate functionality of loratadine resulted in the production of potent MAGL inhibitors. Several urea/carbamate analogues were examined and compound **12a** (JZP-361) was identified as a potent hMAGL inhibitor (IC_{50} = 46 nM) having ~150-fold higher selectivity over FAAH and ~35-fold higher selectivity over ABHD6. In the [^{35}S] GTPγS binding assay, compound **12a** exhibited no activity at the cannabinoid CB_1 and CB_2 receptors. Reversibility studies indicated that the binding mode of JZP-361 was slowly reversible. In addition, in our molecular modeling study, compound **12a** displayed favourable interactions within the active site of hMAGL including the important hydrogen-bonding of the carbonyl oxygen to the oxyanion hole. Moreover, compound **12a** also underwent favourable interactions with aromatic side chain residues $Trp158^{4.56}$, $Phe432^{6.52}$, $Phe435^{6.55}$ and $Trp428^{6.48}$ of the human histamine H_1 receptor.

As compounds **12a** and **16** fully retained H_1 antagonistic activity, these compounds represent novel dual-acting pharmacological tools that could be tested in inflammation models where simultaneous blockade of MAGL and H_1-receptors would be desirable. Although speculative at this point, one could envision conditions where MAGL-dependent 2-AG hydrolysis liberates arachidonic acid, the substrate for COX-2-catalyzed production of the proinflammatory compound, prostaglandin E_2. This lipid mediator could, in turn, evoke inflammation through mast cell activation and subsequent histamine release, thereby increasing both vascular permeability and edema formation. Recent research suggests that such a scenario is possible.[49] Our results also point to the possibility that some of the previously described MAGL inhibitors might also exert H_1-antihistamine activity.

Table 4
Histamine H_1 antagonistic affinity of analogues **12a** and **16** as tested on the in vitro test system on the guinea pig jejunum

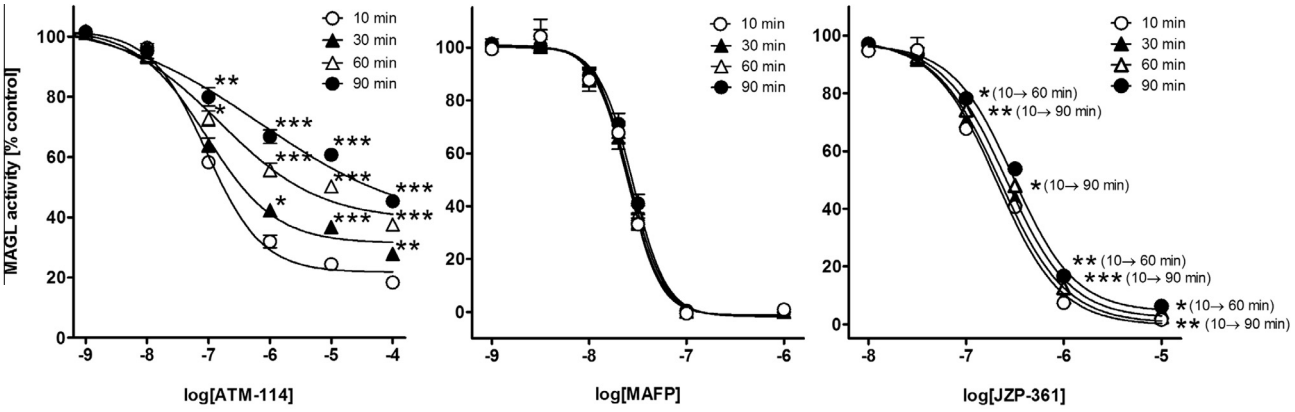
Compound	pA_2 (SEM)[a]	N[b] (caviae)[c]
12a (JZP-361)	6.81 (0.021)	2(2)
16	6.50 (0.189)	3(2)
Loratadine (**9**)	7.14 (0.042)	3(2)
Pyrilamine	8.84 (0.031)	3(2)

[a] SEM—standard error of the mean.
[b] *N*—number of different animal preparations.
[c] caviae—number of animals.

Table 3
Activity of compound **12a** (JZP-361) at CB1 and CB2 receptors

Compound	Agonist activity		CB_1R antagonist activity[a]
	CB_1R[a]	CB_2R[b]	
	[^{35}S]GTPγS binding % basal (mean (range), n = 2)		[^{35}S]GTPγS binding % HU210-evoked response (mean (range), n = 2)
12a (JZP-361)	90 (87–92)	101 (96–107)	80 (80–80)
HU210 (1 μM)	304 (297–311)	–	–
HU210 (10 nM)	–	172 (169–174)	–
AM251 (1 μM)	–	–	35 (33–39)
SR144528 (1 μM)	–	53 (51–57)	–

[a] Rat cerebellar membranes.
[b] hCB_2R-CHO cell membranes.



Incubation time	ATM-114 (pIC_{50} ± SEM)	MAFP (pIC_{50} ± SEM)	JZP-361 (pIC_{50} ± SEM)
10 min	7.04 ± 0.05	7.60 ± 0.02	6.68 ± 0.05
30 min	7.03 ± 0.08	7.60 ± 0.02	6.65 ± 0.04
60 min	6.81 ± 0.14	7.58 ± 0.02	6.59 ± 0.03
90 min	6.17 ± 0.36	7.56 ± 0.02	6.51 ± 0.03 #

Figure 2. Inhibition of MAGL by JZP-361 (**12a**) indicates a slowly reversible mode of inhibition. Unlike the established irreversible MAGL inhibitor MAFP, a rapid 40-fold dilution of inhibitor-treated hMAGL preparation results in a statistically significant time-dependent decline in potency of JZP-361 (90 min compared to 10 min time point, unpaired *t*-test, $p < 0.05^{\#}$). A dramatic time-dependent change in the efficacy (maximal inhibition obtained at different concentrations) of the reversible MAGL inhibitor ATM-114[44] indicates a weaker mode of reversible binding. Note however, that due to ambiguous dose-response curves, the potencies of ATM-114 at early and late time-points are not significantly different from each other. Note also that due to methodological limitations, the IC_{50} values obtained by the dilution method are not directly comparable to those obtained using the routine assay protocol.[32,44] Data are mean ± SEM from three independent experiments. Statistical comparisons between maximal inhibition at various time-points within the same inhibitor concentration (for ATM-114: 10 min vs 30/60/90 min) were conducted using one-way Anova, followed by Tukey's multiple comparison test ($p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$).

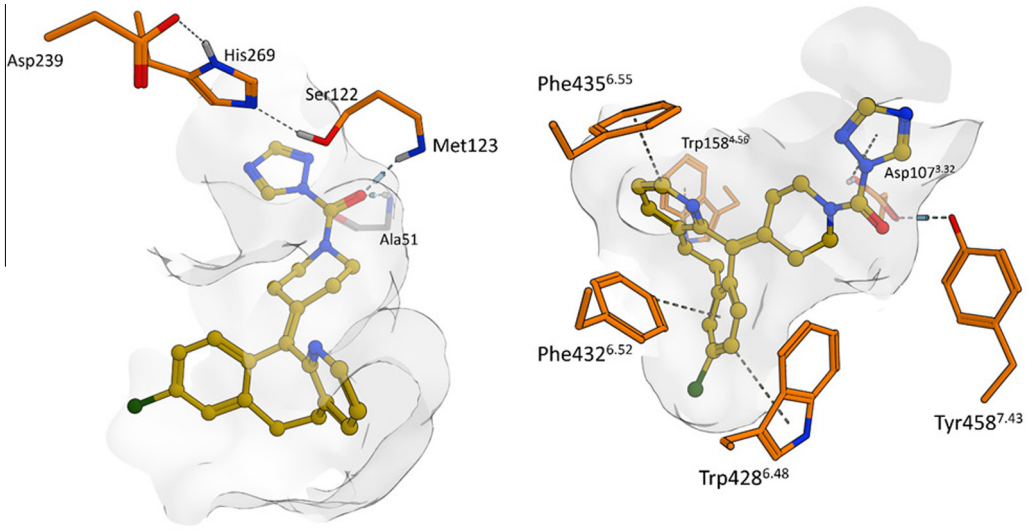


Figure 3. Favourable docking poses of compound **12a** (JZP-361) to the human MAGL active site (left) and to the human H_1 receptor (right). The catalytic triad residues (Ser^{122}-His^{169}-Asp^{239}) of MAGL are shown in orange and the oxyanion hole residues are also depicted (Met^{123}-Ala^{51}). The active site surface is illustrated but residues defining the shape of the cavity are omitted for clarity. For the histamine H_1 receptor, key residues are shown in orange and potent π–π interactions are depicted with dashed lines. Residue numbering is according to Shimamura et al.[48]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.02.037. These data include MOL files and InChiKeys of the most important compounds described in this article.

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