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New trisubstituted 1,2,4-triazoles as ghrelin receptor antagonists

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ABSTRACT

Ghrelin receptor ligands based on a trisubstituted 1,2,4-triazole scaffold were recently synthesized and evaluated for their in vitro affinity for the GHS-R1a receptor and their biological activity. In this study, replacement of the α-aminoisobutyryl (Aib) moiety (a common feature present in numerous growth hormone secretagogues described in the literature) by aromatic and heteroaromatic groups was explored. We found potent antagonists incorporating the picolinic moiety in place of the Aib moiety. In an attempt to increase affinity and activity of our lead compound **2**, we explored the modulation of the pyridine ring. Herein we report the design and the structure–activity relationships study of these new ghrelin receptor ligands.

Ghrelin, the natural ligand of the Growth Hormone Secretagogue Receptor type 1a (GHS-R1a),[1] was recently discovered and characterized.[2] Its main biological activities were found to be the stimulation of both growth hormone release and food intake. Ghrelin also regulates the metabolic balance by decreasing fat use, affecting body weight and adiposity. Thus, ghrelin functions as an orexigenic hormone[3] and it can be of interest to find agonists and antagonists of its receptor.

Before the discovery of ghrelin, research focused on the finding of agonists able to elicit the stimulation of GH secretion. Among many compounds, we can cite the peptidic precursor component GHRP-6 described by Bowers,[4] the non peptidic Merck compound, MK-0677, which allowed the characterization of the GHS-R1a in its S^{35} labeled version,[5] and the CP-424,391 (or Capromorelin), developed by Pfizer.[6] Our team also described the JMV 1843, a pseudo-peptide which was able to stimulate GH secretion by oral administration in man.[7] This compound will be soon commercialized for the diagnostic of GH deficiency in adults. When multiple ghrelin biological activities were exhibited, it appears that antagonist (and/or inverse agonist) compounds of the GHS-R1a receptor could be useful to treat obesity, for example. Ipsen developed ghrelin analogs and identified a competitive GHS-R1a antagonist, BIM 28163,[8] while Tranzyme Pharma has extensively claimed conformationally defined macrocyclic compounds incorporating peptides as modulators of the GHS-R1a, including antagonists.[9] Numerous nonpeptide small molecules such as benzodiazepine,[10] piperazine bisamide,[11] azaquinazolinone[12] and indolinone[13] derivatives have been described as potent antagonists of the ghrelin receptor.[14]

In our effort to find new ghrelin receptor ligands, we recently described a family of peptidomimetics based on a decorated 1,2,4-triazole scaffold that led to potent agonists and antagonists of this receptor.[15–17] These compounds were synthesized from D-tryptophan residue as starting material and present in their final structure only one asymmetric carbon atom, whose configuration was controlled during the synthetic process. Replacement of the α-aminoisobutyryl (Aib) moiety, a common feature often present in GHS-R1a ligands,[5–7] by groups such as glycyl, propyl, isonipecotyl or piperazyl-2-carbonyl groups was first explored.[16] One of our front runners, compound JMV 2959 (compound **1**, Fig. 1), a trisubstituted 1,2,4-triazole comprised of a glycyl group in place of the Aib moiety, was found to be a potent GHS-R1a antagonist. Therefore **1** has been extensively studied in various in vivo animal models[18] and exhibits a large range of interesting properties such as suppression of food intake induced by ghrelin or by fasting[19] and suppression of fat mass accumulation.[20] It also presents in vivo effects on addictive behavior, providing evidence that the central ghrelin signaling system is required in the reward induced

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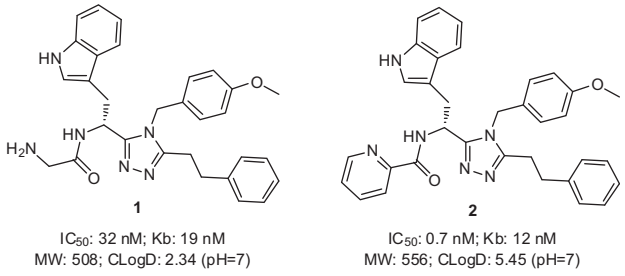


Figure 1. Structures of compounds **1** (JMV 2959) and **2** (JMV 3018).

by addictive drugs such as alcohol,[21] amphetamine[22] and cocaine.[23,24]

When replacing the glycine residue on the N-terminal part of compound **1** by a picolinic moiety, we obtained a very potent antagonist (compound **2**, Fig. 1).[17] As shown in Table 1, the pharmacokinetic studies of the two compounds **1** and **2** exhibited a very different pattern. Indeed, compound **2** showed a much better pharmacokinetic profile than compound **1**, with a high maximal plasma concentration (C_{max}) reached 5 h after administration and a high AUC value reflecting a long drug exposure to the body with a slow clearance.

These results prompted us to conserve the picolinic moiety on the N-terminal amino function of our compounds and introduce various substituents on the pyridine ring in an attempt to modulate the physicochemical properties of the ligands. In this Letter, we report on the consequences of these structural modifications on both the affinity and the biological activity of the new ligands toward the ghrelin receptor.

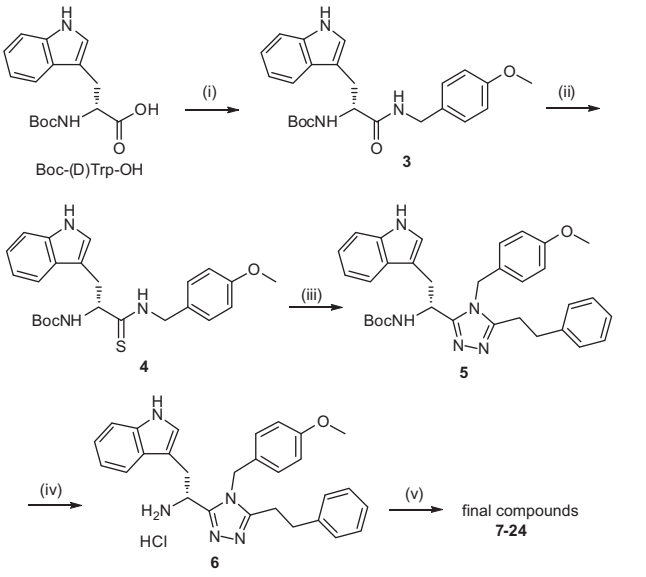
Compounds were synthesized as previously described.[25] Briefly, triazole derivatives were obtained in five steps starting from Boc-D-Trp-OH as shown in Scheme 1. After coupling with 4-methoxybenzylamine, the amide **3** was transformed into the thioamide **4** using the Lawesson's reagent. Cyclization into the triazole heterocycle (compound **5**) was performed in the presence of 3-phenylpropanehydrazide and silver benzoate.[26] After removal of the Boc protecting group using 4N HCl in dioxane, the structural diversity was introduced on compound **6** by coupling various substituted picolinic, isonicotinic and nicotinic acids. All final compounds were purified by reversed-phase preparative HPLC. The purity assessed by analytical reversed phase C18 HPLC was found to be greater than 98% for most of the target compounds and greater than 95% for the rest, and the structures of the compounds were confirmed by MS (electrospray), ^{1}H NMR, and ^{13}C NMR.

The synthesized compounds **7**–**24** along with intermediate salt **6** were first tested for their ability to displace ^{125}I-[His9]-ghrelin from the cloned hGHS-R1a receptor transiently expressed in LLC PK-1 cells as previously described[15] and compared to the parent compound **2** (Table 2). Overall, the binding affinities were in the nanomolar range for the most potent ligands (compounds **13**–**18**) with IC_{50} values between 0.7 and 6 nM while compound **6**, with a free amine, lost its binding affinity (IC_{50} >1000 nM). This indicates that substitution of the N-terminal amino function by an acylating group such as the pyridyl ring remains essential for the

Table 1
Pharmacokinetic profiles of compounds **1** and **2**

Compounds	AUC[a]	C_{max} (ng/mL)[b]	T_{max} (h)[c]
1	15	5	1.2
2	7250	1064	5.3

[a] Area under the curve (integral of the plasma concentration–time curve).
[b] Peak concentration (max plasma concentration after drug administration).
[c] Peak time (time to reach C_{max}).

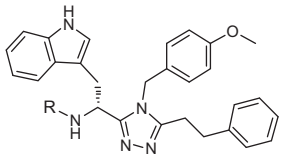


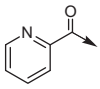
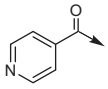
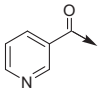
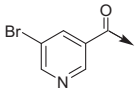
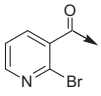
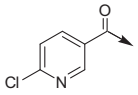
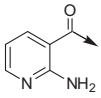
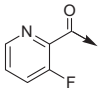
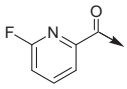
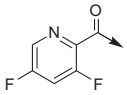
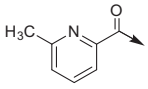
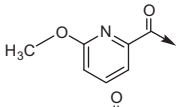
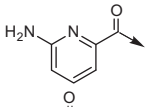
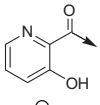
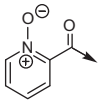
Scheme 1. Reagents and conditions: (i) BOP, DIEA, 4-methoxybenzylamine, DCM, rt, (ii) Lawesson's reagent, DME, 80 °C, (iii) 3-phenyl-propanehydrazide, $PhCO_2^-Ag+$, AcOH, DCM, rt, (iv) 4N HCl/dioxane, rt, (v) substituted pyridyl acid, BOP, DIEA, DCM, rt.

compounds to bind to the receptor. So at first, the effect of the position of the nitrogen atom into that pyridyl ring was investigated. When the picolinamide (compound **2**) was replaced by an isonicotinamide (compound **7**), a loss of binding affinity was observed (IC_{50} = 220 nM). A similar decrease was found with a nicotinamide moiety (compound **8**) even when substituted with 5-bromo, 2-bromo, 6-chloro or 2-amino substituent (compounds **9**–**12**, respectively). The position of the nitrogen atom in the pyridyl moiety has therefore proved to be determinant for the binding to GHS-R1a. Indeed, it has to be in ortho position to the carbonyl to allow the best binding to the receptor. Thus, all the following compounds were designed with a picolinamide moiety decorated with various substituents. Incorporation of an electron withdrawing group (fluorine atom) in position 3 or 6 of the pyridyl ring (compounds **13** and **14**, respectively) or even of 2 fluorine atoms in position 3 and 5 (compound **15**) was well tolerated as it led to very potent compounds with nanomolar affinities ($IC_{50} \leqslant 4$ nM). Various substituents such as methyl-, methoxy- or amino-group were also well tolerated in position 6 as shown with compounds **16**–**18**, respectively ($IC_{50} \leqslant 6$ nM). Unfortunately, the presence of a hydroxyl group in position 3 (compound **19**) led to a significant loss of binding affinity (IC_{50} = 240 nM) while the introduction of a N-oxide into the pyridyl ring (compound **20**) was better tolerated (IC_{50} = 42 nM), which remains interesting as it lowers the ClogD value. At last, in order to explore the size of the binding site of the N-terminal substituent, commercially available picolinic acids substituted by sterically hindered groups were used to design new ligands. Pyrollidin-1-yl group in position 5 of the pyridyl ring (compound **21**) as well as phenylmethanone in position 3 (compound **22**) led to a significant loss of affinity for GHS-R1a (IC_{50} = 65 nM and IC_{50} = 240 nM, respectively) compared to parent compound **2.** This indicates that steric hindrance on this side of the molecule might be a limitation for binding to the receptor as it decreases the binding affinity of the ligands. The same results were observed with isoquinoline-1 or isoquinoline-3. Indeed, compounds **23** and **24**, respectively, exhibited less potent binding affinities than unsubstituted picolinamide **2**.

The biological in vitro activity of the compounds was then evaluated. First, the compounds were tested for their ability to induce intracellular calcium $[Ca^{2+}]_i$ mobilization in GHS-R1a expressing

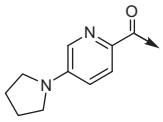
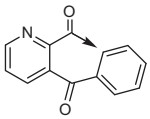
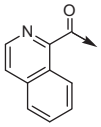
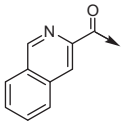
Table 2
SAR study on various substituted pyridine rings



Compounds	R	IC_{50} (nM)			ClogD (pH = 7)[d]
		Competitive binding[a]	CRE/Luc reporter gene assay[b]	Calcium release[c]	
2		0.7 ± 0.2	6.0 ± 3.1	4.5	5.45
6	H (HCl salt)	>1000	nd	nd	3.45
7		220 ± 60	828	nd	5.06
8		122 ± 20	411	337	5.06
9		84 ± 12	222	88	5.83
10		250 ± 52	193	510	6.04
11		130 ± 21	771	1010	5.89
12		380 ± 63	427	671	5.47
13		3 ± 0.6	8.8 ± 1.1	7.0	5.59
14		2 ± 0.2	2.1 ± 0.7	2.1	5.99
15		4 ± 0.8	2.8 ± 0.2	4.6	5.74
16		3.4 ± 1.2	7.5 ± 0.4	5.4	5.58
17		2 ± 0.5	9.0 ± 2.7	7.8	5.89
18		6 ± 1.6	21 ± 6.6	25	5.22
19		240 ± 38	108	165	5.44
20		42 ± 12	79	112	4.24

(*continued on next page*)

Table 2 (*continued*)

Compounds	R	IC_{50} (nM)			ClogD (pH = 7)[d]
		Competitive binding[a]	CRE/Luc reporter gene assay[b]	Calcium release[c]	
21		65 ± 21	1210	2115	5.96
22		240 ± 33	313	230	6.91
23		150 ± 21	73	82	6.44
24		100 ± 12	126	404	6.44

[a] IC_{50} values are the mean of at least two independent experiments, each of them were conducted in triplicate.
[b] IC_{50} values were determined by one experiment for the less efficient compounds and are the mean of at least two independent experiments for compounds in the nanomolar range. Mean data points of each experiment are based on quadruplicate measurements. (nd for not determined).
[c] IC_{50} values were determined by one experiment and the mean data points are based on quadruplicate measurements. (nd for not determined).
[d] ClogD values were calculated using the Marvin software provided by http://www.chemaxon.com.

cells.[15] We considered as potential antagonist any compound unable to elicit at 10^{-5} M an increase superior to 10% of the intracellular calcium level compared to the maximal response (fixed at 100% when elicited by 10^{-7} M ghrelin). In that case, the IC_{50} value was determined using antagonist inhibition curves in the presence of 10^{-7} M ghrelin (submaximal concentration) and calculated as the molar concentration of antagonist that reduced the maximal response of ghrelin by 50%. Then, the compounds were tested in a cyclic AMP response element CRE-luciferase reporter gene assay.[27] This assay was conducted on mouse LTK-cells stably expressing the human GHS-R1a and uses a luciferase reporter gene under the control of CRE elements and the CMV minimal promoter. Receptor-mediated reporter gene expression was calculated in % inhibition according to cells treated with saturating concentrations of ghrelin (0% inhibition), and nontreated cells as positive control (100% inhibition). Results are reported in Table 2 and show a good correlation between the two assays (calcium and CRE). All the new ligands were found to act as ghrelin receptor antagonists except compound **6** which was not tested as it showed no binding affinity for the receptor. As expected, compounds with poor binding affinities revealed low efficacy with IC_{50} values sometimes in the micromolar range. However, compounds designed with the picolinamide moiety decorated with 3-fluoro, 6-fluoro, 3,5-difluoro, 6-methyl or 6-methoxy substituent (compounds **13**–**17**, respectively) revealed great efficacy with IC_{50} values in the nanomolar range (IC_{50} <10 nM). Even the presence of an amino group at position 6 (compound **18**) was well tolerated with only a slight decrease of potency (IC_{50} = 25 nM). These results correlate with the fact that compounds **13**–**18** have high affinity for the GHS-R1a receptor.

Replacement of the glycyl moiety present on compound **1** by a pyridyl-carbonyl group led to antagonists of the GHS-R1a receptor. These compounds were easily synthesized from commercially available materials and contain only one asymmetric center. The most potent compounds in this series were obtained with a picolinamide moiety indicating the nitrogen atom of the pyridyl group must be in ortho position to the carbonyl to obtain good affinity and efficacy. Various substituents on the pyridyl ring were well tolerated, but compounds **13**, **14**, and **15** containing fluorine atoms and compounds **16** and **17** containing respectively a methyl or a methoxy group, showed the best results. As compounds **1** and **2**, our trisubstituted 1,2,4-triazole front runner ligands, are not able to inhibit GH secretion induced by an agonist (hexarelin, ghrelin),[16,17] we have good hopes that this new series of antagonists will present the same properties. This may be particularly interesting for the development of potential anti-obesity (or anti-addiction) treatment. These compounds are currently under further in vivo investigations.

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Supplementary data

Supplementary data (complete set of data related to ^{1}H and ^{13}C NMR, LC/MS and analytical HPLC studies, and copies of ^{1}H and ^{13}C NMR of compounds **2**, **7**–**25**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.031. These data include MOL files and InChiKeys of the most important compounds described in this article.

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27. In the CRE/Luc reporter gene assay, mouse LTK-cells were stably transfected with a plasmid containing the CMV minimal promotor linked to three cAMP response elements (CRE) followed by a luciferase reporter gene. Based on this parental cell line, single cell clones stably overexpressing the human GHS-R1A were established and characterized. The cells were incubated for 6 h with 1 μM rolipram in the presence of different concentrations of the tested compounds and saturating concentration of ghrelin. Subsequently, cells were lysed and ATP bioluminescence was measured in the luminescence mode on FlexStation3 (Molecular Devices). All data were assessed in quadruplicate measurements and calculated in % inhibition according to cells treated with saturating concentrations of ghrelin (Polypeptide #sc1357) as negative (0% inhibition), and nontreated cells as positive control (100%). IC_{50} values were determined by using the GraphPad Prism analysis program (GraphPad Software).