# New Trisubstituted 1,2,4-Triazole Derivatives as Potent Ghrelin Receptor Antagonists. 3. Synthesis and Pharmacological in Vitro and in Vivo Evaluations

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*Received October 15, 2007*

Ghrelin receptor ligands based on trisubstituted 1,2,4-triazole structure were synthesized and evaluated for their in vitro binding and biological activity. In this study, we explored the replacement of the α-aminoisobutyryl moiety by aromatic or heteroaromatic groups. Compounds **5** and **34** acted as potent in vivo antagonists of hexarelin-stimulated food intake. These two compounds did not stimulate growth hormone secretion in rodents and did not antagonize growth hormone secretion induced by hexarelin.

## Introduction

Ghrelin[1] and its receptor (GHS-R1a[a])[2] are nowadays intensively studied. Ghrelin exhibits a wide range of biological activities.[3,4] It not only increases growth hormone (GH) plasma levels, it also stimulates food intake. Ghrelin affects body weight and adiposity. Chronic administration of ghrelin in freely fed mice and rats results in increased body weight and decreased fat utilization.[5] Antagonizing the ghrelin effect with a peptide antagonist results in reduction of food intake and body weight gain in diet-induced obese mice.[6] For these reasons, small non-peptide GHS-R1a antagonists became of great interest as potential drugs for the treatment of obesity.[7–9]

We recently described new series of ghrelin receptor–ligands based on trisubstituted 1,2,4-triazoles of general formula shown in Figure 1. In an early study,[10] we reported the synthesis and biological evaluation of potent GHS-R1a ligands; the structure–activity relationship study on positions $R_1$ and $R_2$ led to the conclusion that a benzyl group in position 4 of the triazole ring or a two-carbon atom chain bearing a phenyl or an indole group in position 5 of the triazole ring, keeping an α-aminoisobutyryl (Aib) moiety as the $R_3$ group (see Figure 1), yields the most potent compounds. Furthermore, the introduction of a 4-methoxybenzyl or a 2,4-dimethoxybenzyl group in position 4 of the triazole ring yielded GHS-R1a antagonists.[10] Thus, compounds JMV2806 and JMV2844 (Figure 2) (respectively, compounds **18a** and **19c** in ref 10) were found to be potent GHS-R1a ligands with agonist and antagonist activities, respectively.

More recently,[11] we investigated the significance of the Aib moiety by replacing it with natural or unnatural α-amino acids and piperidine- and piperazine-carboxyl groups. Contrary to previous unsuccessful attempts with compound JMV1843,[12] a potent GHS-R1a agonist active in man by oral administration,[13,14]

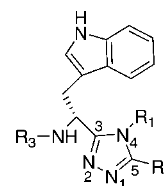


**Figure 1.** General formula for the trisubstituted 1,2,4-triazoles.

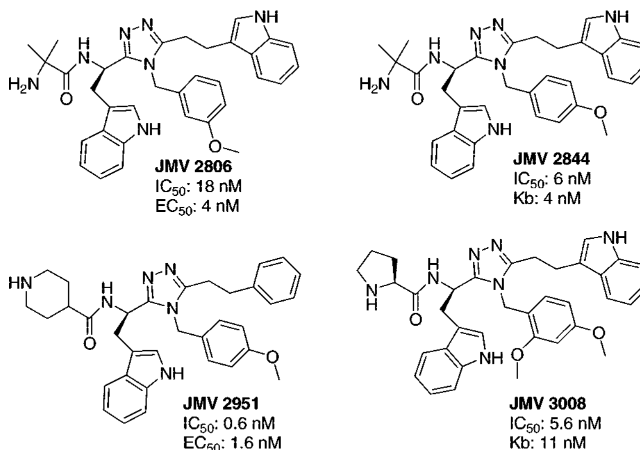


**Figure 2.** Previously described 1,2,4-triazole based GHS-R1a ligands.

we have shown that it was possible to maintain or even enhance GHS-R1a affinity by introducing a large variety of substituents in place of the Aib moiety in this triazole series. We were also able to modulate the agonist or antagonist character of the molecule by modifying this $R_3$ group. We described subnanomolar GHS-R1a agonists such as compound JMV2951 and also potent antagonists such as compound JMV3008 (Figure 2) (respectively **40** and **20** in ref 11).

In our ongoing efforts to target new potent selective and orally active GHS-R1a ligands, and particularly antagonists, we now describe the replacement of the Aib moiety by aromatic or heteroaromatic groups such as benzyl, phenyl-carboxyl, pyridinyl-carboxyl, pyridinyl-acetyl, or pyrazine-carboxyl groups.

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[a] Abbreviations: GHS-R1a, growth hormone secretagogue receptor type 1a; GH, growth hormone; Aib, α-aminoisobutyric acid; CHO, Chinese hamster ovary.

10.1021/jm701292s CCC: $40.75 © 2008 American Chemical Society
Published on Web 01/15/2008

**Table 1.** Binding Affinity Constants and Biological Activities of Trisubstituted 1,2,4-Triazoles

| compd | $R_1$ | $R_2$ | $R_3$ | $IC_{50}$ (nM)[a] | % of maximum $[Ca^{2+}]_i$ response at 10 $\mu$M[b] | $EC_{50}$ (nM)[c] | $K_b$ (nM)[d] |
|---|---|---|---|---|---|---|---|
| **1** | 2,4-dimethoxybenzyl | 1*H*-indole-3-yl-ethyl | aminoisobutyryl | 108 ± 17 | 0 | | 14 ± 2 |
| **2** | 4-methoxybenzyl | phenethyl | aminoisobutyryl | 11 ± 4 | 0 | | 5 ± 1 |
| **3** | 2,4-dimethoxybenzyl | phenethyl | aminoisobutyryl | 60 ± 10 | 0 | | 17 ± 7 |
| **4** | 2,4-dimethoxybenzyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)carboxyl | 1.8 ± 0.4 | 0 | | 19 ± 11 |
| **5** | 2,4-dimethoxybenzyl | phenethyl | (pyridin-2-yl)carboxyl | 1.1 ± 0.5 | 0 | | 25 ± 3 |
| **6** | 2,4-dimethoxyphenyl | phenethyl | (pyridin-2-yl)carboxyl | 33 ± 14 | 0 | | 65 ± 9 |
| **7** | 4-ethylbenzyl | phenethyl | (pyridin-2-yl)carboxyl | 67 ± 15 | 0 | | 513 ± 45 |
| **8** | 4-ethylphenyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)carboxyl | 49 ± 9 | 0 | | 129 ± 27 |
| **9** | 4-methoxybenzyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)carboxyl | 1.9 ± 0.6 | 0 | | 102 ± 20 |
| **10** | 4-methoxybenzyl | phenethyl | (pyridin-2-yl)carboxyl | 0.7 ± 0.2 | 0 | | 12 ± 3 |
| **11** | phenyl | phenethyl | (pyridin-2-yl)carboxyl | 105 ± 19 | 0 | | ND |
| **12** | phenyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)carboxyl | 160 ± 20 | 0 | | ND |
| **13** | 2,4-dimethoxybenzyl | phenethyl | (pyridin-4-yl)carboxyl | 58 ± 1 | 0 | | 93 ± 23 |
| **14** | 4-ethylbenzyl | phenethyl | (pyridin-4-yl)carboxyl | >1000 | 0 | | ND |
| **15** | 4-methoxybenzyl | phenethyl | (pyridin-4-yl)carboxyl | 220 ± 70 | 0 | | ND |
| **16** | 2,4-dimethoxybenzyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)acetyl | 34 ± 6 | 0 | | 14 ± 3 |
| **17** | 2,4-dimethoxyphenyl | phenethyl | (pyridin-2-yl)acetyl | 120 ± 30 | 3 | | 35 ± 2 |
| **18** | 4-ethylbenzyl | phenethyl | (pyridin-2-yl)acetyl | 51 ± 20 | 0 | | 124 ± 44 |
| **19** | 4-ethylphenyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)acetyl | 96 ± 33 | 0 | | 34 ± 11 |
| **20** | 4-methoxybenzyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)acetyl | 7.9 ± 0.0 | 0 | | 12 ± 4 |
| **21** | 4-methoxybenzyl | phenethyl | (pyridin-2-yl)acetyl | 3 ± 1 | 6 | | 6 ± 3 |
| **22** | benzyl | 1*H*-indole-3-yl-ethyl | (pyridin-2-yl)acetyl | 61 ± 34 | 4 | | 41 ± 7 |
| **23** | phenyl | phenethyl | (pyridin-2-yl)acetyl | 700 ± 250 | 0 | | ND |
| **24** | 4-ethylbenzyl | phenethyl | (pyridin-3-yl)acetyl | 480 ± 120 | 3 | | ND |
| **25** | 4-methoxybenzyl | phenethyl | (pyridin-3-yl)acetyl | 29 ± 0 | 3 | | 93 ± 2 |
| **26** | 4-methoxybenzyl | 1*H*-indole-3-yl-ethyl | (pyridin-4-yl)acetyl | 76 ± 1 | 3 | | 10 ± 3 |
| **27** | 4-methoxybenzyl | phenethyl | (pyridin-4-yl)acetyl | 270 ± 50 | 6 | | ND |
| **28** | 4-methoxybenzyl | phenethyl | (pyridin-3-yl)propionyl | 177 ± 78 | 5 | | ND |
| **29** | 4-methoxybenzyl | phenethyl | phenylcarboxyl | 80 ± 20 | 5 | | 23 ± 0.3 |
| **30** | 2,4-dimethoxybenzyl | phenethyl | phenylcarboxyl | 11.8 ± 4.0 | 31 ± 7 | 16 ± 4 | |
| **31** | 2,4-dimethoxybenzyl | phenethyl | benzyl | >1000 | 20 ± 2 | ND | |
| **32** | 2,4-dimethoxybenzyl | 1*H*-indole-3-yl-ethyl | 2-aminophenylcarboxyl | 112 ± 17 | 7 | | ND |
| **33** | 2,4-dimethoxybenzyl | 1*H*-indole-3-yl-ethyl | (cis)2-aminocylcohexylcarboxyl | 140 ± 10 | 4 | | 140 ± 50 |
| **34** | 2,4-dimethoxybenzyl | 1*H*-indole-3-yl-ethyl | pyrazine-2-carboxyl | 18.5 ± 5.0 | 0 | | 97 ± 24 |
| **35** | 2,4-dimethoxybenzyl | phenethyl | pyrazine-2-carboxyl | 5 ± 1 | 0 | | 24 ± 5 |
| **36** | 4-methoxybenzyl | phenethyl | pyrazine-2-carboxyl | 14 ± 8 | 0 | | 530 ± 110 |
| **37** | 4-ethylbenzyl | phenethyl | pyrazine-2-carboxyl | 89 ± 26 | 0 | | 76 ± 11 |
| **38** | 2,4-dimethoxyphenyl | phenethyl | pyrazine-2-carboxyl | 300 ± 70 | 1 | | ND |

[a] Specific binding was determined by incubation of membranes from GHS-R1a transfected LLC PK-1 cells with $^{125}$I-His$^9$-ghrelin in the presence of increasing concentrations of compounds. [b] The activation percentage for each compound ($10^{-5}$ M) was assessed relatively to the maximal response, obtained with ghrelin at $10^{-7}$ M (100%). [c] The signaling through the GHS-R1a, as determined by the accumulation of intracellular calcium and thus fluorescence output, was measured in the presence of increasing concentrations of agonists. [d] The ability of the antagonists to inhibit ghrelin signaling through the GHS-R1a (measurement of flurorescence output) was assessed using Schild plots, with increasing concentrations of ghrelin alone or in the presence of antagonist compound at $10^{-8}$, $10^{-7}$, or $10^{-6}$ M.

## Chemistry

To study the effect of Aib replacement, 1,2,4-triazoles were prepared as previously described[10,15] from Boc-D-Trp-OH and various substitutes of Aib were introduced after deprotection of the primary amine.

All compounds described in this paper were obtained by coupling a carboxylic function on the primary amine, except compound **31** which was obtained by reaction of benzyl chloride with the corresponding amine in dichloromethane in the presence of sodium bicarbonate as a base. Keeping the starting material Boc-D-Trp-OH and in a few steps, we could prepare interesting molecules representing three points of diversity, thus allowing a structure–activity relationship study.

## Results and Discussion

The synthesized compounds were tested for their ability to displace $^{125}$I-His$^9$-ghrelin from the cloned hGHS-1a receptor transiently expressed in LLC PK-1 cells. Binding affinities of human ghrelin and MK-0677 on these cells were in accordance with values reported in the literature. The biological in vitro activity of the compounds ($10^{-5}$ M) was then evaluated on intracellular calcium mobilization $[Ca^{2+}]_i$ using CHO cells transiently expressing GHS-R1a. Results are expressed as a percent of the maximal response induced by $10^{-7}$ M ghrelin (Table 1). Compounds **1**−**3**, containing the Aib moiety, are given as reference compounds.[10] The best compounds were tested in vivo for their ability to stimulate food intake or to inhibit hexarelin-stimulated food intake.

We previously described[11] that the best GHS-R1a ligands of our series had an isonipecotyl moiety in position $R_3$, i.e., a six-atom ring with a nitrogen atom in a position para to the carbonyl group. We so evaluated the influence of the introduction of heteroaryl group containing a nitrogen atom in the same position on the binding affinity and we first introduced a pyridyl moiety. (Pyridin-2-yl)carboxyl group replaced favorably the Aib residue, yielding compounds with high affinity for the ghrelin receptor. When comparing **1**, **2**, and **3** possessing an Aib with **4**, **10**, and **5** having a (pyridin-2-yl)carboxyl group with $R_1$ and $R_2$ unchanged, the affinity constants were found to be 60-, 56-, and 13-fold better for the last compounds with nanomolar affinity (**4**, $IC_{50}$ = 1.8 ± 0.4 nM; **5**, $IC_{50}$ = 1.1 ± 0.5 nM; **10**, $IC_{50}$ = 0.7 ± 0.2 nM) (Table 1). As previously described, 4-methoxybenzyl or 2,4-dimethoxybenzyl groups in position $R_1$ gave the best ligands. When 2,4-dimethoxyphenyl (**6**), 4-ethylbenzyl (**7**), and 4-ethylphenyl (**8**) groups were placed in the $R_1$ position, the affinities decreased from 33 to 67 nM.

**Table 2.** Cumulative Food Intake in Rats after Subcutaneous Administration of 160 μg/kg Compound and/or 80 μg/kg Hexarelin

| compd | cumulative food intake[a] at 2 h for 160 μg of compound (cumulative food intake at 2 h for 80 μg of hexarelin) | cumulative food intake[a] at 2 h for 160 μg compound + 80 μg of hexarelin | cumulative food intake[a] at 6 h for 160 μg compound (cumulative food intake at 6 h for 80 μg of hexarelin) | cumulative food intake[a] at 6 h for 160 μg compound + 80 μg of hexarelin | % variation versus hexarelin at 6 h[b] |
|---|---|---|---|---|---|
| saline | 0.18 ± 0.11 | | 0.49 ± 0.18 | | |
| **1** | 0.02 ± 0.01 (0.83 ± 0.30) | 0.06 ± 0.03 | 0.06 ± 0.04 (0.91 ± 0.38) | 0.33 ± 0.21 | −64 |
| **2** | 0.01 ± 0.0 (0.63 ± 0.23) | 0.53 ± 0.21 | 0.20 ± 0.19 (0.70 ± 0.21) | 0.63 ± 0.20 | −10 |
| **4** | 0.30 ± 0.11 (0.57 ± 0.17) | 0.72 ± 0.16 | 0.32 ± 0.13 (0.98 ± 0.19) | 1.03 ± 0.12 | +5 |
| **5** | 0.01 ± 0.0 (0.43 ± 0.16) | 0.01 ± 0.0 | 0.11 ± 0.10 (0.69 ± 0.20) | 0.02 ± 0.01 | −97 |
| **9** | 0.14 ± 0.14 (1.03 ± 0.21) | 0.47 ± 0.20 | 0.35 ± 0.15 (1.45 ± 0.19) | 0.73 ± 0.24 | −50 |
| **10** | 0.29 ± 0.17 (0.58 ± 0.13) | 0.37 ± 0.11 | 0.43 ± 0.25 (0.88 ± 0.09) | 0.75 ± 0.20 | −15 |
| **13** | 0.01 ± 0.0 (0.41 ± 0.12) | 0.56 ± 0.10 | 0.02 ± 0.01 (0.73 ± 0.21) | 0.87 ± 0.14 | +19 |
| **16** | 0.02 ± 0.01 (0.43 ± 0.16) | 0.37 ± 0.18 | 0.02 ± 0.01 (0.69 ± 0.20) | 0.64 ± 0.23 | −7 |
| **18** | 0.01 ± 0.0 (1.06 ± 0.15) | 0.89 ± 0.32 | 0.62 ± 0.30 (1.39 ± 0.29) | 0.91 ± 0.32 | −34 |
| **21** | 0.01 ± 0.0 (0.26 ± 0.11) | 0.43 ± 0.23 | 0.01 ± 0.0 (0.73 ± 0.11) | 0.87 ± 0.38 | +19 |
| **25** | 0.01 ± 0.0 (0.46 ± 0.15) | 0.13 ± 0.13 | 0.28 ± 0.17 (0.72 ± 0.17) | 0.46 ± 0.25 | −36 |
| **27** | 0.01 ± 0.0 (0.54 ± 0.17) | 0.55 ± 0.34 | 0.02 ± 0.0 (0.59 ± 0.17) | 0.56 ± 0.33 | −5 |
| **33** | 0.01 ± 0.0 (0.28 ± 0.08) | 0.01 ± 0.01 | 0.08 ± 0.06 (0.88 ± 0.26) | 0.48 ± 0.14 | −45 |
| **34** | 0.52 ± 0.28 (0.69 ± 0.27) | 0.11 ± 0.07 | 0.53 ± 0.28 (0.73 ± 0.27) | 0.20 ± 0.09 | −73 |
| **35** | 0.01 ± 0.0 (0.29 ± 0.11) | 0.42 ± 0.15 | 0.14 ± 0.08 (0.60 ± 0.15) | 1.04 ± 0.15 | +73 |
| **36** | 0.41 ± 0.33 (0.58 ± 0.13) | 0.72 ± 0.18 | 0.91 ± 0.17 (0.88 ± 0.09) | 0.81 ± 0.20 | −8 |

[a] Expressed as g of food intake per 100 g of body weight. [b] 100 × (cumulative food intake at 6 h for compound and hexarelin minus cumulative food intake at 6 h for hexarelin)/(cumulative food intake at 6 h for hexarelin).

Substitution with a phenyl group in $R_1$ position (**11** and **12**) resulted in a significant decrease of affinity. When the pyridin-2-yl moiety was replaced with the pyridin-4-yl group, the affinity constant decreased from 1.1 ± 0.5 nM (**5**) to 58 ± 1 nM (**13**) and from 0.7 ± 0.2 nM (**10**) to 220 ± 70 nM (**15**). Compound **14** with a 4-ethylbenzyl group in the $R_1$ position did not present any affinity toward the GHS-1a receptor (compared to **7**). These results pointed out the significance of the nitrogen atom position in the $R_3$ group for affinity to the GHS-R1a.

We then introduced a methylene group between the pyridyl and the carbonyl moieties (**16**−**27**). Clearly, the introduction of more flexibility in this part of the molecule produced less affine compounds (compare **4** and **16** or **5** and **17**, for example). However, **20** and **21** are exceptions because they displayed a nanomolar range affinity. (Pyridin-3-yl)- and (pyridin-4-yl)acetyl containing compounds (**24**−**27**) were found to be less affine toward the GHS-1a receptor, even with the optimized $R_1$ and $R_2$ substituents. As previously observed, replacement of the 4-methoxybenzyl group in the $R_1$ position by phenyl (**23**) or 4-ethylbenzyl groups (**24**) yielded compounds with decreased affinity. Addition of an extra methylene between the heteroaryl and the carbonyl group led to a weak ligand (**28**). Suppression of the heteroatom in $R_3$ produced less affine compounds (compare **29** and **10** or **30** and **5**) showing the significance of the nitrogen atom for interaction with the GHS-R1a. When the carbonyl group in $R_3$ was reduced to a methylene group, the resulting **31** presented an $IC_{50}$ superior to 1000 nM, demonstrating the significance of the carbonyl group for binding to the receptor. Introduction of an amine function as a substituent in position 2 of the aryl ring in $R_3$ moiety did not improve the binding (**32**) while dearomatization did not yield to a significant loss of affinity (**33**). The replacement of the carbonyl group by a sulfonyl moiety in $R_3$ position led to moderate or bad ligands (data not shown in Table 1). Introduction of a second nitrogen atom in the aromatic ring of $R_3$ was well tolerated for the binding to the GHS-1a receptor as assessed by compounds containing a pyrazine ring, the best compound exhibiting an $IC_{50}$ of 5 ± 1 nM (**35** to compare with **2** and **10**). As previously observed, replacement of the 4-methoxybenzyl group by an 4-ethylbenzyl in the $R_1$ position decreased the affinity constant (**37**) while suppression of the methylene moiety in the $R_1$ benzyl group led to **38** with a lower $IC_{50}$.

When tested for their ability to induce intracellular calcium mobilization, most of the compounds were not able to activate the receptor. This ability to activate the GHS-1a receptor and promote a biological response was determined for all compounds by assessing the increase of intracellular calcium levels. The activation percentage for each compound ($10^{-5}$ M) was assessed relative to the maximal response obtained with ghrelin ($10^{-7}$ M, 100%). $EC_{50}$ for agonist compounds with high affinity and $K_b$ for antagonist compounds with high affinity were determined as described,[10,11] and these values are reported in Table 1.

Most of the compounds described in this paper exhibited an antagonist activity toward the GHS-R1a except **30** and **31**. Compound **31** exhibited a very low binding affinity. We only determined the $EC_{50}$ for **30** (Table 1), which was found to be a weak partial agonist with a maximal response of about 30% of the maximal response induced by $10^{-7}$ M ghrelin. Most of the compounds that were synthesized were not able to promote $[Ca^{2+}]_i$ accumulation, although they were able to bind to the GHS-1a receptor with moderate to high affinities. They were able to antagonize the $[Ca^{2+}]_i$ accumulation induced by $10^{-7}$ M ghrelin. Compounds **4** ($K_b$ = 19 ± 11 nM), **5** ($K_b$ = 25 ± 3 nM), **9** ($K_b$ = 102 ± 20 nM), **10** ($K_b$ = 12 ± 3 nM), **16** ($K_b$ = 14 ± 3 nM), **20** ($K_b$ = 12 ± 4 nM), **21** ($K_b$ = 6 ± 3 nM), **29** ($K_b$ = 23 ± 0.3 nM), and **35** ($K_b$ = 24 ± 5 nM) were among the most potent ghrelin receptor antagonists. They were able to antagonize dose-dependently ghrelin-induced $[Ca^{2+}]_i$ accumulation in CHO cells transiently transfected with the GHS-R1a. Interestingly, in the presence of various concentration of these compounds, the dose-response curves of ghrelin on $[Ca^{2+}]_i$ accumulation were shifted rightward in a parallel manner, indicating a competitive antagonism.

Selected compounds were then tested in vivo in rats for their activity on food intake (subcutaneous injection) (Table 2). Compounds were first evaluated alone for their effect on food intake and then for their ability to inhibit hexarelin-stimulated food intake (80 μg of hexarelin). A significant increase in cumulative food intake was found for **34** and **36** when administered alone at 2 and 6 h (Table 2). These compounds behaved as ghrelin antagonists in vitro with $K_b$ values of 97 and 530 nM, respectively. All other compounds did not significantly increase food intake when administered alone. Eight of them were able to inhibit food intake even at 6 h (**1**, **5**, **13**,

**Table 3.** Inhibitory Activity by Graded Doses of Compounds **5** and **34** on Feeding Stimulated by 80 μg/kg Hexarelin[a]

| compd | food intake at 2 h | food intake at 6 h |
|---|---|---|
| hexarelin, 80 μg | 1.00 ± 0.26 | 1.01 ± 0.26 |
| **5**, 20 μg/kg | 0.56 ± 0.11 | 0.56 ± 0.11 |
| **5**, 80 μg/kg | 0.02 ± 0.01 | 0.02 ± 0.01 |
| **5**, 160 μg/kg | 0.21 ± 0.11 | 0.21 ± 0.11 |
| **5**, 320 μg/kg | 1.23 ± 0.37 | 1.23 ± 0.37 |
| hexarelin, 80 μg | 1.25 ± 0.26 | 1.26 ± 0.26 |
| **34**, 20 μg/kg | 0.66 ± 0.23 | 0.66 ± 0.23 |
| **34**, 80 μg/kg | 0.23 ± 0.13 | 0.23 ± 0.13 |
| **34**, 160 μg/kg | 0.29 ± 0.09 | 0.31 ± 0.10 |
| **34**, 320 μg/kg | 0.20 ± 0.09 | 0.21 ± 0.09 |

[a] Cumulative food intake at 2 and 6 h expressed in g of food per 100 g of body weight.

**Table 4.** GH Secretion in Infant Rat (sc Injection)[a]

| compd | [rGH], ng/mL |
|---|---|
| saline | 5.55 ± 1.62 |
| hexarelin | 187.58 ± 22.68 |
| **2** | 5.28 ± 0.60 |
| **2** + hexarelin | 220.52 ± 15.52 |
| saline | 5.24 ± 0.73 |
| hexarelin | 170.10 ± 13.23 |
| **5** | 13.06 ± 2.17 |
| **5** + hexarelin | 138.39 ± 14.58 |
| saline | 10.72 ± 2.02 |
| hexarelin | 253.82 ± 12.27 |
| **34** | 15.32 ± 1.35 |
| **34** + hexarelin | 173.61 ± 18.44 |

[a] Hexarelin was tested at a dose of 80 μg/kg, and compounds were tested at a dose of 160 μg/kg.

**16**, **21**, **27**, **33**, and **35**). When considering the coadministration with hexarelin, only **5** and **34** were able to inhibit hexarelin-induced food intake by −97% and −73%, respectively, at 6 h. Some other compounds were found to be less efficient at inhibiting hexarelin-stimulated food intake (**1**, −64%; **9**, −50%; **18**, −34%; **25**, −36%; **33**, −45%). However, unexpectedly, **35**, without effect when administered alone, was found to be able to potentiate food intake induced by hexarelin (+73%). Compounds **13** and **21**, without effect alone, also seemed to stimulate food intake induced by hexarelin but in a weaker way (+19%).

Although, as it was reported previously,[10,11] some of the discrepancies between in vitro and in vivo results on food intake were difficult to understand and explain (i.e., **35**), it clearly appeared in this study that in vitro potent GHS-R1a antagonists such as **5** and **34** were also efficient in antagonizing hexarelin-stimulated food intake.

A dose-response study for the two most active compounds (**5** and **34**) was conducted with rats at various doses (20, 80, 160, and 320 μg/kg) along with a 80 μg/kg hexarelin-stimulated food intake. Results are in Table 3. Compound **34** displayed a well correlated progressive dose-response curve inhibition of hexarelin stimulated cumulative food intake. The lowest 20 μg/kg dose was able to inhibit 47% of hexarelin-stimulated feeding behavior at 6 h, while higher doses inhibited 75−83% of hexarelin-stimulated food intake. Compound **5** was found to be efficient in suppressing feeding at very low doses: −44% inhibition of food intake at 20 μg/kg and −98% inhibition at 80 μg/kg. Surprisingly, its efficiency decreased at a dose of 160 μg/kg and no effect was obtained at the highest dose (320 μg/kg).

Information on the toxicity of the compounds could be obtained by the observation of adult rats that received subcutaneous doses of the compounds (20–320 μg/kg) for assessing the effect on food intake. We did not obtain mortality with any of the tested compounds, and the observation of the animals during the 6 h of the food intake experiments did not reveal altered locomotion behavior.

The binding affinity of a model compound, **5**, was tested at 10 μM versus a panel of 92 GPCRs including cannabinoids, melanocortins, and NPY receptors (MDS Pharma Services' GPCR screen). Only GHS-R1a, CCKI, motilin, tachykinin 2, and vasopressin 1a receptors showed significant binding with this compound. However, the binding affinity to the ghrelin receptor GHS-R1a was 1000 times higher than the affinity to the other receptors.

Three selected antagonists (**2**, **5**, and **34**) were tested for their ability to stimulate/inhibit GH secretion in infant rats after sc administration, in the presence or not of hexarelin (Table 4). Indeed, it is known that in the baby rats the pulsatility of the GH secretion is not yet activated at the hypothalamic level, and therefore, a constant GH blood level is measured in these young animals.

As expected, when administered alone, these GHS-R1a antagonists did not elicit significant GH release. Unexpectedly, these GHS-R1a antagonists were unable to significantly inhibit GH secretion stimulated by hexarelin.

When regarding both GH secretion and food intake responses, **5** and **34**, both GHS-R1a antagonists of this new series, inhibited food intake induced by hexarelin but not hexarelin-stimulated GH secretion. These results support our previous observations[10,11] showing the possible modulation of the ghrelin effect on appetite without altering GH secretion. To assess the medical relevance of this observation, these compounds will be tested in various obesity animal models.

## Conclusion

Replacement of the Aib moiety, often present in GHS-R1a ligands, by a heteroaryl-carbonyl or a heteroarylalkyl-carbonyl group led to potent antagonists of the GHS-1a receptor. These compounds were easily synthesized from commercially available materials and contain only one asymmetric center. The most potent compounds in this series were tested in vivo for their activity on food intake in the rat. Compounds **5** and **34**, both in vitro antagonists of the GHS-R1a, were found to be the most potent compounds in inhibiting hexarelin-induced food intake in the rat. Furthermore, they did not show any activity on GH secretion in the rat and did not inhibit hexarelin-stimulated GH secretion. It has been previously demonstrated that the ghrelin receptor couples to Gq/11 and to G12/13 G proteins,[16] and a recent study describes compounds able to activate the ghrelin receptor through one or the other signaling pathway.[17] Our results suggest that some of the compounds described in this study selectively target the mechanism of action of the ghrelin receptor that correlated with food intake and not with GH secretion. Further investigations are ongoing to explain these observations.

## Experimental Section

**Chemistry.** Compounds were synthesized as previously described.[10,11] All final compounds were purified by reversed-phase HPLC. The purity assessed by analytical reversed-phase C18 HPLC was found to be greater than 95% for target compounds and greater than 98% for key target compounds (**4**, **5**, **10**, **13**, **16**, **20**, **21**, **34**, **35**, and **36**), and the structures were confirmed by MS (electrospray), $^{1}H$ NMR, and $^{13}C$ NMR for the most interesting compounds.

***(R)*-*N*-(1-(4-(2,4-Dimethoxybenzyl)-5-phenethyl-4*H*-1,2,4-triazol-3-yl)-2-(1*H*-indol-3-yl)ethyl)picolinamide (5).** $^{1}H$ NMR (300 MHz, DMSO-$d_6$, 300 K): δ 2.83 (m, 2H, $\underline{CH_2}$−$CH_2$−phenyl), 2.90 (m, 2H, $CH_2$−$\underline{CH_2}$−benzyl), 3.48 (m, 2H, $CH_2$ βTrp), 3.57 (s, 3H,

$OCH_3$), 3.61 (s, 3H, $OCH_3$), 4.97 (d, 1H, $J = 17$ Hz, $CH_2$ *o*,*p*-dimethoxybenzyl), 5.09 (d, 1H, $J = 17$ Hz, $CH_2$-*o*,*p*-dimethoxybenzyl), 5.56 (m, 1H, CH αTrp), 6.18 (dd, 1H, $J_o = 8$ Hz and $J_m = 2$ Hz, $H_5$ *o*,*p*-dimethoxybenzyl), 6.41 (d, 1H, $J_m = 2$ Hz, $H_3$ *o*,*p*-dimethoxybenzyl), 6.55 (d, 1H, $J_o = 8$ Hz, $H_6$ *o*,*p*-dimethoxybenzyl), 6.87 (t, 1H, $J_o = 8$ Hz, $H_5$ Trp), 7.01 (t, 1H, $J_o = 8$ Hz, $H_6$ Trp), 7.08 (m, 3H, $H_2$ Trp, $H_2$ and $H_6$ phenyl), 7.14 (d, 1H, $J_o = 7$ Hz, $H_4$ Trp), 7.19–7.31 (m, 4H, $H_7$ Trp, $H_3$, $H_4$ and $H_5$ phenyl), 7.56 (t, 1H, $J = 8$ Hz, NH amide), 7.91 (m, 2H, $H_4$ and $H_5$ *o*-pyridyl), 8.57 (d, 1H, $J_{\alpha\beta} = 5$ Hz, $H_6$ *o*-pyridyl), 9.16 (d, 1H, $J_o = 8$ Hz, $H_3$ *o*-pyridyl), 10.80 (s, 1H, NH indole Trp). $^{13}$C NMR (75 MHz, DMSO-$d_6$, 300 K): δ 26.2 ($\underline{C}H_2-CH_2-$phenyl), 28.8 (C βTrp), 32.1 ($CH_2-\underline{C}H_2-$phenyl), 43.0 ($CH_2$ *o*,*p*-dimethoxybenzyl), 45.5 (C αTrp), 55.6 ($OCH_3$), 55.8 ($OCH_3$), 98.9 ($C_3$ *o*,*p*-dimethoxybenzyl), 105.0 ($C_5$ *o*,*p*-dimethoxybenzyl), 109.5 ($C_3$ Trp), 111.8 ($C_7$ Trp), 114.4 ($C_1$ *o*,*p*-dimethoxybenzyl), 118.4 ($C_4$ Trp), 118.8 ($C_5$ Trp), 121.4 ($C_6$ Trp), 122.4 ($C_3$ *o*-pyridyl), 124.4 ($C_2$ Trp), 126.7 ($C_6$ *o*,*p*-dimethoxybenzyl), 127.2 ($C_5$ *o*-pyridyl), 127.5 ($C_9$ Trp), 128.6–128.8 ($C_2$, $C_3$, $C_4$, $C_5$, and $C_6$ phenyl), 136.4 ($C_8$ Trp), 138.1 ($C_4$ *o*-pyridyl), 140.2 ($C_1$ phenyl), 148.8 ($C_6$ *o*-pyridyl), 149.3 ($C_2$ *o*-pyridyl), 155.2 (Cq triazole), 155.4 (Cq triazole), 157.9 ($C_2$ *o*,*p*-dimethoxybenzyl), 161.0 ($C_4$ *o*,*p*-dimethoxybenzyl), 163.9 (CO amide).

**(*R*)-*N*-(1-(5-(2-(1*H*-Indol-3-yl)ethyl)-4-(2,4-dimethoxybenzyl)-4*H*-1,2,4-triazol-3-yl)-2-(1*H*-indol-3-yl)ethyl)pyrazine-2-carboxamide (34).** $^1$H NMR (300 MHz, DMSO-$d_6$, 300 K): δ 3.01 (m, 2H, $C\underline{H_2}-CH_2-$indole), 3.10 (m, 2H, $CH_2-C\underline{H_2}-$indole), 3.51 (m, 2H, $CH_2$ βTrp), 3.55 (s, 3H, $OCH_3$), 3.57 (s, 3H, $OCH_3$), 5.15 (d, 2H, $J = 7$ Hz, $CH_2$ *o*,*p*-dimethoxybenzyl), 5.63 (m, 1H, CH αTrp), 6.08 (dd, 1H, $J_o = 8$ Hz and $J_m = 2$ Hz, $H_5$ *o*,*p*-dimethoxybenzyl), 6.35 (d, 1H, $J_m = 2$ Hz, $H_3$ *o*,*p*-dimethoxybenzyl), 6.53 (d, 1H, $J_o = 8$ Hz, $H_6$ *o*,*p*-dimethoxybenzyl), 6.89 (m, 2H, $H_5$ indole and $H_5$ Trp), 6.99 (m, 2H, $H_6$ indole and $H_6$ Trp), 7.08 (m, 2H, $H_2$ indole and $H_2$ Trp), 7.29 (m, 3H, $H_4$ Trp, $H_4$ and $H_7$ indole), 7.41 (d, 1H, $J_o = 8$ Hz, $H_7$ Trp), 8.61 (t, 1H, $J = 2$ Hz, $H_3$ *o*-pyrazinyl), 8.79 (d, 1H, $J = 2$ Hz, $H_5$ *o*-pyrazinyl), 8.94 (d, 1H, $J = 1$ Hz, $H_6$ *o*-pyrazinyl), 9.43 (d, 1H, $J = 8$ Hz, NH amide), 10.84 (s, 2H, NH indole and NH indole Trp). $^{13}$C NMR (75 MHz, DMSO-$d_6$, 300 K): δ 22.0 ($CH_2-\underline{C}H_2-$indole), 25.3 ($\underline{C}H_2-CH_2-$indole), 27.9 (C βTrp), 44.1 ($CH_2$ *o*,*p*-dimethoxybenzyl), 45.5 (C αTrp), 55.5 ($OCH_3$), 55.8 ($OCH_3$), 98.8 ($C_3$ *o*,*p*-dimethoxybenzyl), 104.9 ($C_5$ *o*,*p*-dimethoxybenzyl), 109.3 ($C_3$ Trp), 111.8 ($C_7$ indole and $C_7$ Trp), 112.1 ($C_3$ indole), 113.4 ($C_1$ *o*,*p*-dimethoxybenzyl), 118.4 ($C_4$ indole), 118.5 ($C_4$ Trp), 118.8 ($C_5$ indole and $C_5$ Trp), 121.5 ($C_6$ indole and $C_6$ Trp), 127.0 ($C_9$ indole), 127.4 ($C_9$ Trp), 136.4 ($C_8$ Trp), 136.6 ($C_8$ indole), 141.2 ($C_6$ *o*-pyrazinyl), 144.1 ($C_2$ *o*-pyrazinyl), 144.3 ($C_3$ *o*-pyrazinyl), 146.8 ($C_5$ *o*-pyrazinyl), 155.4 (Cq triazole), 156.0 (Cq triazole), 157.8 ($C_2$ *o*,*p*-dimethoxybenzyl), 161.0 ($C_4$ *o*,*p*-dimethoxybenzyl), 163.2 (CO amide).

Expression of the receptor, in vitro determination of the binding affinities and intracellular calcium mobilization assay, in vivo experiments in the rat for GH secretion, and food intake experiments were previously described.[10,11]

**Acknowledgment.** The authors thank Aeterna-Zentaris and the CNRS for financial support of this work and for providing a research grant for the Ph.D. thesis project of A.M. (Grant BDI 752776/01).

**Supporting Information Available:** Biological data; LC−MS chromatograms of **4**−**38**; table of physicochemical properties of **1**−**38**; general preparation of thioamides and triazoles; $^1$H and $^{13}$C NMR data of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM701292S