Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

New ligands of the ghrelin receptor based on the 1,2,4-triazole scaffold by introduction of a second chiral center

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ARTICLE INFO

Article history:
Received 16 February 2016
Revised 1 April 2016
Accepted 3 April 2016
Available online xxxx

Keywords:
1,2,4-Triazole scaffold
Ghrelin receptor (GHS-R1a)
Structural modulation
Chirality
Inverse agonist
Antagonist

ABSTRACT

Introducing a second chiral center on our previously described 1,2,4-triazole, allowed us to increase diversity and elongate the 'C-terminal part' of the molecule. Therefore, we were able to explore mimics of the substance P analogs described as inverse agonists. Some compounds presented affinities in the nanomolar range and potent biological activities, while one exhibited a partial inverse agonist behavior similar to a Substance P analog.

Ghrelin,[1] an orexigenic hormone essentially synthesized in the stomach, is the endogeneous ligand of the growth hormone secretagogue receptor (GHS-R1a).[2] Ghrelin is a peptide composed of 28 amino-acids, octanoylated on the seryl residue in position 3. This lipidation is essential for both binding to the receptor and biological activity.[3] Among its various biological functions, ghrelin stimulates the secretion of GH (Growth Hormone),[3] and food intake,[4] controls energy homeostasis[5] and gastrointestinal motility.[6] It has effects on cellular proliferation,[7] cardiovascular,[8] pancreatic, pulmonary and immune functions, memory and sleep.[9] More recently, it was established that ghrelin plays a role in addiction processes.[10] In our effort to find efficient ghrelin receptor ligands, we have developed a pseudo-peptide (JMV 1843),[11] which is a potent in vitro and in vivo agonist of the GHS-R1a. This compound contains a gem-diamino moiety (Fig. 1) and is orally active in man[12] as exemplified by an initial validation study.[13] Named macimorelin, it is evaluated in a test that detects adult growth hormone deficiency. A pivotal confirmatory study is currently recruiting participants.[14] We then focused on the search of GHS-R1a antagonists, which could lead to potential anti-obesity agents. We decided to explore non-peptide ligands of the GHS-R1a through heterocycles bearing the pharmacophore groups included in JMV 1843. We discovered that the 1,2,4-triazole scaffold presented an interesting approach to obtain ligands with high affinity toward the GHS-R1a (Fig. 1).[15,16]

The synthesis of the 1,2,4-triazole scaffold was efficiently performed starting from commercially available compounds. An easy access to tri-substituted 1,2,4-triazoles with four points of diversity was developed and optimized.[17] The first step of the synthesis included a *N*-protected α-amino acid whose configuration was conserved during the entire synthetic route. Several derivatives of these new active non-peptide compounds were synthesized. An intensive SAR study involving the four putative points of diversity was carried out. The following conclusions were made: (i) the preferred amino-acid starting material was D-tryptophan; (ii) on position 4 of the triazole, a 4-methoxy- or 2,4-dimethoxy-benzyl group preferentially led to receptor antagonists; (iii) a 2 carbon chain bearing a phenyl or an indole group was preferred in position 3; (iv) numerous acyl groups including α-aminoisobutyryl (Aib), pyridin-2-ylcarboxyl, and glycyl groups, could be introduced in R^3, modulating both the binding affinity and the biological activity.

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http://dx.doi.org/10.1016/j.bmcl.2016.04.003
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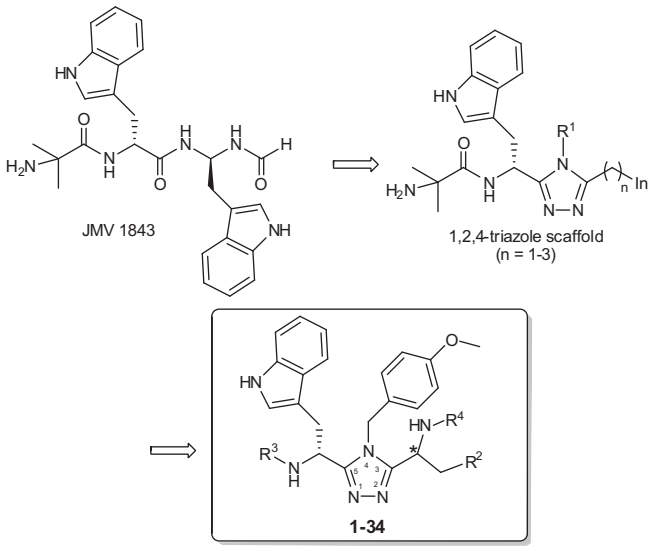

Figure 1. From pseudo-peptide to peptido-mimetic.

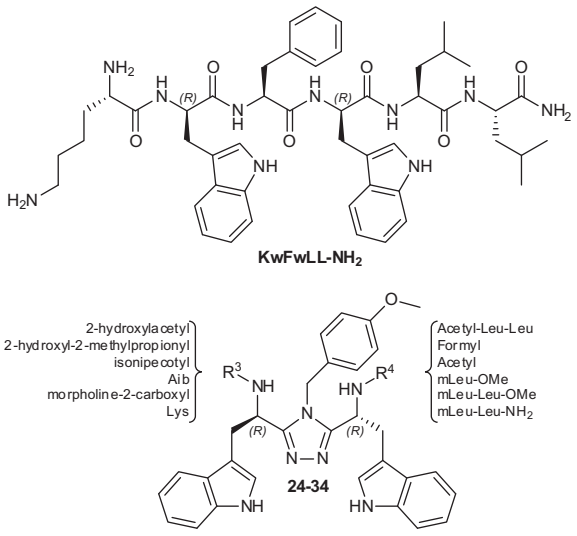

Figure 3. Similarity between KwFwLL-NH_2 and triazoles 24–34.

It is also interesting to notice that a simple atom change could shift an agonist compound into an antagonist (Fig. 2). Indeed, when the piperidine moiety of compound JMV 2951 [EC_{50} (Ca^{2+}) = 1.6 nM] was replaced by a tetrahydro-2*H*-pyran group (compound JMV 3168), the agonist character of the ligand was lost to the benefit of the antagonist character [IC_{50} (Ca^{2+}) = 60 nM].

As compound JMV 1843 includes in its C-terminal part a gem-diamino function, we then decided to introduce a chiral center in position 3 of our 1,2,4 triazole scaffold allowing the presence of an amine function at this position. This modification should lead to closer structures of compound JMV 1843 than the previous tri-substituted triazoles and could allow an additional point of diversity (Fig. 1). Moreover, a characteristic of this receptor is its high constitutive (ligands independent) activity, which reach about 50% of the maximal activity induced by ghrelin.[18] Although ghrelin receptor antagonists are able to reduce meal-associated food intake,[19] inverse agonists of the ghrelin receptor, by blocking the constitutive receptor activity, are expected to lower the set-point for hunger between meals.[20] The first GHS-R1a inverse agonist was reported by Holst et al. as a Substance P analog: [(D)Arg[1],(D)Phe[5],(D)Trp[7,9],Leu[11]]-substance P.[18] Later, extensive SAR studies identified new inverse agonist peptides with better specificity toward GHS-R1a than the Substance P analog.[21] In these papers, a core pentapeptide wFwLL-NH_2 was described as the minimal active sequence maintaining the inverse agonist activity. When a lysine residue was introduced at the N-terminal of this pentapeptide, a potent inverse agonist of the receptor was obtained.[21a] A striking structural similarity could be found between the hexapeptide KwFwLL-NH_2 described by Holst et al. and our JMV 1843 compound: the presence of two (D)tryptophan residues (Fig. 3). When comparing our triazole scaffold with this peptide, we can hypothesize that the benzyl group in position 4 of our scaffold can play the role of the phenylalanine residue. In that case, the new chiral center incorporating an amino function in position 3 of our triazole scaffold could allow the elongation with the Leu-Leu dipeptide sequence.

A new series of triazole derivatives was therefore designed and tested in order to find new efficient ligands, potentially inverse agonists, of the GHS-R1a.

In a first set of experiments (Table 1), the new chiral center was introduced by the reaction of the thioamide Boc-(D)Trp[S]-NH(pOMe)Benzyl with Cbz-(L) or (D)phenylalanine hydrazide or Cbz-(L) or (D)tryptophan hydrazide in the presence of silver benzoate to form the triazole scaffold bearing two chiral centers (Scheme 1). After removal of the Boc protecting group, diverse interesting R^3 acids were introduced to the amine function by conventional coupling. The Cbz protection was then removed by hydrogenolysis and the amino function was acylated or not with a formyl or acetyl group to lead to the final compounds. All final compounds were purified by reversed-phase preparative HPLC.[22] These compounds were tested for their affinity toward the GHS-R1a, their ability to induce intracellular calcium mobilization[15] and for confirmation of their agonist/antagonist character in a cyclic AMP response element CRE-luciferase reporter gene assay.[23]

Compounds **1** to **8** with the indole group as R^2 clearly showed that the R configuration of the new chiral center leads to ligands with a higher affinity than compounds with the S configuration of this carbon. The most potent compounds were obtained with Aib or picolinic acid at the N-terminus (compounds **5** and **8** with K_i values of 12 and 9 nM, respectively). As previously described, the picolinic acid was well tolerated at this position.[24] For compounds **9** to **18**, all containing the phenyl group as R^2, the best affinities were also obtained when the second chiral center was of R configuration. For this reason, compounds **19**–**23** were only synthesized in the R series. Acetylation of the amino function was preferred and only the N-terminal group was modulated. We introduced at this position acyl groups that gave good results in other series: 3-fluoro(pyridin-2-yl)carboxylic acid, 4,6-difluoro(pyridin-2-yl)carboxylic acid, 3,4-dihydro-2*H*-pyran-6-carboxylic acid, 2-hydroxylacetic acid, and (*S*) morpholine-2-carboxylic acid.[15] These modifications led to compounds with a high affinity toward the receptor, particularly compounds **22** and **23**, exhibiting

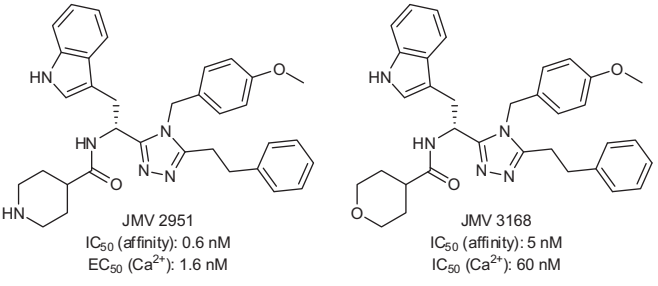

Figure 2. Shift from agonist (JMV2951) to antagonist (JMV3168).