Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1955–1957

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Spiro(indoline-3,4′-piperidine) Growth Hormone Secretagogues as Ghrelin Mimetics

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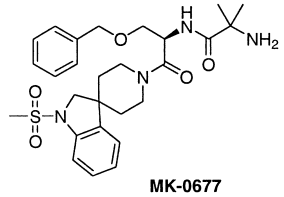
Received 23 April 2001; accepted 9 May 2001

Abstract—A series of small molecules derived from MK-0677, a potent synthetic GHS, mimicking the N-terminal Gly-Ser-*O*-(*n*-octanoyl)-L-Ser-Phe segment of ghrelin was synthesized and tested in a binding and in a functional assay measuring intracellular calcium elevation in HEK-293 cells expressing hGHSR1a. Replacement of Phe in this tetrapeptide with a spiro(indoline-3,4′-piperidine) group, Gly-Ser with 2-aminoisobutyric acid, and *O*-(*n*-octanoyl)-L-Ser with *O*-benzyl-D-Ser provided synthetic GHS agonists with similar functional potency as ghrelin.

Over the past decade, extensive research has been focused on identifying synthetic, nonpeptidyl growth hormone secretagogues (GHSs) as an alternative treatment to growth hormone replacement therapy.[1] For example, research at Merck & Co. has led to the discovery of MK-0677, one of the most potent peptidomimetic GHSs.[2] A comparison of the structural architecture of synthetic MK-0677 with natural endogenous ligand(s) was not previously possible because natural endogenous ligand(s) were not yet identified. A systematic comparison of the structural architecture of natural and synthetic GHSs has the potential to be utilized in the identification of new, perhaps more potent peptidomimetic GHSs.



MK-0677

Ghrelin was recently isolated and identified as an endogenous ligand of the GHS receptor(s).[3] This discovery has sparked interest in the structural features responsible for its interaction with GHSR1a. Evaluation of peptidyl ghrelin analogues revealed that the N-terminal tetrapeptide, Gly-Ser-*O*-(*n*-octanoyl)-L-Ser-Phe, is the smallest segment of ghrelin (28-amino acid peptide) which has agonist activity at hGHSR1a.[4] Based upon these studies, we wanted to examine if its structural features can be used with advantage in synthetic peptidomimetic GHS compounds (i.e., MK-0677).

Human Ghrelin

We speculated that our peptidomimetic GHS compounds may bind to the cloned hGHS1a receptor mimicking the Gly-Ser-*O*-(*n*-octanoyl)-L-Ser-Phe N-terminal sequence of ghrelin [ghrelin(1-4)]. In particular, we envisioned that the spiro(indoline-3,4′-piperidine) group binds to the receptor in the same region as Phe in the truncated N-terminal tetrapeptide of ghrelin, and that the dipeptide part-structure of MK-0677 binds to the receptor in the same region as Gly-Ser-*O*-(*n*-octanoyl)-L-Ser. Herein, we report the synthesis and evaluation of novel MK-0677 analogues containing N-terminal analogues of ghrelin, which show similar functional potency as ghrelin.

The spiro(indoline-3,4′-piperidine) compounds were prepared in a linear fashion as illustrated in Scheme 1.

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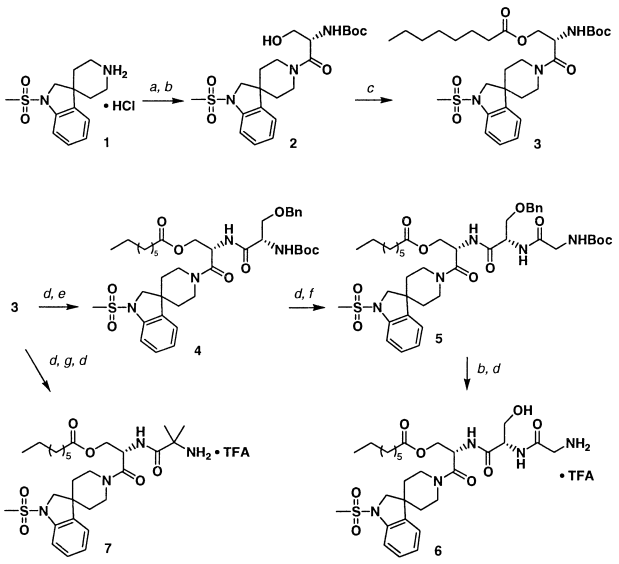
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00324-9



Scheme 1. Reagents and conditions: (a) *N*-Boc-L-serine-*O*-benzyl, EDC, HOBt, DIEA, CH_2Cl_2; (b) H_2, Pd/C, EtOH; (c) capryloyl chloride, pyridine, CH_2Cl_2; (d) 1:1 TFA–CH_2Cl_2; (e) *N*-Boc-L-serine-*O*-benzyl, EDC, HOBt, DIEA, CH_2Cl_2; (f) *N*-Boc-glycine, EDC, HOBt, DIEA, CH_2Cl_2; (g) *N*-Boc-aminoisobutyric acid, EDC, HOBt, DIEA, CH_2Cl_2.

Spiro(indoline-3,4′-piperidine) **1**[5] was coupled to *N*-Boc-L-serine-*O*-benzyl using 1-hydroxybenzotriazole hydrate (HOBt) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide·HCl (EDC). Removal of the benzyl protecting group via hydrogenation provided alcohol **2**, which was acylated with capryloyl chloride to yield ester **3**. Ester **3** was treated with trifluoroacetic acid (TFA) in methylene chloride to give the free amine which was then coupled with *N*-Boc-L-serine-*O*-benzyl to provide compound **4**. Another Boc deprotection reaction followed by a third amino acid coupling reaction with *N*-Boc-glycine afforded compound **5**. Final benzyl removal via hydrogenation and Boc removal with TFA provided spiro(indoline-3,4′-piperidine) **6** as a TFA salt. Analogue **7** was synthesized from ester **3** via removal of the Boc protecting group, coupling with *N*-Boc-aminoisobutyric acid (AIB), and final removal of the Boc protecting group.

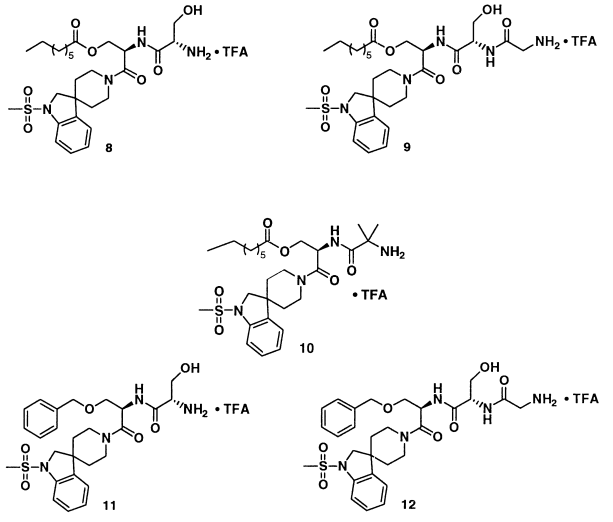


Figure 1. Spiro(indoline-3,4′-piperidine) analogues.

The spiro(indoline-3,4′-piperidine) analogues **8**–**12** shown in Figure 1 were prepared by modifications to the synthetic route described in Scheme 1. For instance, *N*-Boc-D-serine-*O*-benzyl was utilized in the first amino acid coupling reaction. This intermediate provided analogues **8**–**12** via the acylation, coupling, and deprotection methods shown in Scheme 1.

The spiro(indoline-3,4′-piperidine) compounds (**6**–**12**) were evaluated for their binding affinities to the cloned hGHSR1a via a competitive binding assay with [^{35}S]MK-0677 as the radiolabeled ligand. In addition, these compounds were also examined for functional potency via their ability to stimulate inositol triphosphate-coupled mobilization of intracellular calcium in HEK-293 cells expressing hGHSR1a. The data is presented in Table 1.

As shown in Table 1, replacement of Phe with *N*-methanesulfonyl spiro(indoline-3,4′-piperidine) (**6**) led to a 14- and 6-fold decrease in functional potency in comparison to ghrelin and ghrelin(1-4), respectively. MK-0677, however, contains an *O*-benzyl-D-serine amino acid, and this D-amino acid contributes importantly to its binding and functional potency.[2] Likewise, replacement of *O*-(*n*-octanoyl)-L-Ser with *O*-(*n*-octanoyl)-D-Ser provided a GHS agonist (**9**) with similar functional potency as ghrelin and ghrelin(1-4), but with a 7-fold improvement in binding potency in comparison with ghrelin(1-4). Therefore, it appears the spiro(indoline-3,4′-piperidine) group can serve as a replacement for Phe in ghrelin(1-4).

We also examined the binding and functional properties of additional ghrelin(1-4)/MK-0677-hybrid compounds with the cloned hGHS1a receptor. Thus, replacement of *O*-(*n*-octanoyl)-L-Ser in ghrelin(1-4) with the *O*-benzyl-D-serine amino acid in MK-0677 (**12**) led to a notable improvement in both binding and functional potency. The benzyl ether analogue (**12**) is 4- and 9-fold more potent than ghrelin and ghrelin(1-4), respectively, with moderate binding affinity. Interestingly, removal of the

Table 1. Binding and functional data of spiro(indoline-3,4′-piperidine) analogues of the ghrelin tetrapeptide segment

Compound	Binding assay[a] IC_{50} (nM)	Functional assay[b] EC_{50} (nM)
Human ghrelin	0.25[c]	32[c]
Ghrelin(1-4)[c]	889[c]	72[c]
MK-0677	0.63	1.43
6	2670	448.5
7	590.5	378.1
8	828.5	432.2
9	128	68.2
10	8.12	19.6
11	209	69.2
12	36.2	7.95

[a][^{35}S]MK-0677 binding assay.
[b]Aequorin bioluminescence assay.
[c]See ref 4.

glycine amino acid led to a compound (**11**) with similar functional potency to ghrelin and ghrelin(1-4), but with a 4-fold improvement in binding affinity compared to ghrelin(1-4).

Encouraged by the results of the dipeptidyl spiro(indoline-3,4′-piperidine) analogue (**11**), we evaluated whether aminoisobutyric acid (AIB) can be a replacement for Gly-Ser. Indeed, analogue **10** shows similar functional potency as ghrelin, but with a 32-fold decrease in binding affinity. In comparison with ghrelin(1-4), however, the AIB replacement (**10**) improves binding potency about 100-fold. More importantly, this AIB analogue (**10**) binds to the cloned hGHSR1a better than the corresponding Gly-Ser analogue (**9**). As discussed earlier, the *O*-(*n*-octanoyl)-L-Ser analogue (**7**) is less potent than the D-analogue (**10**). These studies emphasize the importance of stereochemistry in truncated versions of ghrelin as was the case with MK-0677 and its analogues.

In summary, we report the synthesis and evaluation of a series of MK-0677/ghrelin(1-4)-hybrid compounds as ghrelin mimetics. Replacement of Phe in the tetrapeptide with a spiro(indoline-3,4′-piperidine) group, Gly-Ser with 2-aminoisobutyric acid, and *O*-(*n*-octanoyl)-L-Ser with *O*-benzyl-D-Ser provided synthetic GHS agonists with similar functional potency as ghrelin. The binding affinities on the cloned hGHSR1a for the D-serine hybrid compounds (**8**–**12**) are equal to or greater than the truncated N-terminal ghrelin tetrapeptide, ghrelin(1-4), which is the smallest segment of ghrelin displaying agonist activity.

References and Notes

1. (a) Nargund, R. P.; Patchett, A. A.; Bach, M. A.; Murphy, M. G.; Smith, R. G. *J. Med. Chem.* **1998**, *41*, 3103. (b) Rosen, T.; Johannsson, G.; Johansson, J.-O.; Bengtsson, B.-A. *Horm. Res.* **1995**, *43*, 93. (c) Strobl, J. S.; Thomas, M. J. *Pharmacol. Rev.* **1993**, *46*, 1.

2. Patchett, A. A.; Nargund, R. P.; Tata, J. R.; Chen, M.-H.; Barakat, K. J.; Johnston, D. B. R.; Cheng, K.; Chan, W. W.-S.; Butler, B.; Hickey, G.; Jacks, T.; Schleim, K.; Pong, S.-S.; Chaung, L.-Y. P.; Chen, H. Y.; Frazier, E.; Leung, K. H.; Chiu, S.-H. L.; Smith, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7001.

3. (a) Kojima, M.; Hosoda, H.; Date, Y.; Nakazato, M.; Matsuo, H.; Kangawa, K. *Nature* **1999**, *402*, 656. (b) Hosoda, H.; Kojima, M.; Matsuo, H.; Kangawa, K. *Biochem. Biophys. Res. Commun.* **2000**, *279*, 909.

4. Bednarek, M. A.; Feighner, S. D.; Pong, S.-S.; McKee, K. K.; Hreniuk, D. L.; Silva, M. V.; Warren, V. A.; Howard, A. D.; Van der Ploeg, L. H. Y.; Heck, J. V. *J. Med. Chem.* **2000**, *43*, 4370.

5. Intermediate **1** was prepared via sulfonylation and then demethylation of *N*′-methyl spiro(indoline-3,4′-piperidine). For the synthesis of *N*′-methyl spiro(indoline-3,4′-piperidine), see: Ong, H. H.; Profitt, J. A.; Fortunato, J.; Glamkowski, E. J.; Ellis, D. B.; Geyer, H. M., III; Wilker, J. C.; Burghard, H. *J. Med. Chem.* **1983**, *26*, 981.