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Synthesis and structure–activity relationships of isoxazole carboxamides as growth hormone secretagogue receptor antagonists

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Abstract—A series of isoxazole carboxamide derivatives has been developed as potent ghrelin receptor antagonists. The synthesis and structure–activity relationship (SAR) are described.

Ghrelin, an octanoylated 28 amino acid peptide, is an endogenous ligand for growth hormone secretagogue receptor (GHS-R), a member of G-protein coupled receptors.[1] Ghrelin is secreted primarily in the stomach and the upper intestinal tract. It stimulates growth hormone secretion and increases food intake in humans.[2] Ghrelin plasma level increases sharply on fasting in rodents[3] as well as prior to each meal in humans and decreases after feeding.[4] A chronic intracerebroventricular (icv) infusion of ghrelin significantly increases food intake and body weight in rats.[5] It has also been shown that subcutaneous administration of ghrelin for 12 days in mice reduces fat utilization.[3] Since endogenous ghrelin appears to play an important role in the long-term regulation of energy balance, blocking the actions of ghrelin is expected to reduce food intake, adiposity, and body weight.

It has been demonstrated that acute or chronic icv administration of *anti*-ghrelin IgG suppresses feeding in lean rats.[5,6] In addition, transgenic rats expressing an antisense ghrelin receptor mRNA have been reported to exhibit ~10% reduction in body weight and up to 80% reduction in adipose tissue than control rats.[7] Recently, Asakawa et al. found that peripherally administered peptidic ghrelin antagonist, [D-Lys-3]-GHRP-6, decreased food intake and body weight gain in *ob*/*ob* obese mice.[8]

These studies have provided some evidence that selective ghrelin receptor antagonists may possibly be useful in the prevention of weight gain and treatment of obesity, which is becoming increasingly prevalent worldwide.

One example of nonpeptidyl ghrelin receptor antagonist, a 3-amino-1,3,4,5-tetrahydro-benzo[*b*]azepin-2-one derivative, was reported in the literature a few years ago.[9] High-throughput screening for small molecule ghrelin receptor antagonists in our labs identified compound **1** (Fig. 1) with an IC_{50} of 130 nM in a binding assay and 180 nM in a cell-based functional assay (FLIPR). Preliminary structure–activity relationship (SAR) revealed that the replacement of the isoxazole core was not successful, while extension at the 5-position of the isoxazole ring led to analogs with >10-fold improvement of potency.[10] The modification of amide linkage was also not tolerated. Accordingly, medicinal chemistry efforts were focused on the variation of substitution on both *N*,*N*-diethylaniline and dichlorophenyl rings.

Synthetic routes to these compounds were relatively straightforward (Scheme 1). Substituted isoxazole acids **9** (commercially available, or prepared by [2 + 3] cyclization between β-keto esters **8** and *N*-hydroxyarylcarboximidoyl chloride followed by hydrolysis) were coupled with anilines by activation of TBTU or HATU to furnish isoxazole carboxamide derivatives (**1**–**7**).

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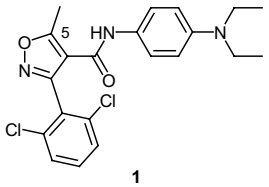

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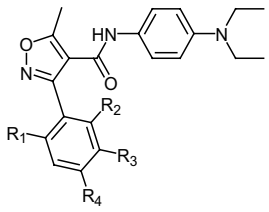
IC_{50} = 130 nM (Binding)
IC_{50} = 180 nM (FLIPR)

Figure 1. Ghrelin antagonist from high-throughput screening.

Alternatively, *ortho*-substituted acid **12**, prepared by acylation of 2-amino benzoate (**10**) and deprotection of allyl eater **11**, could be coupled to various amines to provide analogs **4j**–**n** in a parallel fashion.

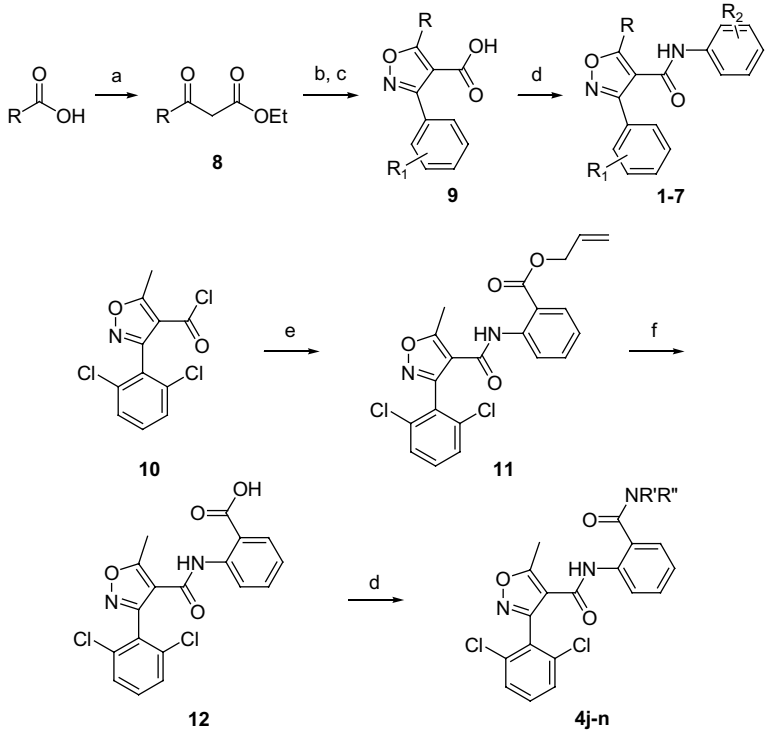
The ghrelin antagonists were assessed in a primary binding assay, monitoring for the displacement of radiolabeled ghrelin from its receptor. Functional activity was determined in a fluorescent calcium indicator assay, measuring compounds' ability to inhibit ghrelin-induced increase of intracellular [Ca^{2+}] in CHO–K cells. The binding and FLIPR data for 2,6-dichlorophenyl modifications are shown in Table 1. Compared to its 2,6-dichloro counterpart **1** (IC_{50} = 0.2 μM), the unsubstituted phenyl analog (**2a**) showed diminished potency (IC_{50} = 8 μM). However, the monochloro-substituted derivative **2b** retained similar FLIPR antagonist activity to the parent **1**. Compounds **2a**–**h** seemed to suggest that *ortho*-substitution, especially with electron-deficient group, would be beneficial to enhance receptor affinity and functional activity, whereas substitution on *meta* or *para* position has little impact on the potency of resultant analogs. Since compound **1** remained the most potent one among these analogs, 2,6-dichlorophenyl moiety was kept for the subsequent SAR studies.

Table 1. SAR of the 2,6-dichlorophenyl group



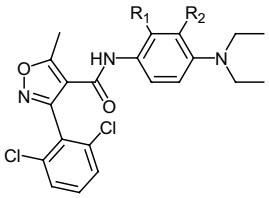
Compd	R_1	R_2	R_3	R_4	Binding IC_{50} (μM)[a]	FLIPR IC_{50} (μM)[a]
1	Cl	Cl	H	H	0.13	0.18
2a	H	H	H	H	8.2	7.6
2b	Cl	H	H	H	0.69	0.25
2c	Br	H	H	H	0.34	0.28
2d	NO_2	H	H	H	0.55	0.62
2e	C_6H_6	H	H	H	1.3	6.3
2f	OMe	H	H	H	4.0	6.8
2g	H	H	Cl	H	18.5	ND[b]
2h	H	H	H	Cl	7.1	6.0

[a] Values are means of at least two experiments against human GHS-R.
[b] For the compounds with binding IC_{50}s > 10 μM, FLIPR data were not determined.



Scheme 1. Reagents and conditions: (a) CDI, MeCN, then potassium ethyl malonate, Et_3N, $MgCl_2$, 38–64%; (b) *N*-hydroxyarylcarboximidoyl chloride, NaOMe, MeOH, rt, 59–73%; (c) NaOH, MeOH, H_2O, 100%; (d) amines, TBTU or HATU, DIPEA, DMF, rt, 78–92%; (e) 2-aminobenzoic acid allyl ester, $NaHCO_3$, THF, rt, 49%; (f) $Pd(PPh_3)_4$, morpholine, dichloromethane, rt, 100%.

Table 2. SAR of substituted phenylenediamine



Compd	R_1	R_2	Binding IC_{50} (μM)	FLIPR IC_{50} (μM)
1	H	H	0.13	0.18
3a	CN	H	9.0	4.5
3b	CF_3	H	4.1	>10
3c	Me	H	0.037	0.039
3d	Et	H	0.37	0.041
3e	OMe	H	0.60	2.9
3f	H	Cl	>10	ND
3g	H	Me	>0.1	>10

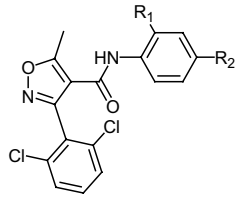
The effect of substitution on *N*,*N*-diethylaniline ring was also explored (Table 2). Compounds **3a** and **3b**, with an *ortho*-electron-withdrawing group (CN or CF_3) next to the amide linker, displayed weaker affinity for ghrelin receptor. In contrast, Compounds (**3c**–**e**) showed little or no loss in binding affinity. In particular, compound **3c** bearing an *ortho*-Me group displayed improved potency (IC_{50} = 0.04 μM, ~4-fold) in both binding and functional activity. The substitution on *meta*-position (**3f**–**g**) seemed to be less favorable.

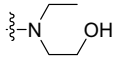
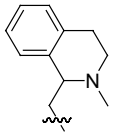
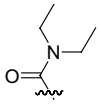
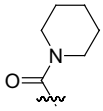
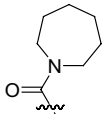
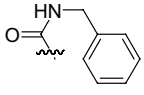
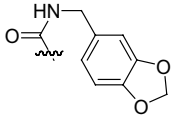
Despite the efforts to increase the potency as ghrelin antagonist, the oral bioavailability of these isoxazole carboxamides remains low (4% for compound **1** and 1% for compound **3c**). A number of factors potentially contribute to the poor bioavailability. Rat liver microsomal incubation results of compound **1** indicated that this compound is rapidly metabolized in 30 min as shown by the rate of 800 pmol/mg protein/minute. The major metabolite seemed to be the de-ethylation of the *N*,*N*-diethylaniline. Therefore, the replacement of the *N*,*N*-diethylaniline group was evaluated.

It was found that *N*,*N*-diethylaniline portion of molecule is very sensitive to modifications (**4a**–**h** in Table 3). Compound **4a**, the metabolite of compound **1**, had no binding affinity for receptor. Only slightly modified *N*,*N*-dialkylaniline groups are tolerated (**4b**–**e**). The attempt to replace one of the ethyl groups by acyl group led to inactive compound **4f**. The replacement of nitrogen with carbon (**4g**) or insertion of an extra carbon between the aniline (**4h**) was also synthesized but led to completely inactive analogs.

We then turned our attention to broadly screen amide derivatives from available amine library without attachment of *N*,*N*-diethylamino moiety. It was found that the *ortho*-substituted analog **4i** displayed modest affinity. Further investigation identified compound **4j** with *ortho*-amide substituent. The improved affinity of **4j** prompted the preparation of numerous analogs with other amide substituents. Compound **4l**, for example, showed submicromolar efficacy in both binding and

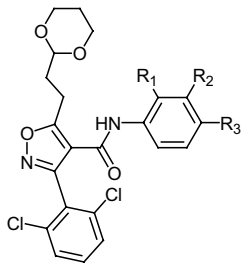
Table 3. Replacement of *N*,*N*-diethylaniline

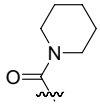


Compd	R_1	R_2	Binding IC_{50} (μM)	FLIPR IC_{50} (μM)
1	H		0.13	0.18
4a	H		>10	ND
4b	H		5.5	4.7
4c	H		0.41	0.51
4d	H		>10	ND
4e	H		9.2	3.1
4f	H		>10	ND
4g	H		>10	ND
4h	H		>10	ND
4i		H	0.98	1.7
4j		H	1.9	2.5
4k		H	2.2	2.3
4l		H	0.86	0.51
4m		H	1.2	10.5
4n		H	0.35	4.9

FLIPR data. While benzylamide analogs displayed improved binding affinity, the functional activity did not demonstrate the same preference.

Table 4. The combination of two sites modification



Compd	R_1	R_2	R_3	Binding IC_{50} (μM)	FLIPR IC_{50} (μM)
5	H	H	NEt_2	0.006	0.014
6	Me	H	NEt_2	0.009	0.010
7		H	H	7.5	7.4

To summarize the SAR from different sites, it was obvious that successful modification comes from the *ortho*-substitution on the aniline ring. We have previously demonstrated that extension of isoxazole side chain at 5-position led to >10-fold boost in potency (compound **5**). The combination of these two optimized modifications was tried (Table 4). To our disappointment, compound **6** only maintained equipotent nanomolar IC_{50} value of **5**. Compound **7** was designed to add 1,3-dioxanepropyl moiety on the top of analog **4k** (~2 μM) to achieve improved potency over **4k**. However, it displayed decreased binding affinity and functional activity (~8 μM). These results implicated that both *ortho*-substituents adjacent to the amide linker may compete for the same binding site. When the size of the substituent on isoxazole ring increased from methyl to 1,3-dioxanepropyl, small groups like Me on aniline ring are just barely tolerated, however, more bulky substituents like amide group in compound **7** will become detrimental to providing favorable binding affinity.

In conclusion, the SAR of isoxazole carboxamides as ghrelin receptor antagonists was explored. The presence of *ortho*-substituent on aniline ring seemed to be beneficial to improve binding affinity and functional activity. However, the combination of substitutions from both sides of the amide linker did not seem to offer any advantage over single substituted analogs. The major challenge in this series of compounds remains to improve PK profile while maintaining the potency of receptor antagonists.

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