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Letters

Discovery of Tetralin Carboxamide Growth Hormone Secretagogue Receptor Antagonists via Scaffold Manipulation

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Abstract: A case study of rational design of an efficient, specific, and proprietary molecular scaffold based on the structure−activity relationship (SAR) information on a screening hit is described. Potent, selective, and orally bioavailable tetralin carboxamide growth hormone secretagogue receptor (GHS-R) antagonists were discovered. Union of rational design and high throughput synthesis provided a quick access to high quality chemical leads.

Identification of molecular scaffolds of biological significance is an important and difficult task in drug discovery.[1] Current approaches include direct screening, structure-based design, ligand-based virtual screening,[2] and combinatorial synthesis.[3] Here we disclose a case study of rational molecular scaffold design guided by structure−activity relationships (SAR). Potent, selective, and bioavailable tetralin carboxamide growth hormone secretagogue receptor (GHS-R) antagonists were discovered using this approach.

Obesity is now recognized as a major health issue in the developed world. Although lack of physical activity and eating habits contribute to much of the obesity epidemic, drug therapy is likely a realistic solution to the problem. Medicines that are capable of reducing food intake can be a potential way of regulating energy balance to achieve weight loss. Ghrelin, a 28 amino acid peptide with an *n*-octanoyl modification on Ser 3[4] is a growth hormone secretagogue and may be also involved in short- and long-term regulation of energy balance. As an orexigenic agent, ghrelin is more potent than melanin-concentrating hormone (MCH) but less potent than neuropeptide Y (NPY).[5] Administration of ghrelin induces food intake (rodent[6] and human[7]), and anti-ghrelin IgG reduces body weight.[8] Antagonizing GHS-R with a peptide antagonist resulted in reduction of food intake and body weight gain in diet-induced obese mice.[9] A small molecule GHS-R antagonist is expected to have similar beneficial effects in obesity therapy.

We have reported the discovery of isoxazole carboxamide-based GHS-R antagonists previously (e.g., compound **1**, Figure 1).[10] The only other nonpeptidyl GHS-R antagonist, a 3-amino-2,3,4,5-tetrahydro-benzo[*b*]azepin-2-one derivative, was reported in 1993.[11] GHS-R agonists, on the other hand, are well documented in the literature. However, most of these compounds were optimized toward stimulating pituitary growth hormone release rather than regulating food intake.[5] An orally active nonpeptidyl GHS-R agonist that stimulates food consumption and adiposity in rats was reported recently.[12]

A high throughput screening of Abbott Laboratories compound collection identified isoxazole carboxamide **1** (Figure 1) as a GHS-R antagonist with a binding IC_{50} of 130 nM. Compound **1** was from a library of amides made via a one-step amide coupling reaction between commercially available carboxylic acids and amines. Extensive SAR exploration led to several low nanomolar antagonists, but no compound that demonstrated a rat oral bioavailability (*F*) higher than 5% was identified (*F* for **1** was 3.9%). The only significant SAR site in **1** is the methyl group in the central isoxazole ring.[10] Analogues that carry certain larger substituents at this position showed increased potency, but at the expense of increased molecular weights and flexibility, which may contribute to their poor pharmacokinetic (PK) profiles.[13] It was clear that modification of the skeleton of these compounds was necessary to improve their PK properties. Besides molecular weight and flexibility, the

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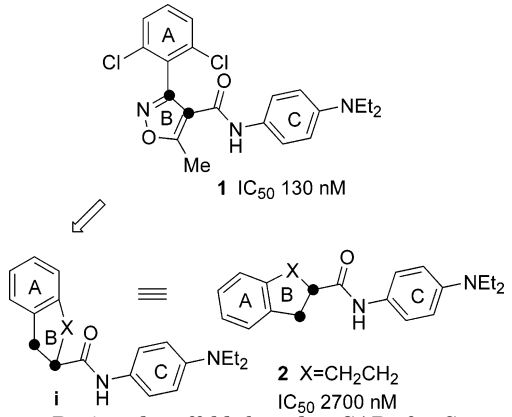


Figure 1. Designed scaffolds based on SAR of **1**. Conservative atoms in B ring during the scaffold hopping are highlighted.

metabolic instability (hydrolysis) of the amide group in compound **1** was also suspected to account for part of its poor PK profile. We decided to modify the central isoxazole ring (B ring, Figure 1) in **1** so that the α-carbon of the carbonyl in the new scaffolds could be quaternized to stabilize the amide bond toward hydrolysis. The SAR study showed that the B ring in **1** could be replaced by several 5- or 6-memebered aromatic ring replacements without losing much potency, which suggested that the role of the B ring in **1** is largely to hold the A ring and C ring in the right orientation.[14] Therefore, the B ring appeared to be the site that would be most likely to tolerate more dramatic skeleton changes. Several aspects should be balanced in the design process in order to identify a high quality hit with a reasonable success rate. First, the new scaffolds should "resemble" that of compound **1** to retain GHS-R antagonism. From the SAR study on compound **1**, the dihedral angle between the A ring and the plane of the amide group appeared important for potency. The distance and relative orientation between the A ring and the amide group were thus the main parameters to optimize in searching for new scaffolds. Second, the properties of designed scaffolds should be tunable, meaning several closely resembled scaffolds should be synthetically accessible so a continuous area in chemical space rather than a point or several widely distributed points could be evaluated. In fact, we regard the tunable nature of the designed scaffolds to accommodate the imperfection of the design hypotheses an important aspect of lead-likeness.[15] Finally, the designed scaffolds should be suitable for further SAR studies. We thus designed 10 closely related scaffolds as shown in general structure **i** in Figure 1. By changing the nature and size of X in **i**, the distance and relative orientation between A ring and the amide group in **i** were systematically evaluated and a tetralin carboxamide was identified as an appropriate hit (**2**, binding IC_{50} 2700 nM, Figure 1).[16] Although antagonist **2** is 20-fold less active than antagonist **1**, it carries some advantageous characteristics. The tetralin 2-carboxamide scaffold has not been exploited much, and its analogues might possess improved PK profiles. Several sites on the tetralin ring can be readily modified to build SAR, and these relatively complex compounds would be less likely to interact with other biological targets and be more selective.[17]

The SAR plan around this new scaffold was directed to further improve the potency and stabilize the amide

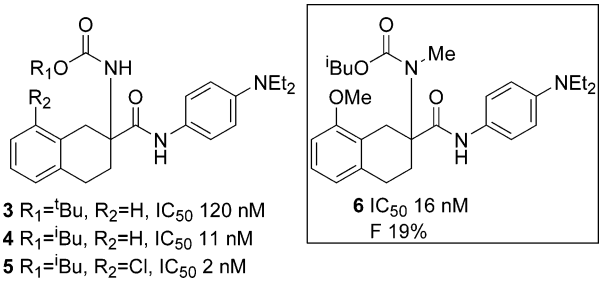


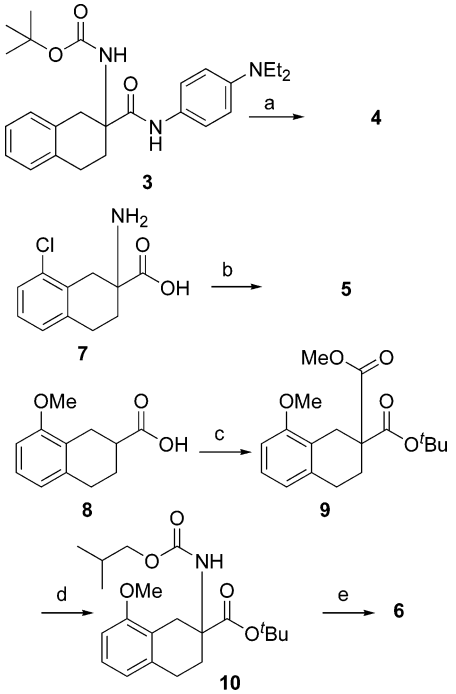
Figure 2. Representative tetralin carboxamides.

group toward hydrolysis. Efforts to quaternize the amide carbonyl α-carbon with eight different classes of substituents [R (alkyls), ROC═O, RNHC═O, RNH, RCONH, RSO_2NH, RNHCONH, and ROCONH] were thus initiated. We were pleased to find that carbamate **3** demonstrated a 22-fold improvement of potency (binding IC_{50} 120 nM, Figure 2) over **2** while the best analogue of each of other classes showed binding activity between 700 nM to 9000 nM. Once the carbamate functional group was found to be the optimal group to branch the amide carbonyl α-carbon, a library of similar carbamates were synthesized and isobutyl carbamate **4** was identified as a more potent compound (binding IC_{50} 11 nM, Figure 2). Routine follow-up SAR studies were then initiated. Noticeably, placing a chloro group at the 8-position of the tetralin moiety in **4** further increased the potency (**5**, binding IC_{50} 2 nM, Figure 2). Other small hydrophobic groups at this position showed similar but less favorable effects on potency. Unfortunately, the rat oral bioavailability for compound **5** was only 3.8%. Replacing the chloro group in **5** with a methoxy group and methylation of the carbamate nitrogen resulted in **6**, which showed good potency (IC_{50} 16 nM) and reasonable rat oral bioavailability (19%, Figure 2). The improved clearance (CLp, 1.3 L/h·kg) for **6** over **1** (3.7 L/hr·kg) suggested **6** might be metabolically more stable than **1**.

In a fluorescent calcium indicator (FLIPR) assay that measures the compounds' ability to inhibit a ghrelin-induced increase in intracellular $[Ca^{2+}]$ in CHO-K cells, compound **6** demonstrated potent functional antagonism of GHS-R (IC_{50} = 29 nM). This compound also showed good selectivity over a panel of GPCR receptors (IC_{50} >33 μM for adrenergic, histaminergic, muscarinic, and dopaminergic receptors) and hERG channel (IC_{50} 6.1 μM), which demonstrated the excellent specificity this unique, relatively complex tetralin scaffold provided.

Compounds **1**−**3** were synthesized from the corresponding commercial carboxylic acids and *N*,*N*-diethylphenylenediamine via a 2-(1*H*-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium tetrafluoroborate (TBTU)-mediated amide formation reaction. The syntheses of **4**−**6** are outlined in Scheme 1. TFA removal of the *tert*-butoxycarbonyl (BOC) group in **3** followed by reacting with ^{i}BuOCOCl produced carbamate **4**. Synthesis of **5** began with known amino acid **7**.[18] Esterification of the carboxylic acid in **7** in acidic methanol followed by carbamate formation with ^{i}BuOCOCl, saponification of the ester, and coupling to *N*,*N*-diethylphenylenediamine gave **5**. Methylation of the carboxyl group in **8** followed by condensation with $(^{t}BuO)_2CO$ provided bis-ester **9**. Selective hydrolysis of the less hindered methyl ester in **9** followed by Curtius rearrangement gave carbamate

Scheme 1. Synthesis of Antagonists **4**−**6**[a]



[a] Reagents and conditions: (a) 1. TFA; 2. ^{i}BuOCOCl, Et_3N; (b) 1. MeOH, HCl; 2. ^{i}BuOCOCl, Et_3N; 3. NaOH(aq); 4. *p*-$NH_2C_4H_6NEt_2$, TBTU, Et_3N; (c) 1. MeI, K_2CO_3; 2. LDA, BOC_2O; (d) 1. LiOH; 2. $(Ph)_2(P{=}O)N_3$; 3. ^{i}BuOH; (e) 1. MeI, NaH; 2.TFA; 3. *p*-$NH_2C_4H_6NEt_2$, TBTU, Et_3N.

10. Methylation of the carbamate nitrogen in **10** followed by TFA removal of the *tert*-butyl group and TBTU coupling to *N*,*N*-diethylphenylenediamine produced **6**.

In conclusion, based on the SAR information on a hit compound (**1**) synthesized in one step from commercially available materials, we have identified a more complex, specific, efficient, and proprietary scaffold. Potent, selective, and bioavailable GHS-R antagonists were discovered. The key to this approach was to use the SAR information as guidance to conceive a cluster of closely resembled and synthetically accessible scaffolds that are suitable for further SAR studies. This approach exhibited a good success rate and synergized the molecular design (complexity) and combinatorial synthesis (throughput). As indicated in a recent analysis, the current drug discovery paradigm relies strongly on the quality of the lead compounds.[19] So the practitioners need to be conscious about evaluating the quality of the hits, and scaffold modifications might frequently be a necessary practice.

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Supporting Information Available: Experimental procedures and spectral characterization of compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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