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Structure–activity relationship studies on tetralin carboxamide growth hormone secretagogue receptor antagonists

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Abstract—The structure–activity relationship studies on a series of tetralin carboxamide growth hormone secretagogue receptor (GHS-R) antagonists are discussed. It was found that certain 2-alkoxycarbonylamino substituted tetralin carboxamides are potent, selective, and orally bioavailable GHS-R antagonists.

Ghrelin is a 28 amino acid growth hormone secretagogue (GHS) with an *n*-octanoyl modification on Ser 3.[1] As an orexigenic agent, ghrelin may be involved in short- and long-term regulation of energy balance. Administration of ghrelin induces food intake in rodents[2] and humans,[3] and anti-ghrelin IgG reduces body weight in rats.[4] Antagonizing growth hormone secretagogue receptor (GHS-R) with a peptide antagonist resulted in reduction of food intake and body weight gain in diet induced obese mice.[5] An orally active non-peptidyl GHS-R agonist that stimulates food consumption and adiposity in rats was reported recently.[6] A small molecule GHS-R antagonist is expected to suppress food intake and reduce body weight.

Relatively few GHS-R antagonists are known in the literature. Only one non-peptidyl GHS-R antagonist, a 3-amino-2,3,4,5-tetrahydro-benzo[*b*]azepin-2-one derivative (**1**, Fig. 1), has been reported[7] before we disclosed the discovery of isoxazole (e.g., **2**, Fig. 1)[8] and tetralin (e.g., **3** and **4**, Fig. 1)[9] carboxamide GHS-R antagonists. As discussed before, the tetralin template was identified via scaffold manipulation based on the structure–activity relationship (SAR) studies on the isoxazole carboxamide GHS-R antagonists.[9] Here we report the results of the SAR studies on both the tetralin and phenylenediamine portion of the lead compound **3**.

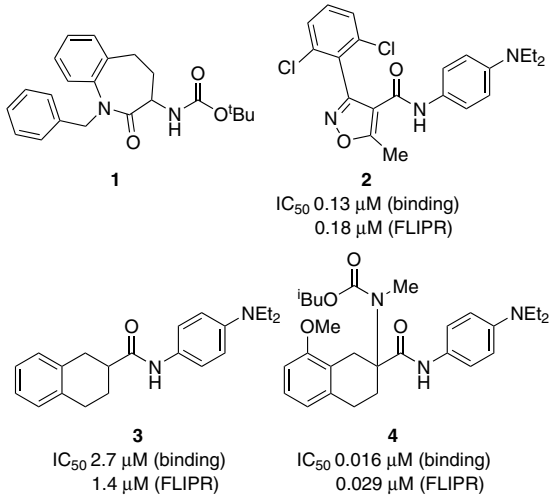

Figure 1. Known GHS-R antagonists.

The syntheses of the tetralin carboxamide GHS-R antagonists are outlined in Scheme 1 (**3**, **4**, **15**–**17**, **23**, **24**, **31**–**33** were prepared using protocols reported before[8,9]). The coupling of carboxylic acid **34** and *N*,*N*-diethylphenylenediamine mediated by 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) provided antagonist **6**. Cleavage of the methyl

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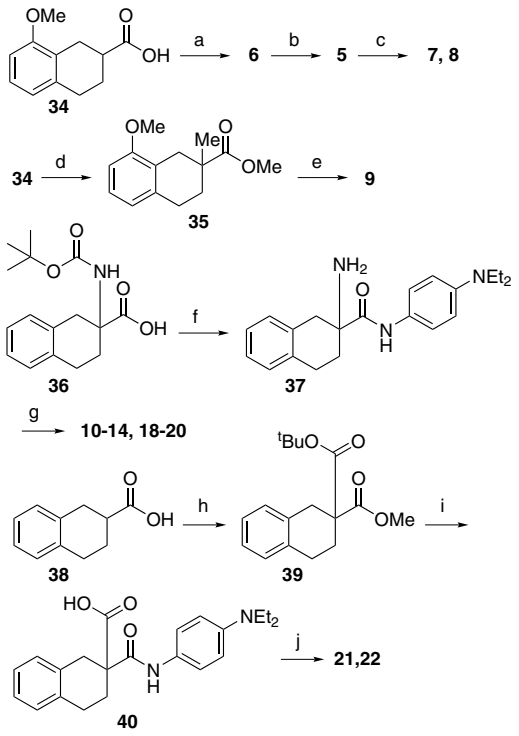
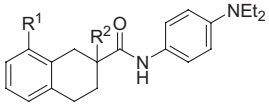


Scheme 1. Reagents and conditions: (a) $NH_2C_6H_4NEt_2$, TBTU, Et_3N; (b) BBr_3; (c) K_2CO_3, EtI (for **7**) or PrI (for **8**); (d) (1) K_2CO_3, MeI, (2) LDA, MeI; (e) (1) LiOH, (2) $NH_2C_6H_4NEt_2$, TBTU, Et_3N; (f) (1) $NH_2C_6H_4NEt_2$, TBTU, Et_3N (2) 4 N HCl, dioxane; (g) for **10**–**14**: corresponding chloroformates, Et_3N; for **18**: isopropyl isocyanate; for **19**: $BuSO_2Cl$, Et_3N; and for **20**: $(CH_3)_2CHCH_2CH_2COCl$, Et_3N; (h) (1) MeI, K_2CO_3, (2) LDA, BOC_2O; (i) (1) LiOH, (2) $NH_2C_6H_4NEt_2$, TBTU, Et_3N, (3) 4 N HCl, dioxane; (j) $(CH_3)_2CHCH_2CH_2NH_2$ (for **21**) or $(CH_3)_2CHCH_2CH_2OH$ (for **22**), TBTU, Et_3N.

group in **6** mediated by BBr_3 yielded antagonist **5**. Alkylation of the phenolic hydroxy group in **5** with ethyl iodide and propyl iodide produced antagonists **7** and **8**, respectively. Methylation of the carboxyl group in **34** followed by methylation of the α-position of the resulting ester carbonyl group gave **35**. Antagonist **9** was obtained by saponification of **35** followed by a TBTU coupling reaction with *N*,*N*-diethylphenylenediamine. Amine **37** could be obtained by a similar TBTU coupling reaction between **36** and *N*,*N*-diethylphenylenediamine followed by the HCl removal of the *tert*-butoxycarbonyl (BOC) group. Antagonists **10**–**14** and **18**–**20** were prepared by coupling **37** with the corresponding chloroformates, isocyanate, sulfonyl chloride, and acid chloride, respectively. Methylation of the carboxyl group in carboxylic acid **38** followed by carbonylation of the α-position of the resulting ester provided diester **39**.[9] Selective saponification of the methyl ester in **39** followed by a TBTU coupling reaction with *N*,*N*-diethylphenylenediamine and the HCl removal of *tert*-butyl group gave carboxylic acid **40**, which was converted to antagonists **21** and **22** under TBTU coupling reaction conditions with 3-methylbutyl amine and 3-methylbutyl alcohol, respectively.

The prototype tetralin carboxamide **3** showed only weak antagonist activity in both receptor binding (IC_{50} 2.7 μM) and cellular function assay [fluorescent calcium indicator (FLIPR), IC_{50} 1.4 μM].[10] The SAR studies on the tetralin portion in **3** identified the 2- and 8-positions as significant SAR sites and the results are summarized in Table 1. Placing a hydroxy group at 8-position (**5**) reduced binding potency while a hydrophobic methoxy group at the same position moderately improved the activity (**6**). A larger ethoxy group further increased potency (**7**), but a propoxy group (**8**) seemed too bulky implying the groups at this position interact with a small hydrophobic binding pocket on the receptor. Halogens such as chloro[9] or bromo groups (e.g., **24**) appeared optimal for binding at this position although the functional (FLIPR) IC_{50} for **24** correlated poorly with its binding IC_{50}.

Table 1. Modifications of the tetralin portion



No.	R^1	R^2	Binding IC_{50} (μM)	FLIPR IC_{50} (μM)
3	H	H	2.7	1.4
5	OH	H	9.5	ND[a]
6	OMe	H	0.60	0.76
7	OEt	H	0.35	0.20
8	OPr	H	1.7	0.80
9	OMe	Me	0.69	0.77
10	H	$NHCO_2Et$	0.77	0.61
11	H	$NHCO_2{}^iPr$	0.15	0.10
12	H	$NHCO_2{}^tBu$	0.12	0.10
13	H	$NHCO_2{}^iBu$	0.011	0.018
14	H	$NHCO_2Bn$	0.12	0.16
15	H	$NMeCO_2{}^tBu$	0.047	0.031
16	H	$NMeCO_2{}^iBu$	0.028	0.039
17	H	$NEtCO_2{}^iBu$	0.060	0.13
18	H	$NHCONH^iPr$	0.37	0.63
19	H	$NHSO_2Bu$	2.7	1.20
20	H	$NHCO(CH_2)_2CH(CH_3)_2$	>10	ND
21	H	$CONH(CH_2)_2CH(CH_3)_2$	2.7	ND
22	H	$CO_2(CH_2)_2CH(CH_3)_2$	0.32	0.75
23	OMe	$NHCO_2{}^iBu$	0.018	0.027
4	OMe	$N(Me)CO_2{}^iBu$	0.016	0.029
24	Br	$NHCO_2{}^iBu$	0.002	0.052

[a] ND: not determined

The study of substitutions at the 2-postion of the tetralin template were directed to further expand the SAR to search for more potent antagonists and to provide steric protection for the amide group toward hydrolysis, which was suspected as a contributing factor to the poor pharmacokinetic (PK) profiles of the tetralin carboxamides without any substitutions at the 2-position [e.g., the rat oral bioavailability (*F*) for **6** was 0.6% (Table 3)]. The carbamate groups proved to be the most efficient substituents at this position. While the potencies of carbamates **10**, **11**, **12**, and **14** demonstrated a general positive correlation with their sizes, isobutyl carbamate **13** showed a sharp increase of both binding and FLIPR potency (IC_{50} 11 and 18 nM, respectively). A library of ~70 similar carbamates were then synthesized and assayed. A variety of carbamates were tolerated but 3–6

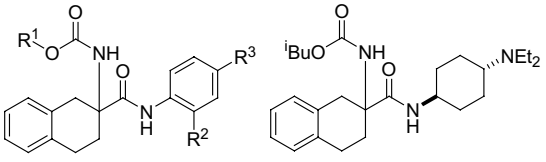
carbon long aliphatic carbamates typically demonstrated better affinity. Methylation of the carbamate nitrogen in **12** led to a more potent antagonist **15**, but the same operation on **13** resulted in a weaker antagonist (**16**) indicating more potent compounds like **13** might induce receptor conformation changes. Increased size of this N-alkyl group further reduced potency (**17**).

A variety of other substituents at the 2-position were exploited and the most active antagonist in each class is shown in Table 1. For the alkyl substituents, a methyl group was best tolerated (**9**) while larger alkyl groups tend to reduce potency. An optimized urea (**18**) showed moderate improvement while even the best sulfonamides (e.g., **19**), amides (e.g., **20**), and reversed amides (e.g., **21**) studied showed neutral or negative effects on the activity.

The beneficial effects of the substituents at the 2- and 8-positions of the tetralin template are not independent. For example, compound **23** showed almost comparable potency as **13** in both binding and FLIPR assays, which again suggests tight binding groups might induce receptor conformation changes.

The SAR results on the phenylenediamine portion are summarized in Table 2. The *ortho*-methylation (**25**, and **32**, **33** from Table 3) was tolerated while *N*,*N*-diethyl group was fairly sensitive toward modifications. A larger *N*,*N*-dipropyl analog **26** barely registered in the binding assay and a yet larger *N*,*N*-dibutyl analog **27** was still 10 times weaker than its *N*,*N*-diethyl counterpart **13**. A closely related *N*,*N*-diisobutyl analog (**28**) was completely inactive. Close analogs such as conformationally restrained *N*-pyrrolidinyl and *N*-morpholinyl compounds **29** and **30** were inactive in binding assay at the highest concentration tested (10 μM). In sharp contrast to the SAR observed in isoxazole carboxamide GHS-R antagonists,[11] *trans*-cyclohexyldiamine analog **31** showed 100-fold loss of potency compared to **13**. However, antagonist **31** should be more water-soluble than **13** due to its increased basicity and flexibility.[12]

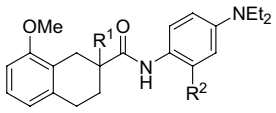
Table 2. Modifications of the phenylenediamine portion



25-30 **31**

No.	R^1	R^2	R^3	Binding IC_{50} (μM)	FLIPR IC_{50} (μM)
25	^{t}Bu	Me	NEt_2	0.092	0.078
26	^{i}Bu	H	NPr_2	9.1	ND
27	^{i}Bu	H	NBu_2	0.19	0.12
28	^{i}Bu	H	N^iBu_2	>10	ND
29	^{t}Bu	H	N-pyrrolidinyl	>10	ND
30	^{t}Bu	H	N-morpholinyl	>10	ND
31	—	—	—	1.4	5.6

Table 3. The rat PK data for selected GHS-R antagonists



No.	R^1	R^2	*F* (%)	CLp (L/h kg)	Binding IC_{50} (μM)	FLIPR IC_{50} (μM)
6	H	H	0.6	3.52	0.60	0.76
4	^{i}BuOCONMe	H	19	1.32	0.016	0.029
23	^{i}BuOCONH	H	18	1.72	0.018	0.027
32	^{i}BuOCONH	Me	15	1.29	0.031	0.020
33	^{i}BuOCONMe	Me	21	1.35	0.010	0.033

Antagonist **31** also lacks an electron rich *N*,*N*-dialkyl-1,4-phenylenediamine group, which could be a metabolically unstable fragment in most other antagonists discussed in this report.

The study of tetralin carboxamide GHS-R antagonists was initiated in response to the poor pharmacokinetic profiles of the first generation isoxazole carboxamides.[9] One strategy was to quaternize the α-position of the amide carbonyl group so that the amide is more resistant toward metabolic hydrolysis. Although oral bioavailability is a difficult parameter to rationally improve,[13] this strategy appeared to work reasonably well. For example, a compound that obeys Lipinski's rules[14] but lacks a quaternized α-carbon to the amide carbonyl group (**6**) showed only a 0.6% rat oral bioavailability (Table 3). In contrast, larger compounds with more hydrogen bond donors and acceptors but containing a quaternized α-carbon to the amide carbonyl group demonstrated significantly improved rat oral bioavailabilities (Table 3). The reduced clearance of these compounds over that of **6** (Table 3) suggests the bulky substituents at the α-carbon to the amide carbonyl group might play a role in stabilizing these molecules.

Several potent antagonists (**4**, **13**, and **24**) were subjected to a GPCR selectivity study and they showed only weak activity toward a panel of receptors (IC_{50} > 33 μM for adrenergic, histaminergic, muscarinic, and dopaminergic receptors). These compounds were also tested for hERG channel blockade and they all demonstrated weak affinity in this assay (IC_{50} 6.1, >10, and 7.7 μM for **4**, **13**, and **24**, respectively).

In conclusion, a series of potent and selective tetralin carboxamide GHS-R antagonists were identified and studied. Some potent GHS-R antagonists also demonstrated reasonable rat oral bioavailability.

Acknowledgements

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10. For assay conditions see Ref. 8.
11. Only a 4-fold loss of potency was observed for the same modification in the isoxazole carboxamide series. See Ref. 8.
12. For the isoxazole carboxamide GHS-R antagonists, replacing the phenylenediamine group with a cyclohexyl diamine group resulted in compounds with much-improved aqueous solubility. See Ref. 8.
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