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Discovery and optimization of novel 4-[(aminocarbonyl)amino]-*N*-[4-(2-aminoethyl)phenyl]benzenesulfonamide ghrelin receptor antagonists

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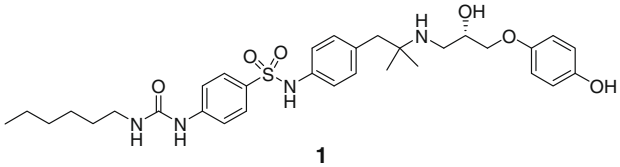
ABSTRACT

This Letter describes optimization of ghrelin receptor antagonists and inverse agonists starting from a screening hit.

Ghrelin receptor antagonists have been proposed as potential therapeutic agents for treatment of obesity,[1] and more recently, type II diabetes.[2] Ghrelin, a 28 amino acid peptide bearing unusual acylation by octanoic acid at Ser 3, was recently identified as the endogenous ligand for the growth hormone secretagogue receptor-1a (GHS-R1a, ghrelin receptor).[3] Activation of the GHS-R1a by ghrelin leads to release of growth hormone (GH) from pituitary tissue, and potently stimulates appetite in rodents[4] and humans.[5] The ghrelin receptor is a member of the seven-transmembrane G-protein-coupled receptor (GPCR) family, and though widely expressed, is most abundant in the hypothalamus and pituitary.[6] The GHS-R1a is a Gq-coupled GPCR and is notable for possessing a high level of ligand-independent, constitutive receptor activity as measured in an inositol phosphate (IP) accumulation assay.[7] Consequently, the ghrelin receptor may not be quiescent during inter-prandial periods even when circulating ghrelin levels are low, and for this reason, it has been suggested that an inverse agonist may be required to most effectively block ghrelin receptor mediated orexigenic signaling.[7c] Despite the enthusiasm surrounding antagonism of the ghrelin receptor as a target for obesity, no small molecule ghrelin antagonist has yet advanced to clinical trials and considerable debate persists with regard to ghrelin-GHS-R1a function and the prospects for treatment of obesity by targeting this pathway.[1b,8] Small molecule ghrelin agonists, antagonists, and inverse agonists should serve as useful tools to address this debate. While GHS-R1a agonists (growth hormone secretagogues) have been widely described in the literature, relatively fewer antagonists are known, and most of these were disclosed recently.[1b,9] Several reports claim decreased food intake and body weight in rodent models following dosing with small molecule ghrelin antagonists,[9,10] although it was not always rigorously established that these effects were totally mechanism based (for example, by showing efficacy in wild type but not in GHS-R1a knockout mice).

As part of our effort to develop ghrelin receptor antagonists for preclinical proof of concept studies, we identified a number of closely related compounds typified by compound **1** as ghrelin receptor ligands.



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Compound **1** was highly potent in our GHS-R1a competitive binding assay employing [^{35}S]-MK-0677 as the radiolabeled probe,[11] (IC_{50} = 13 nM) and was determined to be an antagonist in an aequorin bioluminescence functional assay (IC_{50} = 40 nM; ghrelin at 10 nM) measuring inhibition of ghrelin stimulated intracellular calcium mobilization in HEK-293 cells expressing hGHS-R1a.[6,11] Compound **1** and analogs have previously been described as potent β3

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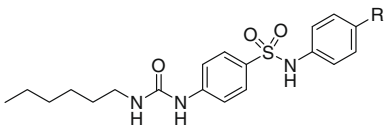
adrenergic receptor agonists,[12] however they suffer from relatively high molecular weights and poor pharmacokinetic properties.[13] In an effort to eliminate the β3 adrenergic receptor agonist activity, as well as decrease molecular weight, we undertook the systematic truncation of lead **1**. We began by attempting to remove the 1-amino-3-(aryloxy)propan-2-ol moiety, known to be a critical pharmacophore required for β3 adrenergic receptor activity.[12a] Analogs were prepared as described previously for the structurally related β3 adrenergic receptor agonists.[12] The analogs were evaluated for their binding affinities to the cloned hGHS-R1a and for aequorin functional agonist activities at the hGHS-R1a,[6,11] in our attempt to minimize agonist liability. Table 1 shows that the entire 1-amino-3-(aryloxy)propan-2-ol moiety is not required for ghrelin receptor binding potency, however the basic amine appears to contribute (**4**). The gem-dimethyl substitution (**5**) improves binding potency compared to the unsubstituted aminoethyl (**4**) by about sixfold. The *N*-ethyl analog of **5** had similar potency (**6**) suggesting no significant contribution of the added ethyl to binding affinity. None of the analogs were agonists based upon our aequorin functional assay where all analogs tested showed little or no ghrelin receptor activation. Importantly, compound **5** showed minimal binding affinity[12] to the human β3 adrenergic receptor (IC_{50} = 5.2 μM), and no functional activation[12] (hβ3 EC_{50} >10 μM), indicating that truncation of lead **1** serves to abolish its original β3 adrenergic agonist activity.

We further evaluated the importance and position of the amino group and several relevant analogs are presented in Table 2. Replacing the amino with a hydroxyl (**7**) and substitution with 2,2,2-trifluoroethyl (**14**) led to dramatic potency loss, supporting that a basic group is required and that reducing basicity is detrimental. Shortening the aryl to amine chain length (**8**) results in potency loss, while increasing the chain length (**9**) is tolerated. Installing the gem-dimethyl substitutions into the 3-carbon chain length analog (**10** and **11**) did not improve potency as it had in the original two-carbon chain length series (**4** to **5**). *m*-Substituted aminoethyl (**12**) and aminopropyl (**13**) analogs were less potent than the original *p*-substituted analogs, and showed increased functional agonism at the ghrelin receptor as measured in the aequorin bioluminescence assay.

We next turned to optimization of the alkyl urea moiety. A library of more than one hundred analogs was prepared. A wide variety of commercially available alkyl and aryl isocyanates were coupled to 1,1-dimethylethyl[2-(4-{[(4-aminophenyl)sulfonyl]amino} phenyl)-1,1-dimethylethyl]carbamate (Scheme 1). This intermediate was prepared starting from phentermine. Nitration gave the *p*-nitro product exclusively. Protection of the amine functionality with a Boc group, and reduction of the nitro gave the corresponding aniline. Coupling of the aniline with 4-nitrophenylsulfonyl chloride, followed by reduction of the nitro group gave the advanced aniline intermediate. After coupling to the isocyanate collection, removal of the Boc-protecting group with TFA afforded the target analogs Binding potencies for selected urea analogs are presented in Table 3. Modifications to the hexyl group (chain length, branching, examples **15**–**20**) led to diminished potencies. Phenyl (**21**), benzyl (**22**), and phenethyl (**23**) ureas had modest potencies; methyl substitution of phenyl urea indicated a slight potency enhancement compared to the unsubstituted phenyl, with *para* (**26**) and *meta* (**25**) isomers appearing most improved. Increasing the substituent size further improved potency in the *p*-substituted series only (**27**–**30**), with the optimal substituent being *iso*-propyl (**28**). 1-Naphthylmethyl urea analog (**31**) had improved potency relative to the parent benzyl urea (**22**). Installation of a benzylic methyl to generate racemic 1-naphthylethyl urea **32** led to a sevenfold boost in potency. The individual enantiomers **33** (*R*), and **34** (*S*) were equipotent. Increasing the benzylic substitution from methyl to ethyl (**36**), or making the gem-dimethyl analog **35** gave no further advantage relative to **33** and **34**. Compound **33** exhibited potent binding to the human ghrelin receptor (IC_{50} = 2 nM), was shown to be a hGHS-R1a antagonist in the aequorin assay (IC_{50} = 1 nM), and was further shown to in-

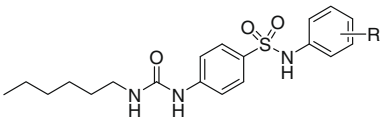
Table 1
Binding affinities of truncated analogs of **1**



Compd	R	hGHS-R1a binding IC_{50}[a] (nM)	Max activation[b] %
2	–H	4,300	—
3	–Et	47%@ 6.25 μM	—
4	$-CH_2CH_2NH_2$	272	5
5	$-CH_2C(CH_3)_2NH_2$	46	4
6	$-CH_2C(CH_3)_2NHEt$	30	1

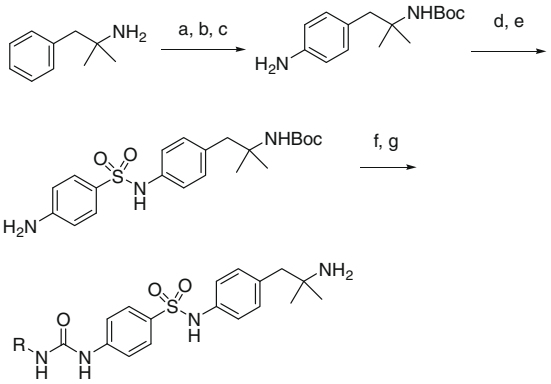
[a] Values are means of two or three experiments, standard deviations were generally less than 20% of the mean values.
[b] Maximum % activation (agonist activity) relative to ghrelin, hGHS-R1a aequorin assay, maximum compound concentration = 10 μM.

Table 2
Binding affinities for SAR focused on the aminoethyl subunit



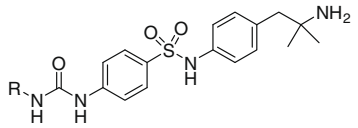
Compd	Position	R	hGHS-R1a binding IC_{50}[a] (nM)	Max activation[b] %
5	*p*	$-CH_2C(CH_3)_2NH_2$	46	4
7	*p*	$-CH_2C(CH_3)_2OH$	48%@ 6.25 μM	—
8	*p*	$-CH_2NH_2$	1715	5
9	*p*	$-CH_2CH_2CH_2NH_2$	108	1
10	*p*	$-CH_2CH_2C(CH_3)_2NH_2$	310	3
11	*p*	$-CH_2C(CH_3)_2CH_2NH_2$	444	2
12	*m*	$-CH_2CH_2NH_2$	625	13
13	*m*	$-CH_2CH_2CH_2NH_2$	429	51
14	*p*	$-CH_2C(CH_3)_2NHCH_2CF_3$	682	1

[a] Values are means of two or three experiments, standard deviations were generally less than 20% of the mean values.
[b] Maximum % activation (agonist activity) relative to ghrelin, hGHS-R1a aequorin assay, maximum compound concentration = 10 μM.



Scheme 1. Reagents and conditions: (a) KNO_3, H_2SO_4, 95%; (b) Boc_2O; (c) H_2, Pd/C, MeOH, 87%, two steps; (d) 4-nitro-benzenesulfonyl chloride, saturated $NaHCO_3$ solution, DCM, vigorous stirring, 89%; (e) H_2, (balloon) Pd/C, MeOH, 100%; (f) alkyl and aryl isocyanates, toluene or dioxane, 60 °C; (g) TFA.

Table 3
Binding affinities of selected ureas



Compds	R	hGHS-R1a binding IC_{50}[a] (nM)	Max activation[b] %
5	*n*-Hexyl	46	4
15	Ethyl	5615	4
16	*n*-Propyl	1649	1
17	*i*-Propyl	846	1
18	*n*-Butyl	474	1
19	*n*-Pentyl	241	6
20	*n*-Heptyl	97	3
21	Ph	263	1
22	Bn	128	4
23	$PhCH_2CH_2$	138	1
24	2-Tolyl	161	1
25	3-Tolyl	69	3
26	4-Tolyl	77	3
27	4-Ethylphenyl	14	1
28	4-*i*-Propylphenyl	8	0
29	4-*n*-Butylphenyl	28	1
30	4-*t*-Butylphenyl	16	1
31	1-Napthylmethyl	21	—
32	(*R*/*S*)-1-Napthylethyl	3	−1
33	(*R*)-1-Napthylethyl	2	1
34	(*S*)-1-Napthylethyl	2	1
35	1-Methyl-1-napthylethyl	2	—
36	(*R*/*S*)-1-Napthylpropyl	4	—
37	(*R*/*S*)-2-Napthylethyl	18	—

[a] Values are means of two or three experiments, standard deviations were generally less than 20% of the mean values.
[b] Maximum % activation (agonist activity) relative to ghrelin, hGHS-R1a aequorin assay, maximum compound concentration = 10 μM.

hibit ghrelin stimulated GH release in rat pituitary cells.[14] Inclusion of a 20 nM concentration of **33** led to a 98-fold right shift in the ghrelin EC_{50} for GH release (ghrelin EC_{50} = 2 nM; EC_{50} = 196 nM with 20 nM of **33**). Compound **33** at 10 μM did not itself increase GH release over basal levels. Compound **33** was further evaluated for inverse agonist activity using an inositol phosphate accumulation-scintillation proximity assay (IP-SPA)[15] in HEK293 cells expressing rat GHS-R1a, which exhibited robust constitutive activity. As shown in Figure 1, compound **33** was observed to be both an inverse agonist (EC_{50} = 1 nM) and a functional ghrelin antagonist (IC_{50} = 11 nM; ghrelin at 1 nM) at the rat ghrelin receptor (rat GHS-R1a binding IC_{50} = 6 nM for **33**). As noted by Holst, et al.,[7a] it is difficult to detect constitutive GPCR signaling using calcium mobilization assays such as aequorin or FLIPR, and inverse agonism of **33** was only observed using the IP-SPA assay which measures receptor dependent, phospholipase C mediated accumulation of IP over a 1 h interval.

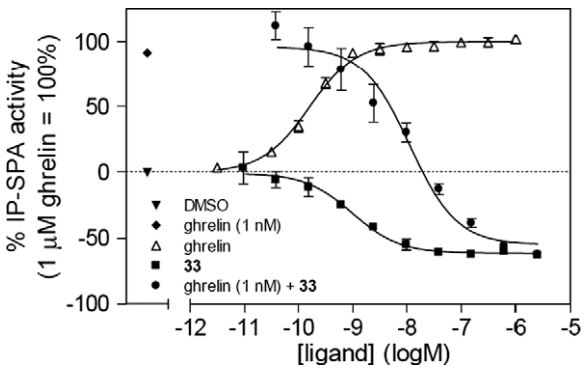


Figure 1. Antagonist and inverse agonist dose–response of **33** determined by IP-SPA in rat GHSR-HEK293 cells.

Compounds **33** and **34** had low binding affinity for the β3 adrenergic receptor (β3 IC_{50} **33** >20 μM, IC_{50} **34** = 5.8 μM), and had no issues with regard to hERG channel binding (**33** hERG IC_{50} = 1.9 μM). Unfortunately, compounds **33** and **34** demonstrated poor oral bioavailability in rat and mouse, precluding their application in oral proof of concept studies. Although compound **33** was not orally bioavailable in rat ($F \sim 0\%$, $t_{1/2}$ = 2.7 h, Clb = 26 mL/min/kg, Vdss = 4.6 L/kg), we decided to explore intravenous (IV) dosing. Dosed at 1 mpk IV in rats the brain/plasma ratio was 0.15 with a 23 nM brain concentration of **33** measured at 4 h post dose. On dosing **33** in lean SD male rats (3, and 10 mg/kg, IV) no effects were observed on overnight food intake compared to control animals. Similarly, no effect on overnight food intake was observed in diet-induced obese mice at 3 and 10 mpk, IV. In a separate study, compound **33** at 10 mg/kg IV was shown to inhibit fasting induced refeeding in lean rats with a 52% reduction in food intake at 1 h post dose compared to control animals. Unfortunately, at higher doses (20 mg/kg, IV) adverse effects were noted that suggest that the inhibition of refeeding may not have been mechanism-based. The lack of efficacy seen in these models may be the result of the poor brain/plasma ratio observed for **33**. Optimization of the pharmacokinetic and brain penetration characteristics within this class was briefly explored, however initial studies which modestly improved oral bioavailability (e.g., by replacing the urea moiety with heterocycles) led to compounds showing undesired agonist activity.

In summary, we have described the discovery and optimization of potent GHS-R1a ligands based on a screening hit. The ligands are antagonists or inverse agonists, but depending on substitution patterns, can also generate partial agonists. Truncation of the original screening hit abolished its potent β3 adrenergic activity, while retaining (and improving) the GHS-R1a binding potency. Milestone analog **33** was a potent inverse agonist of GHS-R1a. It, and most closely related compounds, had little oral bioavailability, limiting in vivo evaluation to the IV route. Compound **33** did not reduce food intake or body weight in several IV dosed animal models, possibly owing to its low brain/plasma ratio; higher doses resulted in the observation of adverse affects. While modifications to improve oral bioavailability were briefly explored, and further work along these lines can be envisioned, we suspended work in this area to follow alternative lead series with better prospects for oral delivery.

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