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Discovery of a new class of ghrelin receptor antagonists

Jeffrey T. Mihalic *, Yong-Jae Kim, Mike Lizarzaburu, Xiaoqi Chen, Jeff Deignan, Malgorzata Wanska, Ming Yu, Jiasheng Fu, Xi Chen, Alex Zhang, Richard Connors, Lingming Liang, Michelle Lindstrom, Ji Ma, Liang Tang, Kang Dai, Leping Li

Amgen Inc., 1120 Veterans Boulevard, ASF2-3, South San Francisco, CA 94080, USA

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ABSTRACT

A series of benzodiazepine antagonists of the human ghrelin receptor GHSR1a were synthesized and their antagonism and metabolic stability were evaluated. The potency of these analogs was determined using a functional aequorin (Euroscreen) luminescent assay measuring the intracellular Ca^{2+} concentration, and their metabolic stability was measured using an in vitro rat and human S9 hepatocyte assay. These efforts led to the discovery of a potent ghrelin antagonist with good rat pharmacokinetic properties.

Obesity has reached an epidemic scale in the US; researchers estimate that one third of adults in the United States are overweight or obese.[1] This disease is responsible for approximately 300,000 deaths a year and is associated with other morbidities such as heart disease, diabetes, asthma, sleep apnea, and some types of cancer.[2] Although the pathogenesis for obesity is often caused by several factors, recent studies identified several promising targets such as the ghrelin receptor to combat this disease.[3] Ghrelin is the endogenous ligand for this G-protein coupled receptor. The ligand is a 28-amino acid acylated peptide hormone produced mainly in the stomach by X/A epithelial cells.[4] It was first identified as the endogenous ligand for the growth hormone secretagogue receptor (GHSR), and later it was recognized to have a role in weight regulation because of its effect on food intake. This effect has been shown to be independent of growth hormone stimulation. Ghrelin's food intake stimulation occurs via its receptor subtype GHSR1a in the hypothalamic arcuate nucleus where it activates NPY and AgRP neurons.[5] The hormone's effect on food intake is well established in the literature. For example, infusion of the hormone has been shown to increase feeding in both mice and humans.[6] Peptide antagonists for the ghrelin receptor have been shown to decrease food intake in lean mice, mice with induced obesity, and *ob*/*ob* mice.[7] Moreover, an ICV injection of anti-ghrelin antibody has been shown to reduce body weight in rats.[8] These studies suggest that a small molecule antagonist for the ghrelin receptor could be a useful therapeutic agent for treating obesity.

In 1993 researchers demonstrated that benzoazepine analog **1** (Fig. 1) can block ghrelin induced growth hormone secretion.[9] This was the first report in the literature of a non-peptidic ghrelin antagonist. Later, a different research group disclosed two classes of GHSR1a antagonists based on the tetralin and isoxazole carboxamide scaffolds (compounds **2** and **3**, Fig. 1).[10] Compounds **2** and **3** were potent ghrelin receptor antagonists with IC_{50} values of 16 nM and 8 nM, respectively, as measured by a blockade of intracellular Ca^{2+} mobilization induced by ghrelin. During a high throughput screen of our compound library, we discovered benzodiazepine **4** (Fig. 1) as a novel antagonist of the GHSR1a receptor. In our aequorin functional assay, benzodiazepine **4** had an IC_{50} of 8 nM for the human ghrelin receptor, and 7 nM for the mouse ghrelin receptor.

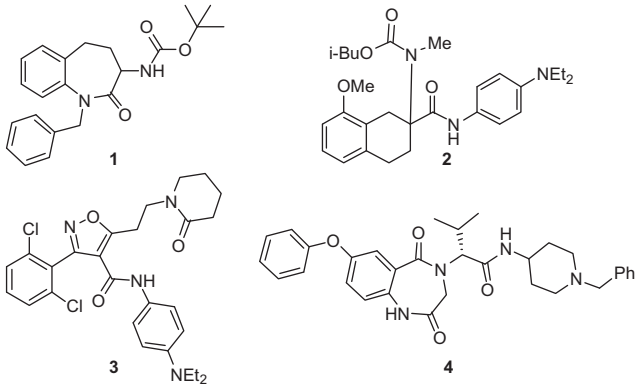

Figure 1. Known ghrelin receptor antagonists.

* Corresponding author.
E-mail address: jmihalic@amgen.com (J.T. Mihalic).

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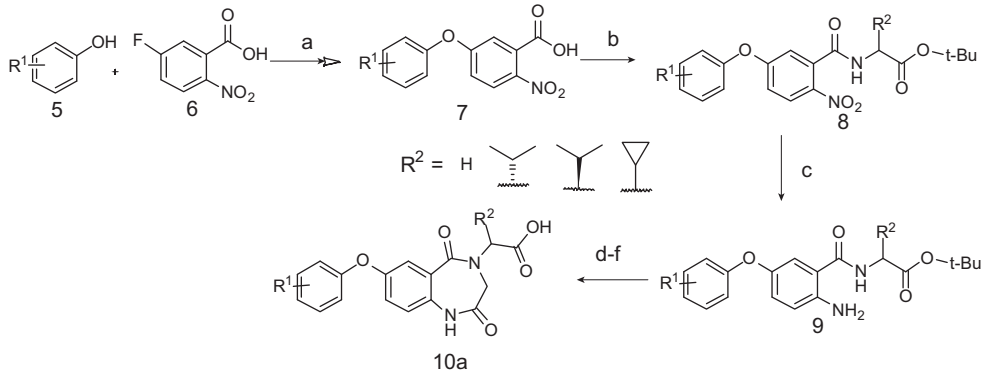
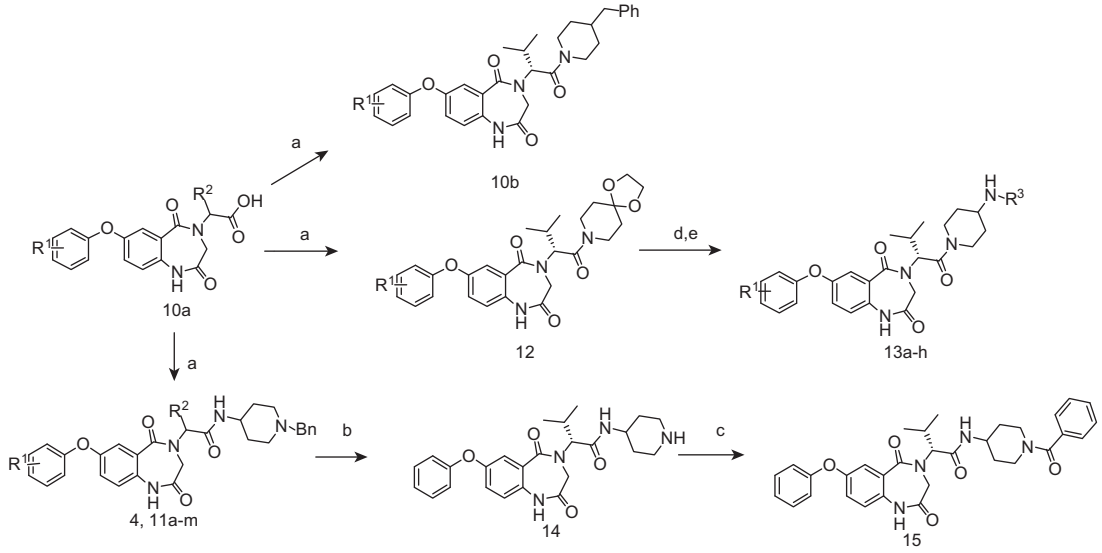
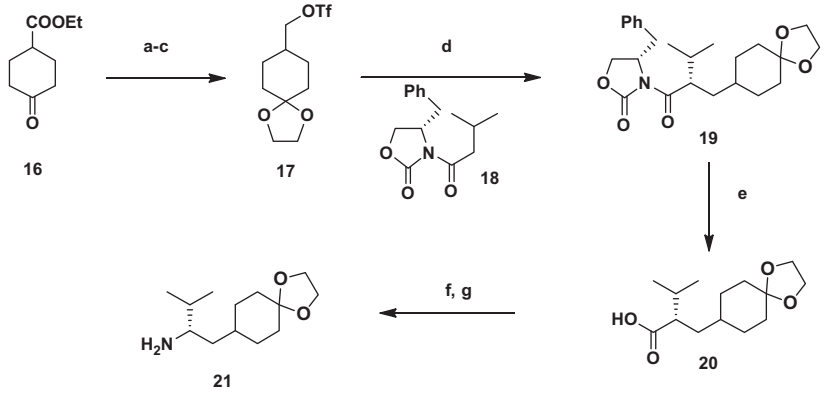


Scheme 1. Reagents and conditions: (a) Cs_2CO_3, DMF, 100 °C; (b) EDC or HBTU, amine, Et_3N, DCM; (c) Pd/C, H_2, EtOH; (d) chloroacetyl chloride, Et_3N, DCM; (e) NaH, DMF; (f) TFA.



Scheme 2. Reagents and conditions: (a) Amine, EDC, Et_3N, DCM; (b) Pd/C, H_2, EtOH; (c) benzoic acid, EDC, Et_3N, DCM; (d) TFA, H_2O; (e) amine, $NaBH(OAc)_3$, DCM.



Scheme 3. Reagents and conditions: (a) Ethylene glycol, pTSA, toluene; (b) LAH, THF; (c) TF_2O, Et_3N, DCM; (d) compound **18**, LDA, then compound **17**, THF; (e) H_2O_2, LiOH, THF, H_2O; (f) DPPA, Et_3N, BnOH, toluene; (g) H_2, Pd/C, MeOH.

This compound was a full antagonist for both ghrelin receptors and had a K_i of 4.4 nM in a human I^{125}-ghrelin competitive binding assay. Unfortunately, **4** suffered from poor metabolic stability; after incubation with S9-hepatocellular fraction for one hour, only 19% (rat) and 30% (human) of **4** remained unchanged. Metabolite identification indicated that debenzylation and aromatic ring oxidation were the major routes of metabolism. This poor stability was reflected in the rat PK with a clearance of 8.6 L/h/kg and an oral bioavailability of 6% (Table 5). Herein we report our studies of the SAR around structure **4** and describe modifications which improve overall potency and reduce metabolic liabilities.

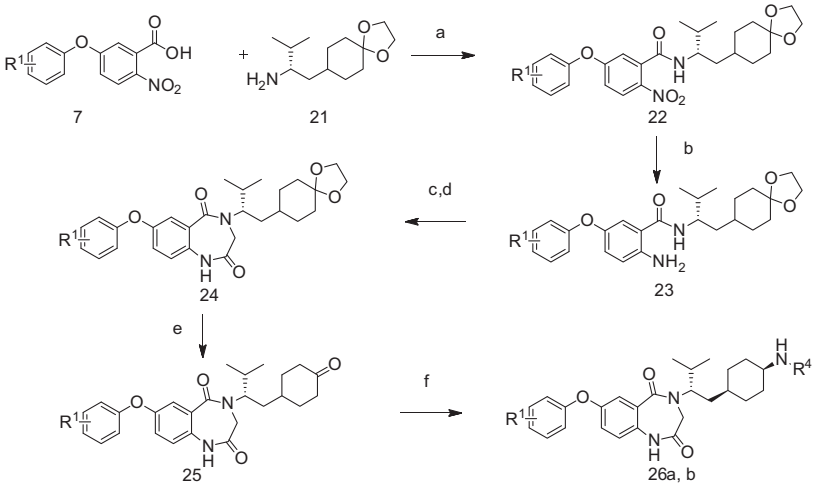
The analogs were synthesized as outlined in Schemes 1–5. The benzodiazapine intermediate **10a** was synthesized according to Scheme 1. Via nucleophillic aromatic substitution, various

substituted phenols **5** were condensed with 5-fluoro-2-nitrobenzoic acid **6** to give the resulting biphenyl ether **7**. This was then condensed with the corresponding amino acid *t*-butyl ester using EDC or HBTU coupling conditions to give amide **8**. Hydrogenation over palladium on carbon reduced the nitro group of **8** to aniline **9**, which was then acylated and cyclized with chloroacetyl chloride under basic conditions followed by deprotection of the *t*-butyl group with TFA to give benzodiazepine **10a**.

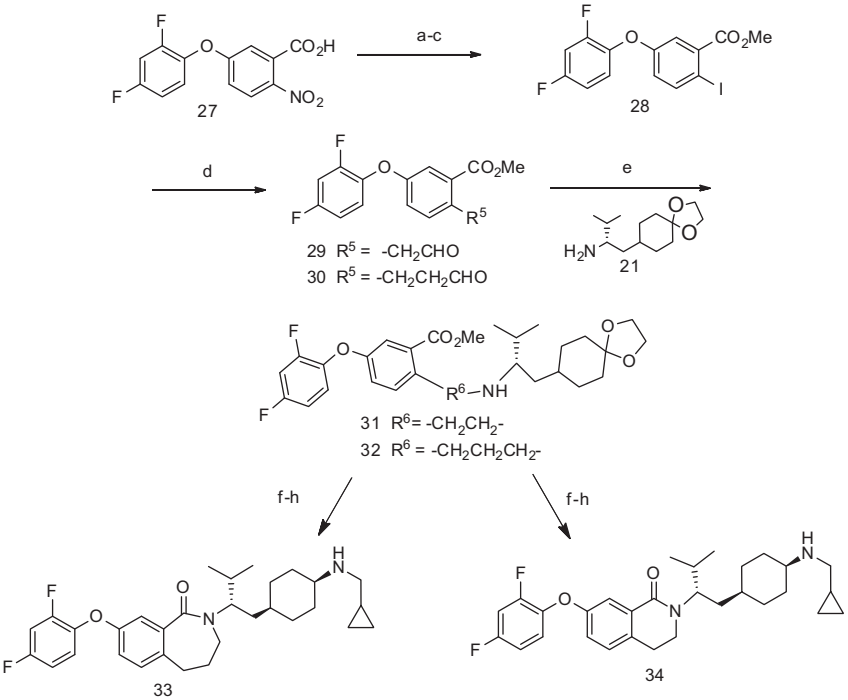
Analogs **4** (Tables 1–3), **10b** (Table 1), **11a–m** (Tables 1 and 2), **13a–h** (Tables 1–3), **14** (Table 3), and **15** (Table 1) were synthesized according to Scheme 2. From intermediate **10a**, EDC mediated amide formation afforded analogs **4**, **11a–m**, **10b**, and intermediate **12**. Hydrogenation was used to remove the benzylic group on **4** yielding **14**. Intermediate **14** was then coupled with benzoic acid using EDC to give amide **15**. Finally, intermediate **12** was converted to the ketone under acidic conditions and was subsequently reductively aminated with various amines in the presence of sodium triacetoxyborohydride to give analogs **13a–h**.

Scheme 3 outlines the synthesis of ketal intermediate **21**. The synthesis begins with the protection of ketone **16** with ethylene glycol and *p*-toluenesulfonic acid followed by the reduction of the ester group with LAH and conversion of the resulting alcohol to triflate **17**. Using Evans' asymmetric alkylation chemistry, oxazolidinone **18** was treated with LDA and alkylated with triflate **17** to give intermediate **19**.[11] The oxazolidinone group was then removed under basic conditions, and the resulting acid **20** was converted to the amine **21** under Curtius rearrangement conditions.[12]

Analogs **26a,b** (Tables 1 and 4) were prepared according to Scheme 4. First, amine **21** was coupled with acid **7** in the presence of EDC. Next, using hydrogenation conditions, the nitro group on

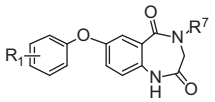


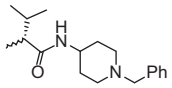
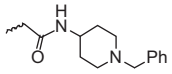
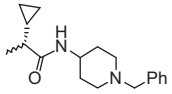
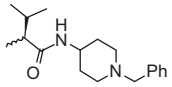
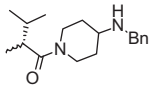
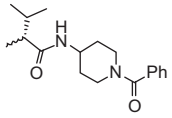
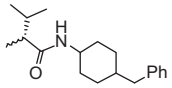
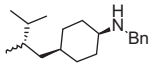
Scheme 4. Reagents and conditions: (a) EDC, Et_3N, DCM; (b) Pd/C, H_2, EtOH; (c) chloroacetyl chloride, Et_3N, DCM; (d) NaH, DMF; (e) TFA, H_2O; (f) cyclopropylmethyl amine or benzylamine, L-selectride, THF.



Scheme 5. Reagents and conditions: (a) TMS–diazomethane, MeOH, ether; (b) Pd/C, H_2, EtOH; (c) HNO_2, KI, EtOH; (d) (1) ethoxyethyne, BH_3, THF; (2) NaOH, $Pd(PPh_3)_4$, THF; or (1) allyl alcohol, $Pd(OAc)_2$ Et_3N, THF; (2) TFA; (e) $NaBH(OAc)_3$, DCM; (f) 50–60 °C, DMF; (g) TFA, H_2O; (h) cyclopropylmethyl amine, L-selectride, THF.

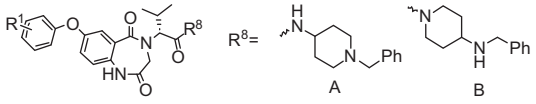
Table 1
SAR of piperidine ring



Compound	R^1	R^7	GHSRIa (Aeq.)[a] IC_{50} (μM)	Rat S9 % remaining	Human S9 % remaining
4	H		0.008	19	30
11k	H		0.314	–	–
11l	H		0.012	–	–
11m	H		0.196	–	–
13a	H		0.008	72	62
15	H		>10	–	–
10b	H		>10	95	23
26a	H		0.006	–	–

[a] Assay values are the means of three experiments. Standard deviation is ±30%.

Table 2
SAR of phenoxy ring



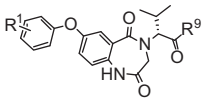
Compound	R^1	R^8	GHSRIa (Aeq.)[a] IC_{50} (μM)	Rat S9 % remaining	Human S9 % remaining
4	A	H	0.008	19	30
11a	A	4-F	0.011	–	–
11b	A	4-Me	0.007	–	–
11c	A	2-Me	0.003	3	5
11d	A	3-Cl	0.171	–	–
11e	A	4-Cl	0.028	–	–
11f	A	2-OMe	0.026	19	26
11g	A	3-OMe	0.143	10	22
11h	A	4-OMe	0.079	–	–
11i	A	4-F,2-Me	0.0002	3	5
11j	A	2,6-Me	0.001	–	–
13h	B	2,4-F	0.0003	65	70

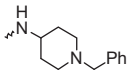
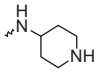
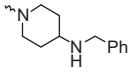
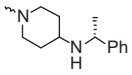
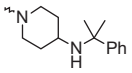
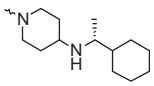
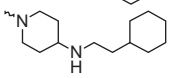
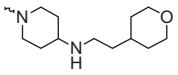
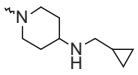
[a] Assay values are the means of three experiments. Standard deviation is ±30%.

22 was reduced to aniline **23** which was then acylated with 2-chloroacetyl chloride and cyclized with sodium hydride yielding benzodiazepine **24**. The ketal group was then removed under acidic conditions, and the resulting ketone **25** was reductively aminated using cyclopropylmethyl amine or benzylamine and L-selectride to yield analogs **26a,b** in a 12:1 ratio of *cis* to *trans* isomers.

Analogs **33** and **34** (Table 4) were synthesized as outlined in Scheme 5. First, the aryl nitrobenzoic acid **27** was esterified with TMS–diazomethane and then hydrogenated over palladium on carbon to give the aniline which was further transformed to aryliodide ester **28** under Sandmeyer conditions.[13] Using palladium-mediated conditions, ester **28** was converted to compounds **29** and **30**. Reductive amination with amine **21** converted these

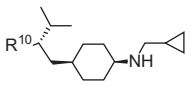
Table 3
SAR of benzyl sidechain

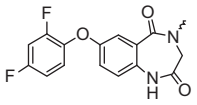
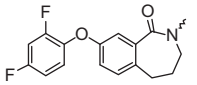
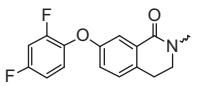


Compound	R^1	R^9	GHSRIa (Aeq.)[a] IC_{50} (μM)	Rat S9 % remaining	Human S9 % remaining
4	H		0.008	19	30
14	H		>10	—	—
13a	H		0.0080	72	62
13b	H		0.0025	46	27
13c	H		0.0165	48	19
13d	H		0.0025	28	30
13e	H		0.0041	—	76
13f	H		0.313	—	—
13g	H		0.050	71	86

[a] Assay values are the means of three experiments. Standard deviation is ±30%.

Table 4
SAR of benzodiazapine ring



Compound	R^{10}	GHSR1a (Aeq.)[a] IC_{50} (μM)	Rat S9 % remaining	Human S9 % remaining
26b		0.020	27	68
33		0.002	97	92
34		0.007	69	90

[a] Assay values are the means of three experiments. Standard deviation is ±30%.

aldehydes to intermediates **31** and **32**. The ketals **31** and **32** were cyclized upon heating to the corresponding quinolinone and azepinone, and then deprotected with acid. The resulting ketones were treated with cyclopropylmethyl amine and L-selectride to give the final products **33** and **34**.

The analogs were tested in a functional aequorin (Euroscreen) luminescent assay measuring the intracellular Ca^{2+} concentration to evaluate overall potency. IC_{50} values are presented in μM concentrations.[14] The metabolic stability for selected compounds was determined in an in vitro metabolic assay using S9 fractions of rat and human hepatocytes.[15] Values are expressed as a percent of parent remaining after 1 h incubation.

The lead optimization utilized a strategy where the initial structure **4** was divided into four regions: the valine piperidine linker (Table 1), the aryl phenoxy region (Table 2), the benzyl substituent (Table 3), and finally the benzodiazepine ring (Table 4). These regions were initially optimized independently of each other, and the best structural features were then combined in an effort to improve potency and metabolic stability. Particular attention was focused on variations that decreased molecular weight, removed or

Table 5
S9 stability and pharmacokinetic parameters[a,b]

Compds	CL (L/h/kg)	%F	AUC PO (μg h/L)	S9 % remaining[c]	
				Rat	Human
4	8.6	6	132	19	30
33	0.6	39	618	97	92

[a] $n = 3$ Sprague-Dawley rats per study.
[b] Dosed at 2 mg/kg po.
[c] Percent of parent remaining after 1 h incubation with rat and human S9.

ameliorated metabolically liable groups, and reduced the number of hydrogen donors and acceptors.

The SAR surrounding the valine and piperidine groups is outlined in Table 1. Removal of the valine isopropyl sidechain **11k** or the enantiomer **11m** caused a significant drop in potency, whereas changing the isopropyl group to the cyclopropyl group **11l** showed potency similar to the lead structure **4**. The regio-isomeric piperidine analog, namely the 4-(benzylamino)piperidin-1-yl amide **13a**, showed potency similar to the lead structure (1-benzylpiperidin-4-yl amide) **4**. Similarly, removal of the amide linkage altogether also resulted in an equipotent analog, **26a**. On the other hand, removal of the basic amine within the piperidine ring resulted in analog **15** and the cyclohexyl analog **10b** which showed complete loss of activity. This demonstrated that the basic amine was essential for potency against the ghrelin receptor. Metabolic stability data was not obtained for all of the structures within this table. However, it should be noted that the regio-isomeric piperidine amide **13a** showed a marked improvement in both rat and human S9 fractions with values of 72% and 62%, respectively, compared to the lead structure **4.**

Table 2 summarizes the SAR of the phenoxy region of the molecule. In general, small electron withdrawing groups maintained or improved the potency, whereas larger electron donating groups as in the analogs **11f–h** generally reduced the potency. Substitution in the *meta* position was less tolerated as shown by the analogs **11d** and **11g**. Disubstituted analogs 4-fluoro-2-methylphenoxy **11i** and 2,6-dimethylphenoxy **11j** showed an improvement in potency. *ortho* Substitutions on the phenoxy ring are believed to restrict the rotation of the ring which allows the compounds (**11i** and **11j**) to bind in a favorable conformation resulting in enhanced potency in the functional assay. Metabolic stability overall remained similar to the lead compound **4** with S9 values ranging from 3% to 26%. The 2-methyl substituted analogs **11c** and **11i** demonstrated a decrease in overall metabolic stability, with S9 values ranging from 3% to 5%. Metabolic identification showed that the 2-methyl group in these structures underwent significant oxidation in the presence of both rat and human S9. In a similar series using the regio-isomeric piperidine linker, structures containing a 2,4-difluorophenoxy substitution such as **13h** were able to ameliorate this metabolic liability while maintaining similar potency.

The SAR surrounding the benzyl group is outlined in Table 3. Removal of the benzyl group as seen in structure **14** resulted in complete loss of activity compared to the lead analog **4**. Introducing substitution in the benzylic position such as in analog **13b** with an IC_{50} of 2.5 nM proved to be beneficial over analog **13a** which had an IC_{50} value of 8 nM. The corresponding *gem*-dimethyl analog **13c** showed twofold loss in potency with an IC_{50} of 16.5 nM compared to **13a**. The aromatic ring was not essential for potency against the ghrelin receptor as demonstrated by the excellent activity of alkyl analogs **13d** (2.5 nM) and **13e** (4.1 nM). Smaller lipophilic substitutions were also tolerated such as the cyclopropyl analog **13g** which had an IC_{50} of 50 nM. Increasing polarity was detrimental to the activity as demonstrated by the THP analog **13f** which showed a 76-fold loss in potency compared to the cyclohexyl analog **13e**. Substitution in this area of the molecule had a significant impact on metabolic stability. The methylcyclopropyl analog **13g** had the best stability with rat and human S9 of 71% and 86%, respectively.

In Table 4, the best attributes from the left and right hand sides of the molecule were combined with various modifications to the central core structure. The difluorophenoxy and cyclopropylmethyl amine appendages were chosen for their marked improvement in overall metabolic stability while maintaining a respectable level of potency and lower molecular weight. When these sidechains were combined with the benzodiazepine core, this resulted in the structure **26b** which had an IC_{50} of 20 nM. This activity could be further improved by replacing the diazepine-2,5-dione in **26b** with the corresponding benzoazepin-1-one lacking the lower amide functionality as in structure **33**. When the seven member ring was replaced with a six member ring (**34**), this resulted in only a minimal loss of potency compared to **33**. Additionally, metabolic stability was markedly improved with these structures. Analog **33** had the best metabolic stability with values in rat and human S9 fractions being 97% and 92%, respectively

Analog **33** was further evaluated in rat PK where it was shown to have a clearance of 0.6 L/h/kg and a bioavailability of 39%. These values were a marked improvement over the initial lead structure **4** (Table 5).

In conclusion, a new class of ghrelin antagonists was identified. Through a systematic approach, key SAR interactions were discovered and metabolic liabilities were removed throughout the structure giving rise to analog **33**. This structure demonstrated excellent potency towards the ghrelin receptor and showed improved pharmacokinetic properties.

References and notes

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