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Letter

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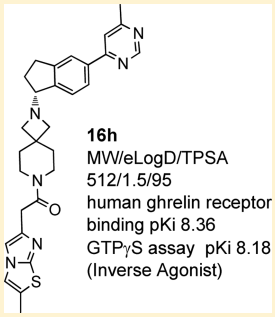
Discovery of PF-5190457, a Potent, Selective, and Orally Bioavailable Ghrelin Receptor Inverse Agonist Clinical Candidate

Samit K. Bhattacharya,*[,†] Kim Andrews,[‡] Ramsay Beveridge,[†] Kimberly O. Cameron,[†] Chiliu Chen,[†] Matthew Dunn,[†] Dilinie Fernando,[†] Hua Gao,[†] David Hepworth,[†] V. Margaret Jackson,[‡] Vishal Khot,[†] Jimmy Kong,[‡] Rachel E. Kosa,[§] Kimberly Lapham,[§] Paula M. Loria,[∥] Allyn T. Londregan,[†] Kim F. McClure,[†] Suvi T. M. Orr,[†] Jigna Patel,[⊥] Colin Rose,[†] James Saenz,[⊥] Ingrid A. Stock,[∥] Gregory Storer,[†] Maria VanVolkenburg,[‡] Derek Vrieze,[†] Guoqiang Wang,[†] Jun Xiao,[†] and Yingxin Zhang[†]

[†]Worldwide Medicinal Chemistry, [‡]Cardiovascular and Metabolic Research Unit, [§]Pharmacokinetics, Dynamics, and Metabolism, [∥]Primary Pharmacology Group, and [⊥]Pharmaceutical Sciences, Pfizer Global Research and Development, 620 Memorial Drive, Cambridge, Massachusetts 02139, United States

Supporting Information

ABSTRACT: The identification of potent, highly selective orally bioavailable ghrelin receptor inverse agonists from a spiro-azetidino-piperidine series is described. Examples from this series have promising in vivo pharmacokinetics and increase glucose-stimulated insulin secretion in human whole and dispersed islets. A physicochemistry-based strategy to increase lipophilic efficiency for ghrelin receptor potency and retain low clearance and satisfactory permeability while reducing off-target pharmacology led to the discovery of **16h**. Compound **16h** has a superior balance of ghrelin receptor pharmacology and off-target selectivity. On the basis of its promising pharmacological and safety profile, **16h** was advanced to human clinical trials.



KEYWORDS: *Ghrelin, ghrelin receptor inverse agonist, ghrelin receptor antagonist, diabetes, PF-5190457*

Type 2 diabetes mellitus (T2DM) is a rapidly expanding public health problem affecting over 285 million people worldwide.[1] The disease is characterized by elevated fasting plasma glucose, insulin resistance, abnormally elevated hepatic glucose production, and reduced glucose-stimulated insulin secretion.[2] Uncontrolled glucose levels can lead to severe downstream complications, such as elevated risks of cardiovascular disease, retinopathy, and nephropathy. While several classes of antidiabetic therapy are available for clinical use, there still remains a significant need for new therapies with improved efficacy, safety, and tolerability to help diabetes patients achieve their treatment goals and avoid long-term complications.[3]

Ghrelin, an agonist of the ghrelin receptor (earlier names for the receptor that have been used are GHS-R1a, GRLN, etc.), stimulates release of growth hormone (GH) from the pituitary gland and increases food intake.[4−8] Through infusion studies in man, ghrelin has been shown to suppress both glucose-dependent insulin secretion and insulin sensitivity.[9−13] The ghrelin receptor is expressed in pancreatic islets, and ghrelin is released into the pancreatic microcirculation. Islet-derived ghrelin has been reported to play an important role in the regulation of insulin release in rodents.[14] Although ghrelin has been shown to cross the blood−brain barrier,[15] several effects of ghrelin appear to be peripherally mediated as shown by the clinical results from the brain-impaired ghrelin receptor agonist capromorelin (CP-424391).[16,17] Treatment with capromorelin led to increases in appetite, fasting glucose, HbA1c levels, and insulin resistance. Thus, ghrelin receptor antagonists/inverse agonists are anticipated to improve glucose homeostasis and insulin sensitivity. Described herein is the discovery of PF-5190457, a potent and selective ghrelin receptor inverse agonist that increases glucose-stimulated insulin secretion (GSIS) in human whole and dispersed islets.

A number of small molecule ghrelin receptor antagonists have been described in the literature (see Figure 1).[18−26] The

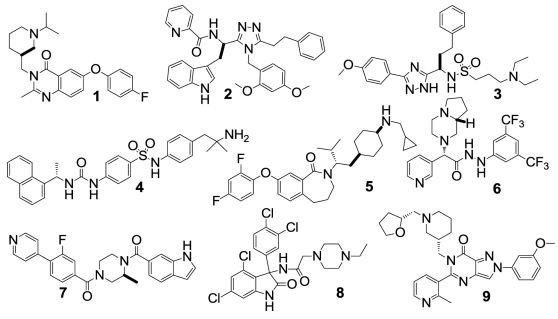


Figure 1. Examples of ghrelin receptor antagonists.

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vast majority of these efforts have focused on centrally acting agents targeted toward the treatment of obesity. We previously reported on a spiro-azetidino-piperidine series that was identified from high-throughput screening (HTS) of our corporate file.[27] In a later report, we described efforts to optimize this series to achieve high central receptor occupancy targeting an obesity indication. Thus, the HTS-hit (**10**) in the aforesaid series was transformed to a centrally acting ghrelin receptor inverse agonist lead (**11**, Table 1).[28]

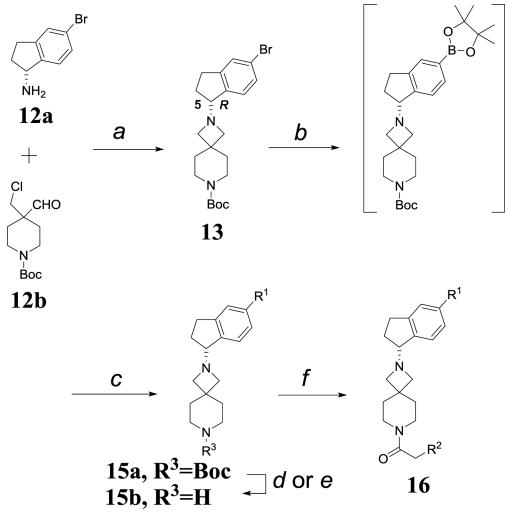
Emerging ghrelin biology suggests that blocking ghrelin receptor signaling has the potential to ameliorate diabetes through direct action on the pancreas.[29] To test this hypothesis, lead **11** was assessed in a human dispersed islet cell assay. As anticipated, **11** induced insulin secretion in a glucose-dependent manner (Supporting Information, Figure S1). These data, in conjunction with other published work linking ghrelin receptor inhibition to improved insulin sensitivity, caused us to redirect our research efforts toward diabetes (T2DM) as the primary indication, and we targeted the peripheral compartment as the key site of action.

The spiro-azetidino-piperidine series was an attractive starting point for optimization for this indication. It provided a rigid core scaffold with high fractional sp^3 content (for leads in series, F_{sp^3} = 0.41−0.53)[30] and two point vectors for introducing orthogonal diversity using simple coupling methodologies. A significant factor in the selection of this series was *consistent inverse agonism of ghrelin receptor*. Functional switching (between antagonism and partial agonism) has been observed by us and others and has demonstrated impact on the in vivo performance of compounds.[26,31] We believed that by focusing on a series where receptor functionality was not an issue, we could accelerate our drug discovery program by eliminating one dimension from our lead optimization efforts and, at the same time, streamline our in vitro pharmacology testing funnel. Furthermore, because of the high constitutive activity of the ghrelin receptor, inverse agonism of ghrelin receptor may provide a greater opportunity for in vivo efficacy through reductions in basal receptor firing.[32]

Although lead **11** showed encouraging insulin secretion effects in islet studies, more extensive profiling highlighted issues that prevented its further development. In preclinical CV safety testing, **11** showed inadequate safety margins to allow progression. The discovery of the indane amine moiety in **11** (built as a less lipophilic surrogate of an *ortho*-chloro benzylic amine) had allowed improvements in selectivity within the spiro-azetidino-piperidine series.[28] However, while selectivity over muscarinic receptors (in particular M2) was improved with the desired chiral indane, in wide-ligand promiscuity profiling (CEREP; tested at a dose of 10 μM, Figure 2) **11** was active at a number of targets including adrenergic α2c and α2a, dopamine D2s, D3, and D4, and histamine H1. Compound **11** also showed human ether-à-go-go-related gene (hERG) activity (IC_{50} = 2.9 μM). Thus, the overall off-target pharmacology profile, coupled with the physicochemical properties of **11** (Table 1), was of concern. An increased risk of adverse safety findings for compounds with higher clogP and lower TPSA has been described.[33] In addition, several groups report that off-target promiscuity leads to elevated safety risk.[34,35] Therefore, our primary objective was to identify a compound in this series with reduced off-target pharmacology in order to achieve broad safety margins to allow advancement to the clinic. Our strategy to reduce off-target pharmacology was to increase the lipophilic efficiency (LipE)[36,37] and retain or improve ghrelin receptor potency while increasing the overall polarity of the inverse agonists. This physicochemistry-based strategy should provide the added benefit of decreasing CNS penetration, leading to a reduction in CNS-based side effects.

Scaffold **13** provided a modular framework for exploring structure−activity relationship (SAR), and earlier work established the preference for the *R*-enantiomer at C(5) as providing robust ghrelin receptor activity. A one-pot sequence reacting chiral amine **12a** with chloroaldehyde **12b** in the presence of $NaCNBH_3$ was developed and used to deliver bulk quantities of **13** (with retention of stereochemistry at the C(5) position), a key chiral intermediate for flexible incorporation of a variety of R^1 and R^2 groups onto scaffold **16**.[28] Suzuki coupling to incorporate R^1 followed by in situ deprotection (Scheme 1) under acidic conditions unmasked the piperidine

Scheme 1. Synthesis of Analogues 16a−h



Reagents and conditions: (a) (i) HOAc, MeOH, 50 °C, 2 h; (ii) $NaCNBH_3$, 70 °C; 99%; (b) bis(pinacolato)diboron, Pd(dppf)Cl_2 (1.6 mol %), KOAc, dioxane, 110 °C, 1 h, >99% conversion; (c) R^1-Cl or R^1-Br, Pd(dppf)Cl_2 (2.5 mol %), K_2CO_3 (aq), dioxane, 110 °C, 3 h, 90% conversion; (d) HCl; (e) TFA; (f) $R^2CH_2CO_2H$ (14), HBTU or CDI, DIEA or TEA, 50−99% (over 2 steps).

nitrogen (**15b**) for incorporation of the R^2 amide. Amide coupling with a variety of carboxylic acids was accomplished with HBTU or CDI as the preferred coupling agent.

We identified two R^2 moieties, pyridyl-3-methoxy (**16a**) and the bicyclic imidazothiazole (**16b**),[27] that maintained ghrelin receptor potency at lower logD with retention of other properties, such as low clearance and satisfactory permeability. Unfortunately, when combined with 2-pyrimidinyl at R^1, neither compound had a positive effect on selectivity over M2 (Table 1). As anticipated, these changes, which were designed to lower lipophilicity, had the effect of increasing efflux ratios in an in vitro MDR assay (*b-a*/*a-b* efflux ratio of >2.5 being generally indicative of reduced CNS exposure; Table 1).[38]

In an attempt to improve M2 selectivity, we switched attention to modification of R^1. Attempts to further increase polarity, as exemplified by carboxamide **16c**, had no effect on selectivity (Table 1). Thus, to complement the property-based design strategy, we investigated the effect of more subtle structural changes on M2 potency. Small changes around the pyrimidinyl group (as exemplified by **16d**, **16e**, and **16f**) did

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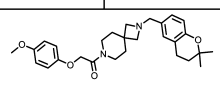
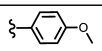
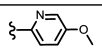
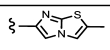
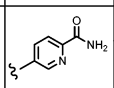
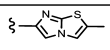
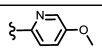
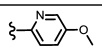
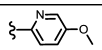
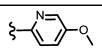
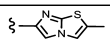
Table 1. Data for Spiro-azetidino-piperidine Analogues 10, 11, and 16a–h

Entry	R^1	R^2	MW	elogD[a]	TPSA[b]	ghrelin receptor binding pK_i[c,d]	ghrelin receptor functional pK_i[c,e] (%Effect)[f]	M2 pK_b[c,g]	M2 K_b/ghrelin receptor K_i ratio[h]	HLM $Cl_{int,app}$[i]	P_{app}[j]	hMDR ba/ab ratio[k]
10			464	(3.0)	51	6.67±0.26	6.39±0.26 (-22%)	n/a	n/a	15	n/a	2.0
11			468	2.3	59	8.20 ±0.17	7.76±0.13 (-39%)	5.94±0.13	44	37	13.2	2.4
16a			469	0.95	71	7.53± 0.45	7.60±0.09 (-42%)	6.18±0.25	32	14	14	3.9
16b			498	1.4	95	8.34 ± 0.44	8.18±0.2 (-31%)	6.41±0.06	47	18	11	5.4
16c			540	1.6	125	7.98 ± 0.19	n/a	6.48±0.26[l]	n/a	n/a	n/a	>18.2
16d			469	1.1	69	7.16±0.34	n/a	6.08±0.17[l]	n/a	82	15	5.4
16e			483	1.2	71	7.81±0.09	7.30±0.03[l] (-92%)	6.09±1.81[l]	20	17	10	3.7
16f			483	1.5	71	8.04±0.14	7.07±0.13 (-40%)	5.86±0.90[l]	17	18	13.5	4.5
16g			483	1.0	71	7.89±0.08	7.77±0.06 (-25%)	5.66±0.05	103	24	13	6.4
16h			512	1.5	95	8.36±0.18	8.18±0.06 (-29%)	5.71±0.08	266	21	5	7.2

[a]elogD = measured logD at pH 7.4 [calculated logD (ACD Laboratories program v12) in parentheses]. [b]TPSA = topological polar surface area. [c]Geometric mean ± standard error of ≥3 measurements (unless noted otherwise). [d]Human ghrelin receptor SPA receptor binding assay as published.[27] [e]Human ghrelin receptor agonist/antagonist/inverse agonist GTP-γ-S functional assay as published.[27] [f]Minimum percent effect in parentheses; a negative value indicates an inverse agonist. [g]Muscarinic M2 β-Arrestin PathHunter assay from Discoverx. K_b for antagonists determined in the presence of EC_{80} concentration of agonist, oxotremorine.[39] [h]Ratio of M2 K_b over human ghrelin receptor functional K_i values. [i]Human liver microsome (HLM) apparent intrinsic clearance, mL/min/kg, uncorrected for fraction unbound. [j]Passive permeability (P_{app} in 10^{-6} cm/s) measured using low-efflux MDCKII cells as described.[40] [k]MDR BA/AB efflux ratio using MDCK cell line transfected with human MDR1.[41] [l]Less than three independent replicates.

not provide significant improvement with the exception of the 6-methyl-4-pyrimidinyl group (as in **16g**), which showed improved ghrelin receptor potency, as compared to **16a**, and a marked enhancement in M2 selectivity. Gratifyingly, combination of the 6-methyl-4-pyrimidinyl group at R^1 with the more polar imidazothiazole moiety at R^2, provided **16h**, which demonstrated significant improvements in both functional potency and selectivity against M2 relative to **11** (Table 1). Indeed, compound **16h** has one of the highest lipophilic efficiencies ($LipE_{elogD}$ = 6.9) in this series, which is a desirable predictor for lowered promiscuity-related developmental attrition risk.[34,36] Compound **16h** maintained a moderate clearance in human liver microsomes (HLM). In addition, the reductions in logP achieved in moving to **16h** had the anticipated and desired effects on MDR efflux ratio, suggesting a high probability of reduced CNS exposure. (Incidentally, **16h** was 11-fold impaired in rat brain after chronic dosing for 14 days.)

Given its promising in vitro pharmacology and ADMET parameters, compound **16h** was profiled in more detail. Compound **16h** showed reduced off-target activity, as assessed by the CEREP panel (screened at 10 μM, Figure 2), with serotonin 5-HT_{2B} (IC_{50} = 3700 nM) being the only target inhibited to >50%. In follow-up screening, **16h** did not demonstrate any agonist (or antagonist) functional effects at this receptor.

Compound **16h** was advanced to a number of ex vivo and in vivo studies. The full pharmacology profile for **16h** (GTP-γ-S functional profile in Supporting Information, Figure S2),

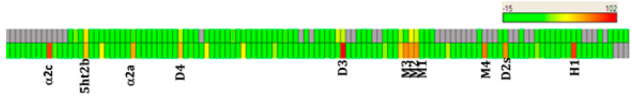


Figure 2. Comparative CEREP profile of **11** (bottom) and **16h** (top).

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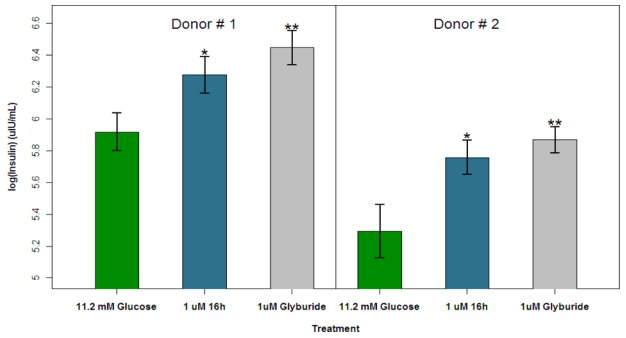

Figure 3. Glucose-stimulated insulin secretion in human whole islet static culture following incubation with **16h** at 1 μM. $^{**}p < 0.001$; $^{*}p < 0.05$. Measurement data are expressed as the arithmetic mean ± standard error.

including a biomarker for target engagement, will be described separately.[42] A human islet assay was used in order to gain confidence in the ability of **16h** to increase insulin secretion in humans (Figure 3). Human whole islets in static culture were incubated at both low (2.8 mM) and high (11.2 mM) glucose concentrations and demonstrated that the islets were glucose responsive.[43] The sulfonylurea, glibenclamide (glyburide), was tested as a positive control. Compound **16h** (1 μM) significantly increased insulin secretion above the 11.2 mM glucose control.

To predict human pharmacokinetics and enable clinical dose setting, the pharmacokinetics of **16h** were evaluated preclinically in three species (Supporting Information, Table S1). Rat pharmacokinetics revealed both high plasma clearance and volume of distribution (consistent with the high clearance value derived from rat liver microsomal in vitro assay). Dog and monkey plasma clearance and volume of distribution were moderate. Urinary and biliary elimination of **16h** contributed minimally to the overall clearance in the species investigated. Because of its high in vivo rat clearance, **16h** was tested in portal vein cannulated rats and demonstrated excellent absorption (F_a = 100%) as anticipated given its high solubility and moderate passive permeability.[44] The human plasma clearance of **16h** was predicted by scaling human liver microsomal data (human hepatocyte data was consistent with microsomal data). When corrected for microsomal ($f_{u,mic}$ = 0.8) and plasma binding (f_u = 0.15), human plasma clearance was predicted to be low (3.3 mL/min/kg). Using preclinical data and physiologically based pharmacokinetic (PBPK) models,[45] **16h** was predicted to have moderate to high human absorption (86%) along with moderate measures of bioavailability (67%), V_{dss} (1.79 L/kg), and half-life (6.3 h). Human free drug concentrations ($C_{eff,u}$) needed to achieve efficacy were based on the equilibrium binding constant (K_d) of **16h**, which was determined using the Motulsky kinetics procedure (3.04 ± 0.91 nM).[42,46] We sought to maintain minimal free drug concentrations of 10 × K_d for the full dosing period (~30 nM). Combining this $C_{eff,u}$ value with predicted human PK parameters provided a projected human oral dose of 35 mg bid. The corresponding predicted $C_{max,u}$ and AUC_u values are 52.4 nM and 1010 nM·h, respectively.

Compound **16h** was evaluated in a variety of assays to ensure robust safety (see Supporting Information). While **16h** showed moderate potential for inhibition of hERG current, the IC_{50} (6.9 μM) represents a significant multiple (>100×) over the predicted human C_{max} required to maintain $C_{eff,u}$ for the full dosing interval (52.4 nM free). Thus, the likelihood of achieving drug exposures in the clinic that would effect QT_c prolongation was considered low.

With the primary design objectives of delivering a peripherally acting ghrelin receptor inverse agonist with improved selectivity satisfied, the nonclinical safety profile of **16h** was assessed in both rats and dogs in 1-month toxicology studies and in safety pharmacology studies. Gratifyingly, the safety profile of **16h** demonstrated *sufficient safety margins* above the projected C_{eff} in humans and supported continued development of the compound.

In summary, we set out to transform a centrally acting ghrelin receptor inverse agonist indane lead (**11**) to a peripherally restricted one, by pursuing a physicochemistry-based strategy to increase LipE for ghrelin receptor potency and reduce off-target pharmacology while retaining low clearance and satisfactory permeability. The addition of the 6-methyl-4-pyrimidinyl indane in the *R* configuration and the imidazothiazole group to the spiro-azetidino-piperidine core led to the discovery of **16h**. Compound **16h** is a potent inverse agonist with excellent selectivity and demonstrated robust increases in glucose-stimulated insulin secretion in human islets. Human pharmacokinetic predictions project a dose of 35 mg bid to achieve 10 × K_d at trough concentrations in the clinic. On the basis of the pharmacological profile and safety results, **16h** (PF-5190457) was advanced to human clinical trials, and results from these trials will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures of analogue preparation and description of biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(S.K.B.) Tel: 617-551-3177. E-mail: samit.k.bhattacharya@pfizer.com.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

TPSA, topological polar surface area; dppf, 1,1′-bis-(diphenylphosphino)ferrocene; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; CDI, 1,1′-carbonyldiimidazole; GHS-R1a, growth hormone secretagogue receptor; GSIS, glucose stimulated insulin secretion; QTc, heart rate-corrected QT interval; PK, pharmacokinetic

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