ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Evaluation and Synthesis of Polar Aryl- and Heteroaryl Spiroazetidine-Piperidine Acetamides as Ghrelin Inverse Agonists

Suvi T. M. Orr,*[,▽] Ramsay Beveridge,[§] Samit K. Bhattacharya,[#] Kimberly O. Cameron,[#] Steven Coffey,[§] Dilinie Fernando,[§] David Hepworth,[#] Margaret V. Jackson,[†] Vishal Khot,[§] Rachel Kosa,[‡] Kimberly Lapham,[‡] Paula M. Loria,[∥] Kim F. McClure,[#] Jigna Patel,[⊥] Colin Rose,[#] James Saenz,[⊥] Ingrid A. Stock,[∥] Gregory Storer,[§] Maria von Volkenburg,[†] Derek Vrieze,[§] Guoqiang Wang,[§] Jun Xiao,[§] and Yingxin Zhang[§]

[#]Worldwide Medicinal Chemistry, Pfizer Global Research and Development, 610 Main St., Cambridge, Massachusetts 02139, United States

[§]Worldwide Medicinal Chemistry, Pfizer Global Research and Development, 550 Eastern Point Road, Groton, Connecticut 06340, United States

[▽]Worldwide Medicinal Chemistry, Pfizer Global Research and Development, 10770 Science Center Drive, San Diego, California 92121, United States

[†]Cardiovascular and Metabolic Research Unit, Pfizer Global Research and Development, 610 Main Street, Cambridge, Massachusetts 02139, United States

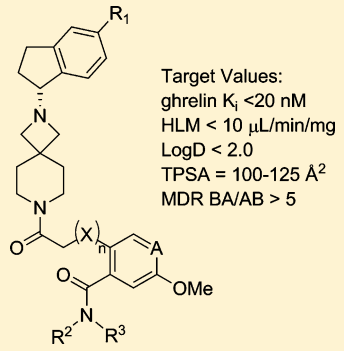
[‡]Pharmacokinetics, Dynamics and Metabolism, Pfizer Global Research and Development, 550 Eastern Point Road, Groton, Connecticut 06340, United States

[∥]Primary Pharmacology Group, Pfizer Global Research and Development, 550 Eastern Point Road, Groton, Connecticut 06340, United States

[⊥]Pharmaceutical Sciences, Pfizer Global Research and Development, 550 Eastern Point Road, Groton, Connecticut 06340, United States

Supporting Information

ABSTRACT: Several polar heteroaromatic acetic acids and their piperidine amides were synthesized and evaluated as ghrelin or type 1a growth hormone secretagogue receptor (GHS-R1a) inverse agonists. Efforts to improve pharmacokinetic and safety profile was achieved by modulating physicochemical properties and, more specifically, emphasizing increased polarity of our chemical series. *ortho*-Carboxamide containing compounds provided optimal physicochemical, pharmacologic, and safety profile. pH-dependent chemical stability was also assessed with our series.



KEYWORDS: *Heteroaryl acetic acid, carboxamide, spiroazetidine-piperidine, growth hormone secretagogue receptor 1a, ghrelin, type 2 diabetes*

Type 2 diabetes mellitus (T2DM) is the more common form of diabetes and millions of people are affected by T2DM, especially in Western countries.[1] The pancreas of type 2 diabetics cannot either produce insulin or the cells in the body are incapable of using insulin effectively. In addition, about 90% of type 2 diabetics are overweight or obese.[2] Ghrelin hormone, an acylated 28-amino acid peptide, is a physiological regulator of insulin release and glucose homeostasis,[1] and it plays a central role as a hunger hormone.[3] Ghrelin is an endogenous ligand of type 1a growth hormone secretagogue receptor (GHS-R1a), also called GRLN (human ghrelin receptor), which is expressed in the pancreatic islets, and is released in the pancreatic microcirculation.[4] Small molecule agonists of ghrelin have been shown to induce hyperglycemia and insulin resistance,[5,6] whereas antagonists (and inverse agonists) have been shown to improve glucose tolerance, suppress appetite, and promote weight loss.[7−18]

We have previously disclosed our efforts to identify centrally and peripherally acting inverse agonists of ghrelin from the

Received: October 9, 2014
Accepted: December 14, 2014
Published: December 14, 2014

spiropiperidine-azetidine series.[19−21] Our discovery efforts focused on the identification of inverse agonists over antagonists in order to reduce basal receptor firing, as there is a high constitutive activity of ghrelin receptor.[22] Our centrally acting and orally bioavailable lead compound **2** (Figure 1)

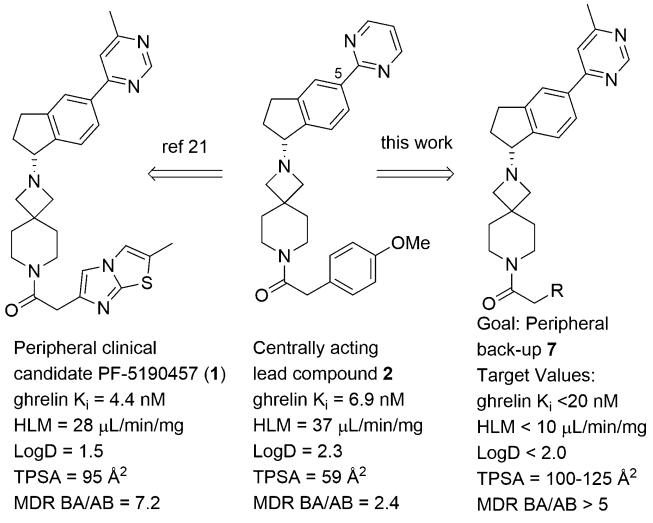
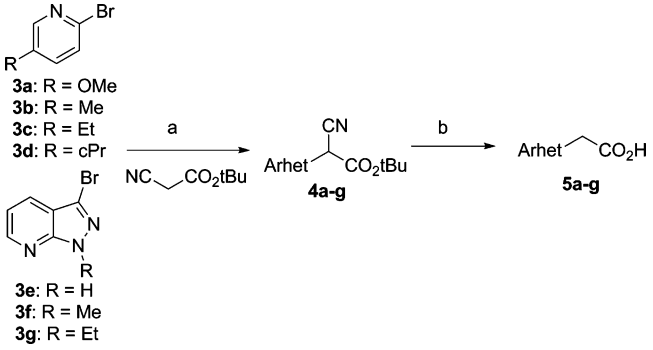


Figure 1. Pfizer ghrelin inverse agonists.

Scheme 1[a]

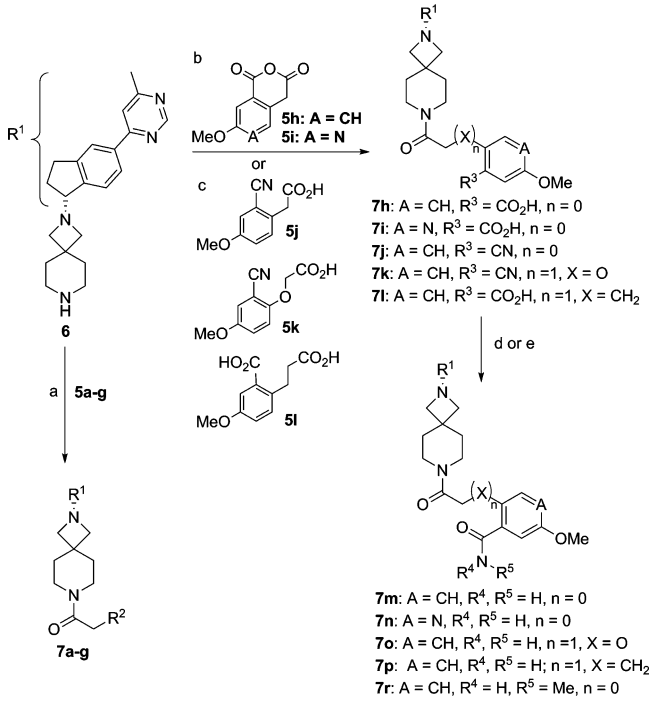


[a]Reagents and conditions: (a) $Pd(PPh_3)_4$ or $Pd(dppf)_2Cl_2$, THF or 1,4-dioxane, reflux, 18 h; (b) conc. HCl, reflux, 6−18 h.

showed significant glucose-dependent insulin secretion in a human dispersed islet cell assay. However, compound **2** suffered from several off-target activities in a Cerep safety panel, and the physicochemical properties were not ideal for further development.[20] The project team noted that the requisite effect should be derived from action on the islet cells in pancreas; as such, peripheral restriction will have advantages in safety and side effect profile.[20]

Variations of **2** at both the C(5) position of the indane ring and the piperidine acetamide portion to access compounds of increased lipophilic efficiency (LipE)[23,24] were investigated and compound **1** (PF-5190457) was discovered as a peripherally acting potent ghrelin inverse agonist.[21] This compound had excellent off-target selectivity and good predicted human pharmacokinetic properties (projected 35 mg bid dose), and it was advanced to human clinical studies. Concurrently, an alternative strategy was investigated to increase polarity and TPSA of the lead compound **2** with further variations at the acetamide portion. Herein, we describe efforts to identify an improved derivative of compound of **2** with reduced off-target

Scheme 2[a]



[a]Reagents and conditions: (a) EDCI, HBTU or CDI, DIEA or Et_3N; (b) Et_3N; (c) EDCI or CDI, DIEA or Et_3N; (d) $Pt(PMe_2OH)_3H$, water, EtOH; (e) CDMT·NMM, NH_4Cl or $MeNH_2$.

pharmacology, optimal physicochemical properties, and increased polarity.

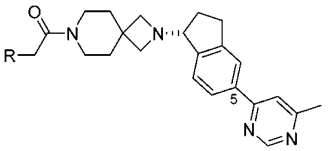
To achieve the desired improvements, we set our goals to achieve at least 20 nM ghrelin potency, lower than 10 μL/min/mg intrinsic clearance in human liver microsomes (HLM), logD lower than 2, total polar surface area (TPSA) between 100 and 125 Å^2, and efflux ratio (BA to AB) greater than 5 in the PgP efflux assay in MDR-MDCK1 (MDR) cell line.[25] The latter two goals were set to minimize brain exposure.[26,27] With the discovery of **1**, we had optimized the indane C(5) heteroaryl group as the 6-methyl-4-pyrimidine and chose to investigate polar replacements of the 4-methoxyphenyl group of **2**.

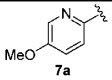
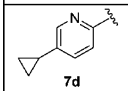
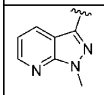
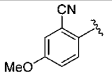
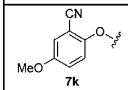
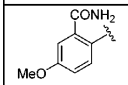
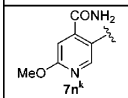
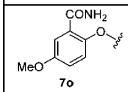
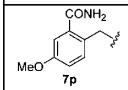
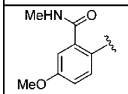
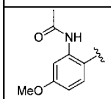
The synthesis of several polar heteroaryl acetic acids is shown in Scheme 1 starting from commercially available heteroaryl bromides **3**. Treatment of **3** in the presence of palladium tetrakistriphenylphosphine ($Pd(PPh_3)_4$) or palladium 1,1′-bis(diphenylphosphino)ferrocene dichloride ($Pd(dppf)_2Cl_2$) and an anion generated from *tert*-butylcyanomalonate and KO*t*Bu (or LiO*t*Bu) provided intermediates **4**. Acidic hydrolysis and decarboxylation provided acids **5**. It is worth noting that a second decarboxylation of the final compounds **5b** and **5c** occurs very rapidly (1−2 h) at room temperature and over time (∼2 weeks) in a refrigerator. Decarboxylation of similar substrates under basic conditions and heat are known.[28] The cyclopropyl substituted acid **5d** was slightly more stable than the methyl and ethyl substituted acids **5b** and **5c**, but even **5d** underwent decarboxylation over 1−2 months at +4 °C. These three acids were freshly prepared and used immediately in the subsequent amide coupling reactions. The other acids (**5a,e-g**) were stable at ambient temperature.

The acids **5a−g** were reacted with amine **6**[21,29,30] in the presence of a coupling reagent (such as HBTU, TBTU, EDCI,

dx.doi.org/10.1021/ml500414n | ACS Med. Chem. Lett. 2015, 6, 156−161

Table 1. In Vitro Data for Selected Ghrelin Inverse Agonists 7a−s



R	SFLogD[a]	ghrelin binding K_i (nM)[b,c]	ghrelin functional K_b (nM)[c,d] (%effect)[e]	HLM (μL/min/mg)[f]	TPSA (Å^2)[g]	MDR (BA/AB)[h]	Passive permeability (10^{-6} cm/s)[i]	Stable at pH 7.4 (yes/no)[j]
 7a	1.6	14±2	17±3 (-25%)	19	71.4	6.4	13	yes
 7b	(1.1)	49±4	N/A	20	62.2	4.5	10	yes
 7d	(1.8)	6.4±0.4	14±7 (-15%)	48	62.2	N/A	10	yes
 7f	2.1	166±25	N/A	32	80.4	6.9	12	yes
 7j	2.4	13±2	28±8 (-16%)	20	82.3	1.16	14	yes
 7k	2.3	4.0±0.6	5.0 (n=1) (-14%)	39	91.6	3.9	5.0	yes
 7m	1.4	9.2±4.0	14±3 (-18%)	<8.3	101.6	>17	2.2	no
 7n[k]	(0.8)	263±6	328 (n=1) (-15%)	N/A	114.5	N/A	N/A	no
 7o	2.0	127±17	81 (n=1) (-12%)	11	110.9	>17	4.0	yes
 7p	1.9	139±24	95 (n=1) (-13%)	71.7	101.6	N/A	3.1	yes
 7r	(0.9)	20±1	N/A	13	87.7	>17	13	no
 7s	1.6	28±4	57±18 (-16%)	33.0	87.7	12.0	15	yes

[a]SFLogD, shake-flask logD at pH 7.4; if not measured, calculated (ACD Laboratories program v12) LogD at pH 7.4 is given in parentheses. [b]All human ghrelin receptor SPA binding data were generated as previously described in ref 19. [c]Geometric mean ± standard error of ≥3 measurements, unless noted otherwise. [d]human ghrelin receptor agonist/antagonist/inverse agonist GTP-γ-S functional assay as published in ref 20. [e]Negative % effect indicates an inverse agonist. [f]HLM, human liver microsome clearance; not corrected to fraction unbound. [g]TPSA, topological polar surface area. [h]MDR efflux ratio (BA/AB) in MDCK/MDR1 cell line as described in ref 25. [i]Passive permeability measured using low efflux MDCKII cells as described in ref 34. [j]UPLC-MS, ultra pressure liquid chromatography mass spectrometry determination of disappearance of the parent compound and appearance of compound **6** at pH 7.4 over 24 h time period; "yes" indicating stability and "no" indicating instability (see Supporting Information). [k]Compound **7n** has indane C(5) 4-methyl-2-pyrimidine substituent (see Supporting Information) instead of 6-methyl-2-pyrimidine substituent that all other analogues have.

or CDI) to form amides **7a−g** that were tested in the human ghrelin binding assay,[19] in HLM and MDR assays (Scheme 2, arrow a; and Table 1). Even though compounds **7a** and **7d** were very potent ghrelin binders (14 and 6 nM, respectively) and inverse agonists in the functional assay (17 and 14 nM, respectively),[21] they either did not achieve the required stability in HLM (48 μL/min/mg for **7d**) or they suffered from off-target activities (i.e., >50% inhibition of M1, M2, MCH1, and

dx.doi.org/10.1021/ml500414n | ACS Med. Chem. Lett. 2015, 6, 156−161

Table 2. Comparison of Physical Chemical, Pharmacological, ADME, and Safety Properties of Compounds 7m and 2

property	compd **7m**	compd **2**
MW	525.6	468.6
cLog P	1.76	2.82
SFLogD (pH 7.4)[a]	1.37	2.30
TPSA (Å^2)	102	59
binding K_i (nm)[b,c]	9 ± 4	7 ± 2
functional K_b (nM) (% effect)[d,e]	14 ± 3 (−18%)	17 ± 7 (−39%)
RLM Cl_{int} (μL/min/mg)[f]	77.4	55.4
DLM Cl_{int} (μL/min/mg)[g]	16.8	29.0
HLM Cl_{int} (μL/min/mg)[h]	<8.6	36.8
CYP450 IC_{50} (1A2, 2C9, 2C19, 2D6, 3A4)	all >30 μM	3A4: 2.5 μM
kin sol (pH 6.5, μg/mL)[i]	185	187
permeability AB (P_{app} cm/s)[j]	2.2 × 10^{-6}	13.2 × 10^{-6}
permeability (MDCK/MDR1, BA/AB)[k]	>17	2.4
hERG (patch clamp) IC_{50} (μM)	47	2.9
CEREP panel (82 assays) IC_{50}	>10 μM (81 assays)	several hits at 10 μM (ref 21)
CEREP hit (5-HT_{2B}) IC_{50} (μM)	7.6	4.3

[a]SFLogD, shake-flask logD at pH 7.4. [b]Geometric mean ± standard error of ≥3 measurements. [c]Human ghrelin receptor SPA binding assay as published in ref 19. [d]Human ghrelin receptor inverse agonist GTP-γ-S functional assay as published in ref 20. [e]Negative % effect indicates an inverse agonist. [f]Rat liver microsome (RLM) intrinsic clearance. [g]Dog liver microsome (DLM) intrinsic clearance. [h]Human liver microsome (HLM) intrinsic clearance. [i]Analiza kinetic solubility (kin sol) from 10 mM DMSO solution at pH 6.5 (2% sodium phosphate buffer) after 24 h. [j]Passive permeability using low efflux MDCKII cells as described in ref 34. [k]MDR efflux ratio (BA/AB) in MDCK/MDR1 cell line as described in ref 25.

5HT_{2B} receptors at 10 μM concentration in the Cerep panel for **7a**). Compounds **7b−c** and **7e−g** did not pass our potency or clearance requirements (all >50 nM in the ghrelin binding assay).

An alternative approach to improve clearance via further increases in polarity (logD and TPSA) involved installing a polar substituent, such as a carboxamide or a nitrile, to the aromatic ring of **2**. Synthetic routes to these polar amides are depicted in Scheme 2 (arrows b−e). Direct coupling of CN-aryl acids **5j** and **5k** with amine **6** under EDCI or CDI amide coupling conditions delivered compounds **7j** and **7k** that could be further transformed to the carboxamides via Pt catalyzed hydrolysis[31] or via urea-hydrogen peroxide mediated hydrolysis[32] to provide compounds **7m** and **7o** in quantities that were suitable for structure−activity relationship (SAR) purposes.

Compounds **7j**, **7m**, and **7r** (Table 1) exhibited the required target values for ghrelin potency (13, 9.2, and 20 nM, respectively), low clearance (20, <8.3, and 13 μL/min/mg, respectively), high polarity, and high efflux ratios (>17 MDR BA/AB for **7m** and **7r**). Compound **7j** was among the most potent, but suffered from low MDR BA/AB ratio (1:1). As compound **7m** started to emerge as a potential back-up compound, more material needed to be synthesized. The overall yield via the nitrile intermediate **7j** was low and not suitable for scale up work. An alternative way to generate the *ortho*-amides was to treat the amine **6** with anhydrides **5h** or **5i** or *ortho*-acid **5l** to generate *ortho*-carboxylic acid intermediates **7h**, **7i**, and **7l** that were then treated with NH_4OH in the presence of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT)[33] and *N*-methylmorpholine (NMM) to give **7m**, **7n**, and **7p** in high and scalable (**7m** up to 10 g) yields. The intermediate **7h** could also be treated under the CDMT conditions in the presence of methylamine to access **7r** or in the presence of dimethylamine to access the dimethyl analogue ($R_4 = R_5 = Me$) in high yields and high purity.

Compound **7m** exhibited the optimal combination of potency/clearance/polarity, and it was profiled further in ADME, safety assays, and in vivo pharmacokinetic experiments (Tables 2 and 3). Significant improvements compared to our lead compound **2** were achieved (Table 2). Compound **7m** had relatively low microsomal clearance in dog and human liver microsomes (16.8 and <8.6 μL/min/mg, respectively) and elevated clearance in rat liver microsomes (77.4 μL/min/mg). It did not inhibit any of the cytochrome P450 enzymes at 3 μM concentration, and the major clearance mechanism was CYP3A4/3A5 mediated. Compound **7m** had very little off-target activity in the Cerep panel and only hit 1 out of 82 targets at 10 μM concentration. The receptor that was inhibited by **7m** was 5-HT_{2B} with an IC_{50} of 7.6 μM. Compound **7m** had an MDR-MDCKI BA/AB ratio of higher than 17 indicating potential for impairment through the blood−brain barrier. Passive permeability was relatively low as judged from the permeability value of 2.2 × 10^{-6} cm/s (passive permeability (P_{app}) measured using low-efflux MDCKII cells).[34] Pharmacokinetic data of **7m** was collected in three preclinical species (rat, dog, and monkey; Table 3). Good oral bioavailability was observed in dogs and monkeys (67% and 32%, respectively). It is worth noting that despite high clearance observed in rats, a reasonable oral bioavailability was achieved (15%). This is likely due to "flip-flop" kinetics where the rate of absorption is much slower than disposition.[35] Compound **7m** had no liabilities in the transformed human liver epithelial (THLE > 248 μM) cell assay nor in a genotoxicity assay (no effect in the AMES bacterial reverse mutation assay up to 600 μM). It had minimal liability in the hERG (human ether-a-go-go gene) channel with an IC_{50} of 47 μM. With the optimal pharmacological, ADME, and pharmacokinetic profiles, compound **7m** was scaled up for toxicology and safety studies in rat.

Table 3. In Vivo Pharmacokinetics of 7m[a]

	dose (mg/kg)[g]	Cl (mL/min/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	oral F (%)
rat[b,c]	IV, 1; PO, 5	115 ± 29	8.1 ± 1.2	1.1 ± 0.1	15 ± 7
dog[b,d]	IV, 1; PO, 4	21.9	7.3	5.5	67
monkey[e,f]	IV, 1; PO, 5	7.6	2.2	6.4	32

[a]CL, V_{ss}, and $t_{1/2}$ calculations were derived from IV data for all species, and standard error is given for $n \geq 3$ measurements. [b]5% DMSO/95% 50 mM lactic acid in saline IV formulation and 0.5% methylcellulose PO formulation. [c]Wistar−Han rats (n = 3 animals per dose group). [d]Beagle dogs (n = 2 animals per dose group). [e]Cynomolgus monkey (n = 2 animals per dose group). [f]12% sulfobutyl ether beta-cyclodextrin in 50 mM citrate buffer IV formulation and 0.5% methylcellulose PO formulation. [g]IV, intravenous; PO, oral administration.

dx.doi.org/10.1021/ml500414n | ACS Med. Chem. Lett. 2015, 6, 156−161

During the scale up process (beyond 30 g), which involved an acid−base workup, the team discovered that a substantial amount of starting material **6** was regenerated during the workup. Compound **7m** was found to be unstable in both plasma (human and rat) and under aqueous conditions at physiological pH (7.4) and above. The analogous compounds **7j**, **7n**, and **7r** (Table 1) were tested in a pH stability assay at three different pHs (1.5, 6.5, and 7.4). Pyridyl amide **7n** and *N*-methylcarboxamide **7r** were unstable at basic pH. Conversely, compound **7j** was stable under both acidic and basic media. Presumably deprotonation and intramolecular cleavage of the piperidine amide bond by the *ortho*-amide of **7m**, **7n**, and **7r** generates the starting material piperidine **6** (observed by LCMS and ^{1}H NMR).

It was reasoned that, if the position of the primary *ortho*-amide moiety was moved further away from the piperidine amide, the compounds should be chemically more stable, disrupting the 1,6 relationship between the carbonyl of the piperidine amide and the Lewis basic groups of the pendant carbamide resulting in subsequent piperidine amide cleavage. A set of *ortho*-amides with longer linkers (compounds **7p** and **7o**) were prepared as described above in Scheme 2. These compounds were stable at pH 7.4 but did not meet our potency criteria in the ghrelin binding assay (>100 nM). Interestingly, the nitrile precursor to **7o**, compound **7k**, was one of the most potent compounds in this series with a K_i of 4 nM in the binding assay and 5 nM in the functional assay. However, the clearance and MDR ratio did not meet our criteria for advancement. In addition, the reverse *ortho*-amide, **7s**, was prepared from readily available 4-methoxy-2-amino-phenylacetic acid and **6** for stability testing purposes. Compound **7s** had reasonable ghrelin binding potency (26 nM) and was stable at pH 7.4, but unfortunately suffered from formation of a reactive metabolite, presumably due to acetamide hydrolysis revealing an aniline that gets bioactivated in liver microsomes.[36] It is also worth noting that all compounds **7a**−**7g** were stable at pH 7.4.

While trying to gain an understanding of the severity of the pH-dependent chemical instability of **7m**, compound **1** progressed to clinical studies and development of compound **7m** was discontinued. In summary, we have described parallel efforts to find a peripherally acting ghrelin inverse agonist via physical chemical property optimization of a centrally acting lead compound **2**. Clear improvements with regards to polarity, potency, clearance, and safety were achieved. Synthesis of polar and heterocyclic piperidine acetamide derivatives delivered several highly potent ghrelin inverse agonists with optimal ADME properties. Compound **7m** was profiled further in various in vitro and in vivo experiments. Chemical instability of the piperidine acetamides bearing an *ortho*-amide functional group was observed. Further optimization of back-up compound **7m** was put on hold pending feedback on the clinical studies with PF-5190457 (**1**).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: (858)-622-8831. E-mail: suvi.orr@pfizer.com.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

HLM, human liver microsome; RLM, rat liver microsome; DLM, dog liver microsome; SFLogD, shake-flask log D; GHS-R1a, growth hormone secretacogue receptor 1a; TPSA, topological polar surface area; MDR, Madin−Darby canine kidney (MDCK) cell line expressing the human MDR-1 gene; EDCI, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; CDI, 1,1′-carbonyldiimidazole; CDMT, 2-chloro-4,6-dimethoxy-1,3,5-triazine; TD, thermodynamic; THLE, transformed human liver epithelial cell assay; hERG, human ether-a-go-go-related gene

■ REFERENCES

(1) Tong, J.; Prigeon, R. L.; Davis, H. W.; Bidlingmaier, M.; Kahn, S. E.; Cummings, D. E.; Tschöp, M. H.; D'Alessio, D. Ghrelin Suppresses Glucose-Stimulated Insulin Secretion and Deteriorates Glucose Tolerance in Healthy Humans. *Diabetes* **2010**, *59*, 2145−2151.

(2) Bray, G. A. Obesity: The Disease. *J. Med. Chem.* **2006**, *49*, 4001−4007.

(3) Kojima, M.; Hosoda, H.; Date, Y.; Nakazato, M.; Matsuo, H.; Kangawa, K. Ghrelin Is a Growth-Hormone-Releasing Acylated Peptide from Stomach. *Nature* **1999**, *402*, 656−660.

(4) Sato, T.; Nakamura, Y.; Shiimura, Y.; Ohgusu, H.; Kangawa, K.; Kojima, M. Structure, regulation and function of ghrelin. *J. Biochem.* **2012**, *151*, 119−128.

(5) Carpino, P. A.; Lefker, B. A.; Toler, S. M.; Pan, L. C.; Hadcock, J. R.; Cook, E. R.; DiBrino, J. N.; Campeta, A. M.; DeNinno, S. L.; Chidsey-Frink, K. L.; Hada, W. A.; Inthavongsay, J.; Mangano, F. M.; Mullins, M. A.; Nickerson, D. F.; Ng, O.; Pirie, C. M.; Ragan, J. A.; Rose, C. R.; Tess, D. A.; Wright, A. S.; Yu, L.; Zawistoski, M. P.; DaSilva-Jardine, P. A.; Wilson, T. C.; Thompson, D. D. Pyrazolinone-piperidine dipeptide growth hormone secretagogues (GHSs): Discovery of capromorelin. *Bioorg. Med. Chem.* **2003**, *11*, 581−590.

(6) Hoveyda, H. R.; Marsault, E.; Gagnon, R.; Mathieu, A. P.; Vézina, M.; Landry, A.; Wang, Z.; Benakli, K.; Beaubien, S.; Saint-Louis, C.; Brassard, M.; Pinault, J.-F.; Ouellet, L.; Bhat, S.; Ramaseshan, M.; Peng, X.; Foucher, L.; Beauchemin, S.; Bhérer, P.; Veber, D. F.; Peterson, M. L.; Fraser, G. L. Optimization of the Potency and Pharmacokinetic Properties of a Macrocyclic Ghrelin Receptor Agonist (Part I): Development of Ulimorelin (TZP-101) from Hit to Clinic. *J. Med. Chem.* **2011**, *54*, 8305−8320.

(7) Liu, B.; Liu, G.; Xin, Z.; Serby, M. D.; Zhao, H.; Schaefer, V. G.; Falls, H. D.; Kaszubska, W.; Collins, C. A.; Sham, H. L. Novel isoxazole carboxamides as growth hormone secretagogue receptor (GHS-R) antagonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5223−5226.

(8) Rudolph, J.; Esler, W. P.; O'Connor, S.; Coish, P. D. G.; Wickens, P. L.; Brands, M.; Bierer, D. E.; Bloomquist, B. T.; Bondar, G.; Chen, L.; Chuang, C.-Y.; Claus, T. H.; Fathi, Z.; Fu, W.; Khire, U. R.; Kristie, J. A.; Liu, X.-G.; Lowe, D. B.; McClure, A. C.; Michels, M.; Ortiz, A. A.; Ramsden, P. D.; Schoenleber, R. W.; Shelekhin, T. E.; Vakalopoulos, A.; Tang, W.; Wang, L.; Yi, L.; Gardell, S. J.; Livingston, J. N.; Sweet, L. J.; Bullock, W. H. Quinazolinone Derivatives as Orally Available Ghrelin Receptor Antagonists for the Treatment of Diabetes and Obesity. *J. Med. Chem.* **2007**, *50*, 5202−5216.

(9) Serby, M. D.; Zhao, H.; Szczepankiewicz, B. G.; Kosogof, C.; Xin, Z.; Liu, B.; Liu, M.; Nelson, L. T. J.; Kaszubska, W.; Falls, H. D.; Schaefer, V.; Bush, E. N.; Shapiro, R.; Droz, B. A.; Knourek-Segel, V. E.; Fey, T. A.; Brune, M. E.; Beno, D. W. A.; Turner, T. M.; Collins, C. A.; Jacobson, P. B.; Sham, H. L.; Liu, G. 2,4-Diaminopyrimidine Derivatives as Potent Growth Hormone Secretagogue Receptor Antagonists. *J. Med. Chem.* **2006**, *49*, 2568−2578.

(10) Xin, Z.; Serby, M. D.; Zhao, H.; Kosogof, C.; Szczepankiewicz, B. G.; Liu, M.; Hutchins, C. W.; Sarris, K. A.; Hoff, E. D.; Falls, H. D.;

Lin, C. W.; Ogiela, C. A.; Collins, C. A.; Brune, M. E.; Bush, E. N.; Droz, B. A.; Fey, T. A.; Knourek-Segel, V. E.; Shapiro, R.; Jacobson, P. B.; Beno, D. W. A.; Turner, T. M.; Sham, H. L.; Liu, G. Discovery and Pharmacological Evaluation of Growth Hormone Secretagogue Receptor Antagonists. *J. Med. Chem.* **2006**, *49*, 4459−4469.

(11) Zhao, H.; Xin, Z.; Liu, G.; Schaefer, V. G.; Falls, H. D.; Kaszubska, W.; Collins, C. A.; Sham, H. L. Discovery of Tetralin Carboxamide Growth Hormone Secretagogue Receptor Antagonists via Scaffold Manipulation. *J. Med. Chem.* **2004**, *47*, 6655−6657.

(12) Demange, L.; Boeglin, D.; Moulin, A.; Mousseaux, D.; Ryan, J.; Bergé, G.; Gagne, D.; Heitz, A.; Perrissoud, D.; Locatelli, V.; Torsello, A.; Galleyrand, J.-C.; Fehrentz, J.-A.; Martinez, J. Synthesis and Pharmacological in Vitro and in Vivo Evaluations of Novel Triazole Derivatives as Ligands of the Ghrelin Receptor. 1. *J. Med. Chem.* **2007**, *50*, 1939−1957.

(13) Moulin, A.; Demange, L.; Bergé, G.; Gagne, D.; Ryan, J.; Mousseaux, D.; Heitz, A.; Perrissoud, D.; Locatelli, V.; Torsello, A.; Galleyrand, J.-C.; Fehrentz, J.-A.; Martinez, J. Toward Potent Ghrelin Receptor Ligands Based on Trisubstituted 1,2,4-Triazole Structure. 2. Synthesis and Pharmacological in Vitro and in Vivo Evaluations. *J. Med. Chem.* **2007**, *50*, 5790−5806.

(14) Moulin, A.; Demange, L.; Ryan, J.; Mousseaux, D.; Sanchez, P.; Bergé, G.; Gagne, D.; Perrissoud, D.; Locatelli, V.; Torsello, A.; Galleyrand, J.-C.; Fehrentz, J.-A.; Martinez, J. New Trisubstituted 1,2,4-Triazole Derivatives as Potent Ghrelin Receptor Antagonists. 3. Synthesis and Pharmacological in Vitro and in Vivo Evaluations. *J. Med. Chem.* **2008**, *51*, 689−693.

(15) Pasternak, A.; Goble, S. D.; deJesus, R. K.; Hreniuk, D. L.; Chung, C. C.; Tota, M. R.; Mazur, P.; Feighner, S. D.; Howard, A. D.; Mills, S. G.; Yang, L. Discovery and Optimization of Novel 4-[(Aminocarbonyl)amino]-*N*-[4-(2-aminoethyl)phenyl]-benzenesulfonamide Ghrelin Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6237−6240.

(16) Mihalic, J. T.; Kim, Y.-J.; Lizarzaburu, M.; Chen, X.; Deignan, J.; Wanska, M.; Yu, M.; Fu, J.; Chen, X.; Zhang, A.; Connors, R.; Liang, L.; Lindstrom, M.; Ma, J.; Tang, L.; Dai, K.; Li, L. Discovery of a New Class of Ghrelin Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2046−2051.

(17) Yu, M.; Lizarzaburu, M.; Beckmann, H.; Connors, R.; Dai, K.; Haller, K.; Li, C.; Liang, L.; Lindstrom, M.; Ma, J.; Motani, A.; Wanska, M.; Zhang, A.; Li, L.; Medina, J. C. Identification of Piperazine-Bisamide GHSR Antagonists for the Treatment of Obesity. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1758−1762.

(18) Hanrahan, P.; Bell, J.; Bottomley, G.; Bradley, S.; Clarke, P.; Curtis, E.; Davis, S.; Dawson, G.; Horswill, J.; Keily, J.; Moore, G.; Rasamison, C.; Bloxham, J. Substituted Azaquinazolinones as Modulators of GHSr-1a for the Treatment of Type II Diabetes and Obesity. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2271−2278.

(19) Kung, D. W.; Coffey, S. B.; Jones, R. M.; Cabral, S.; Jiao, W.; Fichtner, M.; Carpino, P. A.; Rose, C. R.; Hank, R. F.; Lopaze, M. G.; Swartz, R.; Chen, H.; Hendsch, Z.; Posner, B.; Wielis, C. F.; Manning, B.; Dubins, J.; Stock, I. A.; Varma, S.; Campbell, M.; DeBartola, D.; Kosa-Maines, R.; Steyn, S. J.; McClure, K. F. Identification of Spirocyclic Piperidine-Azetidine Inverse Agonists of the Ghrelin Receptor. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4281−4287.

(20) McClure, K. F.; Jackson, M.; Cameron, K. O.; Kung, D. W.; Perry, D. A.; Orr, S. T. M.; Zhang, Y.; Kohrt, J.; Tu, M.; Gao, H.; Fernando, D.; Jones, R.; Erasga, N.; Wang, G.; Polivkova, J.; Jiao, W.; Swartz, R.; Ueno, H.; Bhattacharya, S. K.; Stock, I. A.; Varma, S.; Bagdasarian, V.; Perez, S.; Kelly-Sullivan, D.; Wang, R.; Kong, J.; Cornelius, P.; Michael, L.; Lee, E.; Janssen, A.; Steyn, S. J.; Lapham, K.; Goosen, T. Identification of Potent, Selective, CNS-Targeted Inverse Agonists of the Ghrelin Receptor. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5410−5414.

(21) Bhattacharya, S. K.; Andrews, K.; Beveridge, R.; Cameron, K. O.; Chen, C.; Dunn, M.; Fernando, D.; Gao, H.; Hepworth, D.; Jackson, V. M.; Khot, V.; Kong, J.; Kosa, R. E.; Lapham, K.; Loria, P. M.; Londregan, A. T.; McClure, K. F.; Orr, S. T. M.; Patel, J.; Rose, C.; Saenz, J.; Stock, I. A.; Storer, G.; VanVolkenburg, M.; Vrieze, D.; Wang, G.; Xiao, J.; Zhang, Y. Discovery of PF-5190457, a Potent, Selective, and Orally Bioavailable Ghrelin Receptor Inverse Agonist Clinical Candidate. *ACS Med. Chem. Lett.* **2014**, *5*, 474−479.

(22) Els, S.; Beck-Sickinger, A. G.; Chollet, C. Ghrelin Receptor: High Constitutive Activity and Methods for Developing Inverse Agonists. *Methods Enzymol* **2010**, *485*, 103−121.

(23) Edwards, M. P.; Price, D. A. Role of Physicochemical Properties and Ligand Lipophilicity Efficiency in Addressing Drug Safety Risks. In *Annual Reports in Medicinal Chemistry*; John, E. M., Ed.; Academic Press: New York, 2010; Vol. *45*, Chapter 23, pp 380−391.

(24) Leeson, P. D.; Springthorpe, B. The Influence of Drug-Like Concepts on Decision-Making in Medicinal Chemistry. *Nat. Rev. Drug Discovery* **2007**, *6*, 881−890.

(25) Feng, B.; Mills, J. B.; Davidson, R. E.; Mireles, R. J.; Janiszewski, J. S.; Troutman, M. D.; de Morais, S. M. In Vitro P-glycoprotein Assays to Predict the in Vivo Interactions of P-glycoprotein with Drugs in the Central Nervous System. *Drug Metab. Dispos.* **2008**, *36*, 268−275.

(26) Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. Defining Desirable Central Nervous System Drug Space through the Alignment of Molecular Properties, in Vitro ADME, and Safety Attributes. *ACS Chem. Neurosci.* **2010**, *1*, 420−434.

(27) Hitchcock, S. A. Structural Modifications that Alter the P-Glycoprotein Efflux Properties of Compounds. *J. Med. Chem.* **2012**, *55*, 4877−4895.

(28) Hartz, R. A.; Ahuja, V. T.; Zhuo, X.; Mattson, R. J.; Denhart, D. J.; Deskus, J. A.; Vrudhula, V. M.; Pan, S.; Ditta, J. L.; Shu, Y.-Z.; Grace, J. E.; Lentz, K. A.; Lelas, S.; Li, Y.-W.; Molski, T. F.; Krishnananthan, S.; Wong, H.; Qian-Cutrone, J.; Schartman, R.; Denton, R.; Lodge, N. J.; Zaczek, R.; Macor, J. E.; Bronson, J. J. A Strategy to Minimize Reactive Metabolite Formation: Discovery of (*S*)-4-(1-Cyclopropyl-2-methoxyethyl)-6-[6-(difluoromethoxy)-2,5-dimethylpyridin-3-ylamino]-5-oxo-4,5-dihydropyrazine-2-carbonitrile as a Potent, Orally Bioavailable Corticotropin-Releasing Factor-1 Receptor Antagonist. *J. Med. Chem.* **2009**, *52*, 7653−7668.

(29) Orr, S. T. M.; Cabral, S.; Fernando, D. P.; Makowski, T. One-Pot Synthesis of Chiral Azetidines from Chloroaldehyde and Chiral Amines. *Tetrahedron Lett.* **2011**, *52*, 3618−3620.

(30) Fernando, D. P.; Jiao, W.; Polivkova, J.; Xiao, J.; Coffey, S. B.; Rose, C.; Londregan, A.; Saenz, J.; Beveridge, R.; Zhang, Y.; Storer, G. E.; Vrieze, D.; Erasga, N.; Jones, R.; Khot, V.; Cameron, K. O.; McClure, K. F.; Bhattacharya, S. K.; Orr, S. T. M. Spiroazetidine−Piperidine Bromoindane as a Key Modular Template to Access a Variety of Compounds via C−C and C−N Bond-Forming Reactions. *Tetrahedron Lett.* **2012**, *53*, 6351−6354.

(31) Cobley, C. J.; van den Heuvel, M.; Abbadi, A.; de Vries, J. G. Platinum Catalysed Hydrolytic Amidation of Unactivated Nitriles. *Tetrahedron Lett.* **2000**, *41*, 2467−2470.

(32) Balicki, R.; Kaczmarek, Ł. Mild and Efficient Conversion of Nitriles to Amides with Basic Urea-Hydrogen Peroxide Adduct. *Synth. Commun.* **1993**, *23*, 3149−3155.

(33) Garrett, C. E.; Jiang, X.; Prasad, K.; Repič, O. New Observations on Peptide Bond Formation Using CDMT. *Tetrahedron Lett.* **2002**, *43*, 4161−4165.

(34) Di, L.; Whitney-Pickett, C.; Umland, J. P.; Zhang, H.; Zhang, X.; Gebhard, D. F.; Lai, Y.; Federico, J. J.; Davidson, R. E.; Smith, R.; Reyner, E. L.; Lee, C.; Feng, B.; Rotter, C.; Varma, M. V.; Kempshall, S.; Fenner, K.; El-kattan, A. F.; Liston, T. E.; Troutman, M. D. Development of a New Permeability Assay Using Low-Efflux MDCKII Cells. *J. Pharm. Sci.* **2011**, *100*, 4974−4985.

(35) Boxenbaum, H. Pharmacokinetic Tricks and Traps: Flip-Flop Models. *J. Pharm. Pharm. Sci.* **1998**, *1*, 90−91.

(36) Orr, S. T. M.; Ripp, S. L.; Ballard, T. E.; Henderson, J. L.; Scott, D. O.; Obach, R. S.; Sun, H.; Kalgutkar, A. S. Mechanism-Based Inactivation (MBI) of Cytochrome P450 Enzymes: Structure−Activity Relationships and Discovery Strategies To Mitigate Drug−Drug Interaction Risks. *J. Med. Chem.* **2012**, *55*, 4896−4933.