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Brief Article

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Discovery of a Novel Series of Orally Bioavailable and CNS Penetrant Glucagon-like Peptide-1 Receptor (GLP-1R) Noncompetitive Antagonists Based on a 1,3-Disubstituted-7-aryl-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione Core

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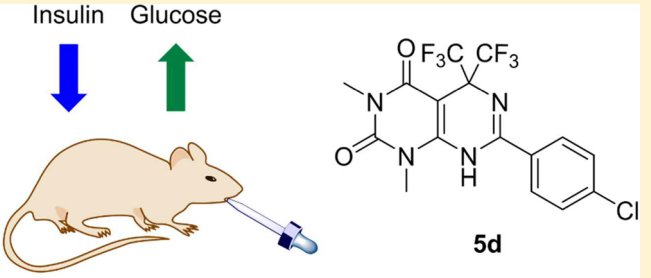
Supporting Information

ABSTRACT: A duplexed, functional multiaddition high throughput screen and subsequent optimization effort identified the first orally bioavailable and CNS penetrant glucagon-like peptide-1 receptor (GLP-1R) noncompetitive antagonist. Antagonist **5d** not only blocked exendin-4-stimulated insulin release in islets but also lowered insulin levels while increasing blood glucose in vivo.



INTRODUCTION

The glucagon-like peptide-1 receptor (GLP-1R) is a family B G-protein-coupled receptor (GPCR) and a critical incretin hormone-responsive receptor with diverse physiological functions in the periphery.[1,2] GLP-1R is a major focus of therapeutic discovery for type II diabetes, where it plays a critical role in the potentiation of insulin secretion and suppression of glucagon secretion.[3,4] However, GLP-1R is also located within the central nervous system (CNS) in multiple brain regions important for appetite, movement, and cognition.[5−7] As a family B GPCR, GLP-1R is activated by four endogenous GLP-1 peptides (GLP-1 (1−37), GLP-1 (7−37), GLP-1 (1−36) amide, and GLP-1 (7−36) amide) and a fifth structurally related peptide, oxyntomodulin.[8,9] To date, the majority of agonists and antagonists ligands to study GLP-1R function are peptides, and several synthetic peptidic drugs, such as exenatide and liragulitide administered by subcutaneous injection, have been developed.[10−12] A peptide-based antagonist, exendin (9−39) or EX-9, has also been employed in proof-of-concept studies wherein it has demonstrated effects on feeding in rodents; however, EX-9 cannot be administered orally.[13,14] Recently, clinical studies have demonstrated efficacy for GLP-1R antagonism by EX-9 in patients suffering from hyperinsulinemia,[15] as well as infants with congenital hyperinsulinism.[16] Disruption of GLP-1R signaling in the CNS, via knockout of the GLP-1R in Sim1 neurons, supports a role for GLP-1R inhibition in acute and chronic stress as well as anxiety.[16] While synthetic peptide ligands, such as EX-9, address the short half-life of endogenous GLP-1, adverse events, iv administration, and limited CNS penetration limit their utility for target validation studies and recapitulation of genetic studies.[18,19] To address these issues, efforts have been directed toward the development of orally bioavailable, non-peptide ligands, particularly small molecule positive allosteric modulators (PAMs) and noncompetitive antagonists/negative allosteric modulators (NAMs), for use as in vivo proof of concept tool cpompunds.[20−22]

We recently reported on a duplexed (GLP-1R and glucagon receptor (GCGR)) triple-add, functional high-throughput screen that identified both small molecule PAM and antagonist leads, leading to the development of the in vivo active GLP-1R PAM **1** (Figure 1).[23,24] As our screen also identified antagonist hits, we were surprised to find that very few small molecule GLP-1R antagonists, such as **2** (IC_{50} = 21 μM) and **3**, have been described in the primary literature (another, PNU-

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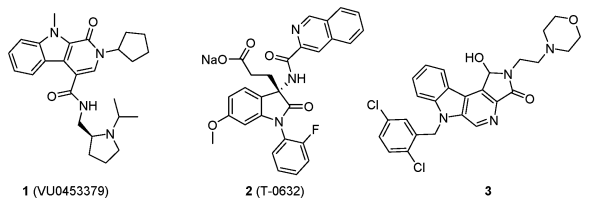


Figure 1. Structures of our GLP-1R PAM **1** and reported small molecule antagonists **2** and **3**. Very little data (pharmacology or DMPK) are known regarding the reported GLP-1R antagonists.

126814, has only been disclosed in an abstract without structure) and without detailed drug metabolism and pharmacokinetics (DMPK) or in vivo efficacy data.[17] Thus, we decided to pursue the discovery of small molecule GLP-1R noncompetitive antagonists and report here on the discovery of a novel series that is highly selective, orally bioavailable, and CNS penetrant with robust efficacy in vivo for use as in vivo tool compounds.

RESULTS AND DISCUSSION

Chemistry. The HTS campaign afforded one small molecule chemotype **4** (Figure 2), based on an unusual 1,3-

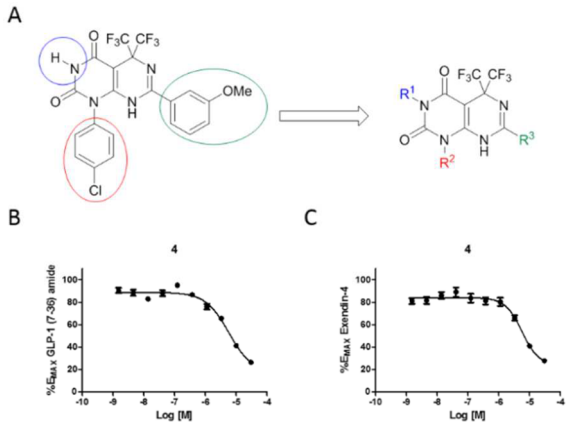
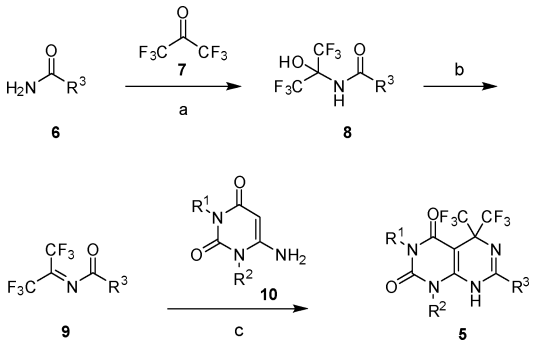


Figure 2. (A) Structure of GLP-1R antagonist HTS hit **4** and the four areas to evaluate in the optimization campaign (**5**). (B) Concentration−response curve (CRC) for inhibition of GLP-1 activation of GLP-1R by **4** (IC_{50} = 5.9 μM, pIC_{50} = 5.23 ± 0.09, 16.8 ± 6.3 GLP-1 min). (C) CRC for inhibition of exendin-4 (EX-4) activation of GLP-1 by **4** (IC_{50} = 5.8 μM, pIC_{50} = 5.24 ± 0.08, 23.9 ± 5.5 GLP-1 min). GloSensor cAMP assay in TREx293 HEK cells was conducted; n = 3.

disubstituted-7-aryl-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione core that proved to be a weak partial GLP-1R antagonist for the native agonist GLP-1 (IC_{50} = 5.9 μM, pIC_{50} = 5.23 ± 0.09, 16.8 ± 6.3 GLP-1_{min}) and the synthetic agonist exendin-4, EX-4 (IC_{50} = 5.8 μM, pIC_{50} = 5.24 ± 0.08, 23.9 ± 5.5 GLP-1 min), employing native cAMP as a readout. Moreover, **4** was inactive at the closely related glucagon receptor (IC_{50} > 30 μM). On the basis of nonpeptidic structure, we assumed the hit was a noncompetitive antagonist, but we would require more potent analogs to confirm the mode of inhibition. Moreover, early DMPK profiling in rat showed that **4** was a moderately cleared compound in vivo (CL_p = 36.4 mL min^{-1} kg^{-1}) with a 2.4 h half-life and possessed exceptional oral bioavailability (F = 100%). Figure 2 also highlights key areas of **4** to be evaluated in the chemical optimization program. Analogs **5**, wherein the *gem*-di-CF_3 is maintained, could be accessed in three synthetic steps (Scheme 1). The R^3 substituent is introduced in the first

Scheme 1. Synthesis of GLP-1R Antagonists 5[a]

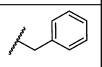
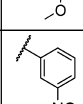


[a]Reagents and conditions: (a) DCM, 95−98% (crude); (b) $(CF_3O)_2O$, pyridine, ether, 94−97% (crude); (c) triethylamine, DMF, 24−36%.

step in the form of primary carboxamide **6**. Condensation with 1,1,1,3,3,3-hexafluoropropan-2-one **7** affords intermediate **8** which then eliminates to provide the substituted *N*-(perfluoropropan-2-yllidine)amide **9**. Both **8** and **9** were unstable and were carried on crude into the condensation step. Thus, condensation of crude **9** with an appropriately substituted 6-amino-1,3-substituted-pyrimidine-2,4(1*H*,3*H*)-dione **10** delivers analogs **5**. Overall yields were modest (~24%) for the three-step sequence, and all requisite intermediates were commercially available.[25]

Structures and GLP-1R antagonist activities of representative analogs **5** are highlighted in Table 1. Limited examples are highlighted, as SAR was steep, and the majority of analogs were inactive (IC_{50} > 10 μM). When R^1 = H (e.g, **4**, **5a**, and **5b**), weak partial antagonists resulted, but **5a**, wherein R^2 is benzyl and R^3 is 2-thienyl, delivered a GLP-1R antagonist more potent (GLP-1 (7−36) amide, IC_{50} = 1.3 μM, pIC_{50} = 5.89 ± 0.04,

Table 1. Structures and Activities of Analogs 5

Entry	R^1	R^2	R^3	GLP-1R IC_{50} (μM)[a,b]	GLP-1R pIC_{50}	GLP-1 min (%)
4	H			5.9[a] 5.8[b]	5.23±0.09[a] 5.24±0.08[b]	16.8±6.3[a] 23.9±5.5[b]
5a	H			1.3[a] 1.5[b]	5.89±0.04[a] 5.82±0.06[b]	36.2±1.9[a] 32.0±2.4[b]
5b	H			7.2[a] 7.2[b]	5.14±0.08[a] 5.14±0.08[b]	22.7±6.2[a] 26.4±6.2[b]
5c	Me	Me		0.61[a] 0.69[b]	6.22±0.03[a] 6.16±0.04[b]	16.4±1.5[a] 15.5±1.7[b]
5d	Me	Me		0.65[a] 0.69[b]	6.19±0.03[a] 6.16±0.05[b]	20.9±1.4[a] 19.7±2.1[b]

[a]GLP-1R IC_{50}, pIC_{50}, and GLP-1 min (%) in TREx293 HEK cell line with GloSensor cAMP assay upon activation with an EC_{80} of GLP-1 (7−36) amide; n = 3. [b]GLP-1R IC_{50}, pIC_{50}, and GLP-1 min (%) in TREx293 HEK cell line with GloSensor cAMP assay upon activation with an EC_{80} of EX-4; n = 3. Data reported as average ± SEM.

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J. Med. Chem. XXXX, XXX, XXX−XXX

36.2 ± 1.9 GLP-1 min; exendin-4, IC_{50} = 1.5 μM, pIC_{50} = 5.82 ± 0.06, 32.0 ± 2.4 GLP-1 min) than the lead **4**. Replacing H with Me at R^1 led to a significant increase in GLP-1 antagonist potency, as highlighted by submicromolar analogs **5c** (GLP-1 (7−36) amide, IC_{50} = 0.61 μM, pIC_{50} = 6.22 ± 0.03, 16.4 ± 1.5 GLP-1 min; exendin-4, IC_{50} = 0.69 μM, pIC_{50} = 6.16 ± 0.04, 15.5 ± 1.7 GLP-1 min) and **5d** (GLP-1 (7−36) amide, IC_{50} = 0.65 μM, pIC_{50} = 6.19 ± 0.03, 20.9 ± 1.4 GLP-1 min; exendin-4, IC_{50} = 0.69 μM, pIC_{50} = 6.16 ± 0.05, 19.7 ± 2.1 GLP-1 min). However, neither analog completely inhibited the GLP-1 response, suggesting that they are partial antagonists, or antagonists with limited negative cooperativity, in this cAMP assay. Alkyl moieties larger than methyl at R^1 lost 5- to 20-fold potency. Thus, we advanced **5c** and **5d** in an effort to rapidly identify an in vivo tool compound for proof-of-concept studies.[25]

Molecular Pharmacology. As mentioned previously, SAR was driven in human TREx293 HEK cells by measuring cAMP levels using a GloSensor cAMP reporter.[25] Responses were assessed in response to inhibition of an ~EC_{80} concentration of either GLP-1(7−36) amide or exendin-4. Importantly, this new series of GLP-1R.antagonists did not display probe dependence (Figure 3), affording comparable IC_{50} values with the native

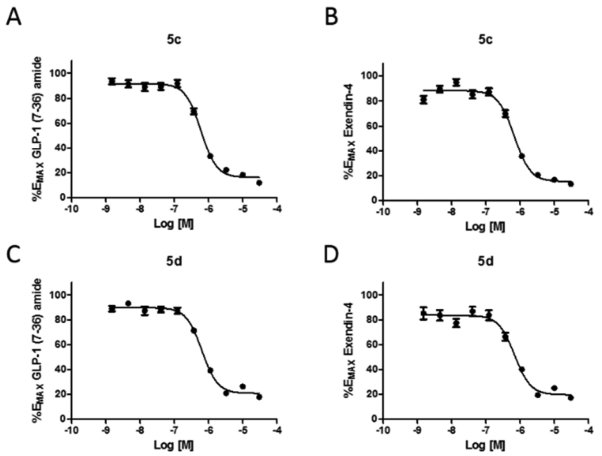

Figure 3. Molecular pharmacology of **5c** and **5d**. (A) Concentration−response curve (CRC) for inhibition of GLP-1 (7−36 amide) activation of GLP-1 by **5c** (IC_{50} = 0.60 μM, pIC_{50} = 6.22 ± 0.03, 16.4 ± 1.5 GLP-1 min). (B) CRC for inhibition of EX-4 activation of GLP-1 by **5c** (IC_{50} = 0.69 μM, pIC_{50} = 6.16 ± 0.04, 15.5 ± 1.7 GLP-1 min). (C) CRC for inhibition of GLP-1 (7−36 amide) activation of GLP-1 by **5c** (IC_{50} = 0.6 μM, pIC_{50} = 6.19 ± 0.03, 20.9 ± 1.4 GLP-1 min). (D) CRC for inhibition of EX-4 activation of GLP-1 by **5c** (IC_{50} = 0.69 μM, pIC_{50} = 6.16 ± 0.05, 19.7 ± 2.1 GLP-1 min). GloSensor cAMP assay in TREx293 HEK cells was conducted; n = 3.

agonist or the synthetic agonist EX-4. As **5c** and **5d** displayed only partial antagonism and due to the steep SAR and low molecular weight (MWs of 440−450) for a class B GPCR ligand, we assumed that these were noncompetitive antagonists. Thus, we performed progressive fold-shift experiments to determine if the antagonists engender a parallel-rightward shift of the agonist CRC (competitive inhibition) or a decrease in GLP-1 max (noncompetitive). Here (Figure 4), a GLP-1 (7−36) amide CRC was subjected to increasing concentrations of **5c** or **5d**, and a dose-dependent decrease in the GLP-1 max response was noted, consistent with a noncompetitive/NAM mechanism of action. Furthermore, both were inactive against the closely related glucagon receptor (IC_{50} > 30 μM). EX-4 and

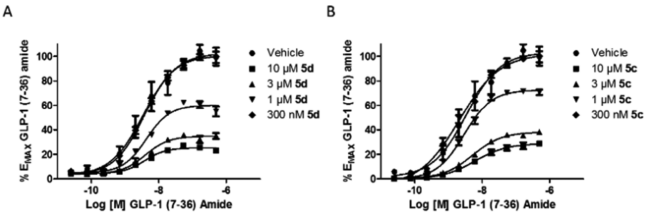

Figure 4. Progressive fold-shift studies with **5c** and **5d**. (A) Increasing concentrations of **5c** in the presence of a fixed CRC of GLP-1 (7−36 amide) inducing a dose-dependent decrease in the GLP-1 max, consistent with noncompetitive inhibition. (B) Increasing concentrations of **5d** in the presence of a fixed CRC of GLP-1 (7−36 amide) inducing a dose-dependent decrease in the GLP-1 max, consistent with noncompetitive inhibition, n = 3.

other GLP-1R agonists, as well as our previously reported GLP-1R PAM (**1**), have been shown to potentiate glucose-induced insulin secretion in primary mouse pancreatic islets.[22,26,27] On the basis of the ability of **5c** and **5d** to inhibit EX-4 activation of GLP-1R, we next examined if the antagonist could block EX-4 stimulated insulin secretion in this model (Figure 5). As

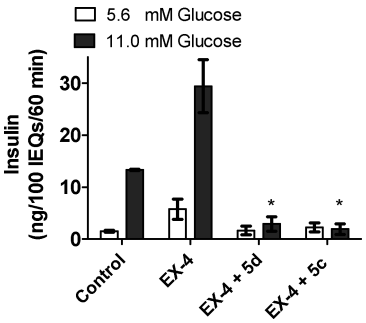

Figure 5. Glucose-stimulated insulin secretion in primary mouse islets in the presence of 10 nM EX-4 under low and high glucose conditions. Both antagonists, **5c** and **5d** (at 1 μM), blocked the potentiation of insulin secretion by EX-4: (*) significantly different from 11.0 mM EX-4 by two-way ANOVA followed by Sidak's multiple comparison test.

expected, EX-4 potentiates insulin secretion upon low and high glucose stimulation, and this effect is completely blocked by **5c** and **5d**. Therefore, the inhibitory activity measured in our cell-based assay translated into the standard ex vivo model.

Drug Metabolism and Disposition. With robust activity in cells and in primary tissue, we next evaluated **5c** and **5d** in a panel of DMPK assays to assess their utility as in vivo probes (Table 2).[25] In general, **5c** and **5d** displayed attractive in vitro DMPK properties (reasonable cLogPs, measurable free drug levels and predicted hepatic clearance). Of note, **5d** was stable in rat microsomes and possessed low predicted hepatic clearance in human microsomes (2.03 mL min^{-1} kg^{-1}). For first generation tool compounds, the CYP450 profiles were acceptable. For example, **5d** showed no inhibition of 3A4 or 2D6 (IC_{50} > 30 μM), weak activity against 1A2 (IC_{50} = 4 μM), but potent inhibition of 2C9 (IC_{50} = 410 nM). In general, this chemotype displayed inhibition of CYP 2C9. In a 3 mg/kg oral plasma/brain distribution study, **5d** was highly CNS penetrant (K_p = 1.45) and clearly orally bioavailable from the high total plasma (45 ng/mL) and brain (65.1 ng/g) concentrations. In an in vivo rat PK study, **5d** showed a good in vitro/in vivo correlation (IVIVC) with a CL_p of 4.79 mL min^{-1} kg^{-1}, a long

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J. Med. Chem. XXXX, XXX, XXX−XXX

Table 2. DMPK Profiles of 4, 5c, and 5d

parameter	4	5c	5d
MW	518.80	451.29	440.73
TPSA	83.03	116.82	65.01
cLogP	4.93	3.63	3.63
P450 inhibition (μM)			
P450 (1A2, 2C9, 3A4, 2D6)	>30, 3.78, 16.44, 8.13	25.04, 0.47, >30, 21.20	4.01, 0.41, >30, >30
in vitro PK			
rat CL_{HEP} (mL min^{-1} kg^{-1})	27.8	35.9	0[a]
human CL_{HEP} (mL min^{-1} kg^{-1})	8.9	0[a]	2.03
rat plasma f_u	0.002	0.005	0.011
human plasma f_u	0.003	0.005	0.007
in vivo rat distribution (po, 3 mg/kg, 1 h)			
plasma (ng/mL)	1145	76.7	45.0
brain (ng/mL)	37.1	2.4	65.1
brain/plasma (K_p)	0.03	0.02	1.45
in vivo rat PK (iv, 1 mg/kg; po, 3 mg/kg)			
CL_p (mL min^{-1} kg^{-1})	36.4	3.64	4.79
$t_{1/2}$ (min)	145	42	587
V_{ss} (L/kg)	0.69	0.22	3.57
F (%)	100	ND	50

[a]Stable in hepatic microsomes.

half-life ($t_{1/2}$ = 9.8 h), and a modest volume of distribution (V_{ss} = 3.57 L/kg). Importantly, in a discrete rat oral (po) study, **5d** was found to be highly orally bioavailable (F = 50%). A Eurofins lead profiling panel was performed against a radioligand binding panel of 68 GPCRs, ion channels, and transporters, and **5d** displayed no significant off-target activity (no inhibition of >50% at 10 μM).[25,28] Thus, **5d** emerged as the ideal in vivo tool compound to probe the pharmacodynamics efficacy of GLP-1R inhibition.[25]

In Vivo Pharmacology. With **5d** in hand, an orally bioavailable GLP-1R antagonist with excellent rat PK and efficacy in primary tissues, we elected to determine if inhibition of GLP-1R by **5d** in vivo would impact glucose and insulin levels.[29] For this study, male Sprague-Dawley rats were administered 10 mg/kg po of **5d** and after 30 min were challenged with glucose or vehicle po in an oral glucose tolerance test (OGTT). As shown in Figure 6, **5d**-treated animals displayed a significant increase in blood glucose levels, with a concomitant decrease in blood insulin levels, thereby validating the inhibitors in vivo.[25]

CONCLUSION

In summary, we have developed the first reported CNS-penetrant and orally bioavailable small molecule GLP-1R antagonist **5d**, VU0650991. Molecular pharmacology studies showed that **5d** did not possess probe dependence, as it blocked the activation of GLP-1R by endogenous agonist, GLP-1 (7−36) amide, as well as the synthetic agonist, EX-4. This new small molecule GLP-1R antagonist blocked the activity of EX-4 in potentiating insulin secretion in primary mouse pancreatic islets and reduced blood insulin levels while increasing blood glucose levels in rats upon oral dosing. There are a number of exciting therapeutic indications (hyperinsulinemia, acute/chronic stress and anxiety) for a centrally active GLP-1R antagonist, and these studies are in progress and will be reported in due course.

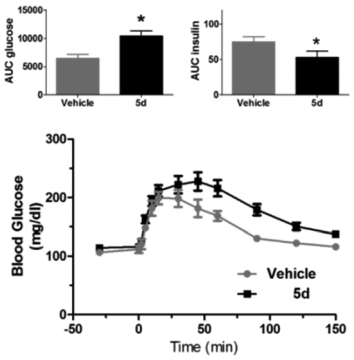

Figure 6. Effect of **5d** on whole blood glucose and insulin levels in rats upon oral dosing at 10 mg/kg. Rats with carotid cannulae surgically implanted were randomized to receive **5d** or vehicle 30 min prior to glucose dose (2 g/kg) given by gavage at time 0. Carotid blood samples were obtained at given intervals to assess whole blood glucose and insulin. Rat were crossed over to other treatment 7 days later and restudied. Area under the curve (AUC) was calculated for glucose and insulin excursion, and the mean values were compared by Student's t test: (*) $p < 0.05$. Glucose excursions were assessed by two-way ANOVA, $p < 0.0001$ for drug interaction, (*) Bonferroni post-test $p < 0.01$ at these time points.

EXPERIMENTAL SECTION

Chemistry. All compounds were purified to ≥95% as determined by analytical LC/MS (214 nm, 254 nm, and ELSD) and ^{1}H NMR. The general chemistry, experimental information, and syntheses of all other compounds are supplied in the Supporting Information.

4-Chloro-*N*-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-benzamide (8d). To an oven-dried three-necked round-bottom flask were added at room temperature 4-chlorobenzamide (311 mg, 2.0 mmol) and CH_2Cl_2 (5 mL). To one neck of the flask was added a coldfinger with dry ice/isopropanol. The second neck was sealed with a septum, and the last neck was attached to a lecture bottle of hexafluoroacetone. The reaction was heated to 30 °C with stirring, at which point hexafluoroacetone was bubbled into the mixture until a vigorous reflux of the gas was observed. The resultant mixture was stirred for 3 h until reaction completion was confirmed by LC/MS. Upon completion, the reaction was purged with air to remove excess hexafluoroacetone and concentrated in vacuo without heating to afford the desired product as a white solid (635 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.84 (s, 1H), 7.60 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.14 (s, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 171.99, 140.45, 130.24, 129.47, 128.63, 121.11 (q, $^1J_{C-F}$ = 288.9 Hz), 84.41 (sep, $^2J_{C-F}$ = 33.0 Hz). LRMS (ES+) found for $C_{10}H_6ClF_6NO_2$ (M + H), 322.1.

4-Chloro-*N*-(perfluoropropan-2-ylidene)benzamide (9d). To a round-bottom flask was added at room temperature **8d** (635 mg, 1.97 mmol), which was dissolved in ether (10 mL). This was cooled with stirring to 0 °C before adding trifluoroacetic anhydride (282 μL, 2.0 mmol) and pyridine (338 μL, 4.2 mmol) dropwise simultaneously. The resultant mixture was slowly warmed to room temperature with stirring over 2 h, and reaction completion was verified by LC/MS. Upon completion, the mixture was diluted with ether (20 mL), filtered to remove organic salts, and concentrated in vacuo without heating to afford the desired product as a colorless oil (580 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (d, J = 8.7 Hz, 2H), 7.44 (d, J = 8.7 Hz, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 171.65, 162.83 (d, $^2J_{C-F}$ = 36.8 Hz), 140.27, 130.33, 129.43, 128.86, 121.11 (q, $^1J_{C-F}$ = 289.5 Hz). LRMS (ES+) found for $C_{10}H_4ClF_6NO$ (M + 1 + MeOH), 336.0.

7-(4-Chlorophenyl)-1,3-dimethyl-5,5-bis(trifluoromethyl)-5,8-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (5d). To a vial were added at room temperature **9d** (580 mg, 1.91 mmol), 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (235 mg, 1.91 mmol), and triethylamine (39 μL, 0.29 mmol), which was then

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J. Med. Chem. XXXX, XXX, XXX−XXX

dissolved in *N*,*N*-dimethylformamide (2 mL). This mixture was heated to 100 °C with stirring for 3 h. Upon completion by LC/MS, the mixture was diluted with 1:1 water/CH_2Cl_2 (20 mL) and extracted three times with CH_2Cl_2 (5 mL). The combined organic layers were washed twice with a 5% LiCl solution (10 mL), passed through a phase separator to dry, and concentrated in vacuo. The brown residue was purified by automated flash chromatography using 40−70% ethyl acetate in hexanes to elute. Desired product was afforded as a pale yellow solid (202 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83 (d, J = 8.7 Hz, 2H), 7.54 (d, J = 8.7 Hz, 2H), 6.70 (s, 1H), 3.65 (s, 3H), 3.37 (s, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 159.60, 157.76, 153.32, 151.52, 140.55, 129.87, 129.80, 128.76, 122.51 (q, $^1J_{C-F}$ = 290.2 Hz), 80.96, 65.67−65.03 (m), 30.49, 28.41. HRMS (TOF, ES+) calcd for $C_{16}H_{11}ClF_6N_4O_2$ (M + 1), 440.0477; found, 440.0475.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01706.

Experimental procedures and spectroscopic data for selected compounds and detailed pharmacology and DMPK methods (PDF)

Molecular formula strings and some data (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

GLP-1, glucagon-like peptide-1; CRC, concentration−response curve; PAM, positive allosteric modulator; PBL, plasma/brain level

■ REFERENCES

(1) Koole, C.; Pabreja, K.; Savage, E. E.; Wootten, D.; Furness, S. G.; Miller, L. J.; Christopoulos, A.; Sexton, P. M. Recent advances in understanding GLP-1R (glucagon-like peptide-1 receptor) function. *Biochem. Soc. Trans.* **2013**, *41*, 172−179.

(2) Dillon, J. S.; Tanizawa, Y.; Wheeler, M. B.; Leng, X. H.; Ligon, B. B.; Rabin, D. U.; Yoo-Warren, H.; Permutt, M. A.; Boyd, A., III. Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. *Endocrinology* **1993**, *133*, 1907−1910.

(3) Calanna, S.; Christensen, M.; Holst, J. J.; Laferrere, B.; Gluud, L. L.; Vilsboll, T.; Knop, F. K. Secretion of glucagon-like peptide-1 with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. *Diabetologia* **2013**, *56*, 965−972.

(4) Meier, J. J. GLP-1 receptor agonists for individual treatment of type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **2012**, *8*, 728−742.

(5) Knauf, C.; Cani, P. D.; Kim, D.-H.; Iglesias, M. A.; Chabo, C.; Waget, A.; Colom, A.; Rastrelli, S.; Delzenne, N. M.; Drucker, D. J.; Seeley, R. J.; Burcelin, R. Role of central nervous system glucagon-like peptide-1 receptors in enteric glucose sensing. *Diabetes* **2008**, *57*, 2603−2612.

(6) Perry, T.; Haughey, N. J.; Mattson, M. P.; Egan, J. M.; Greig, N. H. Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 881−888.

(7) During, M. J.; Cao, L.; Zuzga, D. S.; Francis, J. S.; Fitzsimons, H. L.; Jiao, X.; Bland, R. J.; Klugmann, M.; Banks, W. A.; Drucker, D. J.; Haile, C. N. Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat. Med. (N. Y., NY, U. S.)* **2003**, *9*, 1173−1179.

(8) Estall, J. L.; Drucker, D. J. Glucagon and glucagon-like peptide receptors as drug targets. *Curr. Pharm. Des.* **2006**, *12*, 1731−1750.

(9) Baggio, L. L.; Drucker, D. J. Biology of incretins: GLP-1 and GIP. *Gastroenterology* **2007**, *132*, 2131−2157.

(10) Edwards, C. M.; Stanley, S. A.; Davis, R.; Bynes, A. E.; Frost, G. S.; Seal, L. J.; Ghatei, M. A.; Bloom, S. R. Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *Am. J. Physiol.: Endocrinol. Metab.* **2001**, *281*, E151−E161.

(11) Knudsen, L. B.; Nielsen, P. F.; Huusfeldt, P. O.; Johansen, N. L.; Madsen, K.; Pedersen, F. Z.; Thogersen, H.; Wilken, M.; Agerso, H. Potent derivative of glucagon-like peptide-1 with pharmacokinetic properties suitable for once daily dosing administration. *J. Med. Chem.* **2000**, *43*, 1664−1669.

(12) Derosa, G.; Maffioli, P. GLP-1 agonists exenatide and liraglutide: a review about their safety and efficacy. *Curr. Clin. Pharmacol.* **2012**, *7*, 214−228.

(13) Turton, M. D.; O'Shea, D.; Gunn, I.; Beak, S. A.; Edwards, C. M. B.; Meeran, K.; Choi, S. J.; Taylor, G. M.; Heath, M. M.; Lambert, P. D.; Wilding, J. P. H.; Smith, D. M.; Ghatei, M. A.; Herbert, J.; Bloom, S. R. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* **1996**, *379*, 69−72.

(14) Meeran, K.; O'Shea, D.; Edwards, C. M. B.; Turton, M. D.; Heath, M. M.; Gunn, I.; Abusnana, S.; Rossi, M.; Small, C. J.; Goldstone, A. P.; Taylor, G. M.; Sunter, D.; Steere, J.; Choi, S. J.; Ghatei, M. A.; Bloom, S. R. Repeated intracerebroventricular administration of glucagon-like peptide-1-(7−36) amide or exendin-(9−39) alters body weight in the Rat. *Endocrinology* **1999**, *140*, 244−250.

(15) Salehi, M.; Gastaldelli, A.; D'Alessio, D. A. Blockade of glucagon-like peptide 1 receptors corrects postpranidial hypoglycemia after gastric bypass. *Gastroenterology* **2014**, *146*, 669−680.

(16) Calabria, A. C.; Li, C.; Gallagher, P. R.; Stanley, C. A.; De Leon, D. D. GLP-1 receptor antagonist exendin-(9−39) elevates fasting blood glucose levels in congenital hyperinsulinism owing to inactivating mutations in the ATP-sensitive K+ channel. *Diabetes* **2012**, *61*, 2585−2591.

(17) Ghosal, S.; Packard, A. E. B.; Mahbod, P.; McKlveen, J. M.; Seeley, R. J.; Myers, B.; Ulrich-Lai, Y.; Smith, E. P.; D'Alessio, D. A.; Herman, J. P. Disruption of glucagon-like peptide 1 signaling in Sim1 neurons reduces physiological behavior reactivity to acute and chronic stress. *J. Neurosci.* **2017**, *37*, 184−193.

(18) Gupta, V. Glucagon-like peptide-1 analogues: an overview. *Indian J. Endocrinol. Metab.* **2013**, *17*, 413−421.

(19) Gallwitz, B.; Ropeter, T.; Morys-Wortmann, C.; Mentlein, R.; Siegel, E. G.; Schmidt, W. E. GLP-1-Analogues resistant to degradation by dipeptidyl-peptidase IV in vitro. *Regul. Pept.* **2000**, *86*, 103−111.

(20) Willard, F. S.; Bueno, A. B.; Sloop, K. W. Small molecule discovery at the glucagon-like peptide-1 receptor. *Exp. Diabetes Res.* **2012**, *2012*, 1−9.

(21) Koole, C.; Savage, E. E.; Christopoulos, A.; Miller, L. J.; Sexton, P. M.; Wootten, D. Minireview: Signal bias, allosterism, and

DOI: 10.1021/acs.jmedchem.6b01706
J. Med. Chem. XXXX, XXX, XXX−XXX

polymorphic variation at the GLP-1R: Implications for drug discovery. *Mol. Endocrinol.* **2013**, *27*, 1234−1244.
(22) de Graaf, C.; Rein, C.; Piwnica, D.; Giordanetto, F.; Rognan, D. Structure-based discovery of allosteric modulators of two related class B G-protein-coupled receptors. *ChemMedChem* **2011**, *6*, 2159−2169.
(23) Morris, L. C.; Days, E. L.; Turney, M.; Mi, D.; Lindsley, C. W.; Weaver, C. D.; Niswender, K. D. A duplexed high-throughput screen to identify allosteric modulators of the glucagon-like peptide 1 and glucagon receptor. *J. Biomol. Screening* **2014**, *19*, 847−858.
(24) Morris, L. C.; Nance, K. D.; Gentry, P. R.; Days, E. L.; Weaver, C. D.; Niswender, C. M.; Thompson, A. D.; Jones, C. K.; Locuson, C. W.; Morrison, R. D.; Daniels, J. S.; Niswender, K. D.; Lindsley, C. W. Discovery of (*S*)-2-Cyclopentyl-*N*-((1-isopropylpyrrolidin2-yl)-9-Methyl-1-Oxo-2,9-Dihydro-1 *H*-Pyrrido[3,4-*b*]Indole-4-Carboxamide (VU0453379): A novel, CNS penetrant glucagon-like peptide 1 receptor (GLP-1R) positive allosteric modulator (PAM). *J. Med. Chem.* **2014**, *57*, 10192−10197.
(25) For full experimental details (chemistry and pharmacology), see the Supporting Information.
(26) Schmidt, W. E.; Siegel, E. G.; Creutzfeldt, W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. *Diabetologia* **1985**, *28*, 704−707.
(27) Göke, R.; Fehmann, H. C.; Linn, T.; Schmidt, H.; Krause, M.; Eng, J.; Göke, B. Exendin-4 is a high potency agonist and truncated exendin-(9−39)-amide an antagonist at the glucagon-like peptide 1-(7−36)-amide receptor of insulin-secreting beta-cells. *J. Biol. Chem.* **1993**, *268*, 19650−19655.
(28) For full details, see www.eurofins.com.
(29) Vahl, T. P.; Paty, B. W.; Fuller, B. D.; Prigeon, R. L.; D'Alessio, D. A. Effects of GLP-1-(7−36)NH2, GLP-1-(7−37), and GLP-1- (9−36)NH2 on intravenous glucose tolerance and glucose-induced insulin secretion in healthy humans. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 1772−1779.

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J. Med. Chem. XXXX, XXX, XXX−XXX