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Discovery of novel, potent, selective, and orally active human glucagon receptor antagonists containing a pyrazole core

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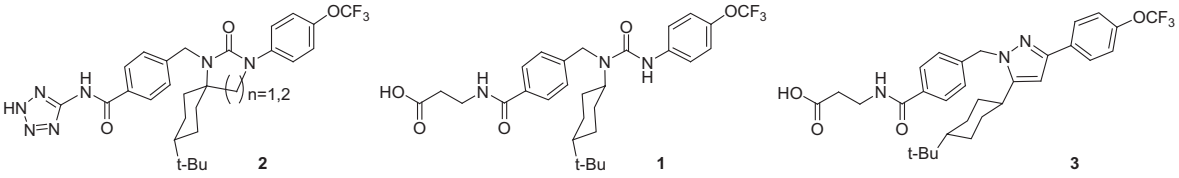
ABSTRACT

A novel class of 1,3,5-pyrazoles has been discovered as potent human glucagon receptor antagonists. Notably, compound **26** is orally bioavailable in several preclinical species and shows selectivity towards cardiac ion channels, other family B receptors such hGIP and hGLP1, and a large panel of enzymes and additional receptors. When dosed orally, compound **26** is efficacious in suppressing glucagon induced plasma glucose excursion in rhesus monkey and transgenic murine pharmacodynamic models at 1 and 10 mpk, respectively.

The ever-increasing disease burden from type 2 diabetes mellitus (T2DM) has prompted continued search for new therapies.[1,2] Glucagon is a 29-amino acid peptide hormone secreted from pancreatic α-cells. It acts through the glucagon receptor (GCGR) in the liver to stimulate gluconeogenesis and glycogenolysis thereby counteracting the role of insulin in maintaining glucose homeostasis.[3] Many studies have validated glucagon antagonists as a potential new therapy for treating T2DM. For example, peptide glucagon antagonists, antibodies, and antisense oligonucleotides against the glucagon receptor all significantly decreased blood glucose levels in animal models of diabetes.[4] A small molecule hGCGR antagonist blocked glucagon-induced hyperglycemia in human subjects.[5] Thus, glucagon receptor antagonism has become a very active area of research for potential T2DM therapy, especially using small molecule glucagon receptor antagonists.[6]

In an earlier Letter,[7] we reported that cyclic ureas such as **2** represent a novel pharmacophore for hGCGR antagonists as conformationally constrained analogs of trisubstituted ureas such as **1**.[8] In a parallel investigation, we also discovered that the urea core of **1** can also be replaced by a pyrazole group as in **3**. Here we report our initial results on the synthesis of glucagon receptor antagonists in this novel series and their biological activity.[9]

During the same modeling experiment that identified **2** as a novel replacement for **1**,[7] we were also pleased to find that a pyrazole core, which had been the subject of our investigations in previous programs,[10] is also a reasonable replacement for the urea as shown in Figure 1. AM1 calculations indicated excellent overlap between the truncated versions of compounds **3** and **1** such that the three atoms bearing substituents in **3** (marked with arrows in Fig. 1) were within 0.6 Å of the corresponding atoms in **1**. The carbonyl oxygen atom in **1** was also about 0.7 Å apart from the pyrazole N2 nitrogen in **3**.



These computational results encouraged us to prepare and test these pyrazoles as potential novel glucagon receptor antagonists. For synthetic considerations, we began by preparing pyrazoles

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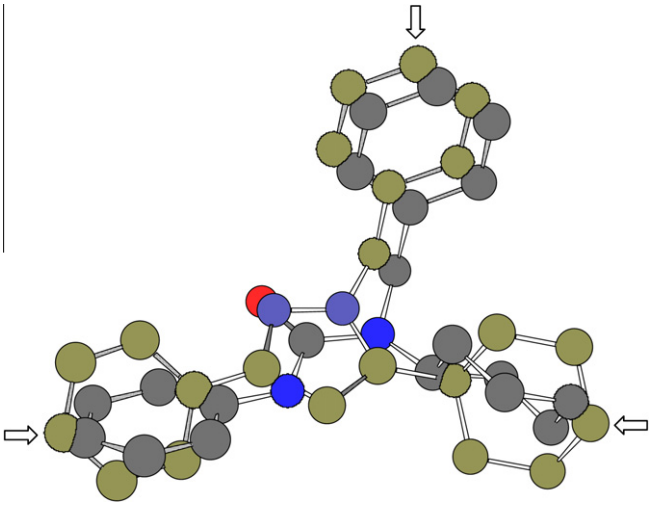
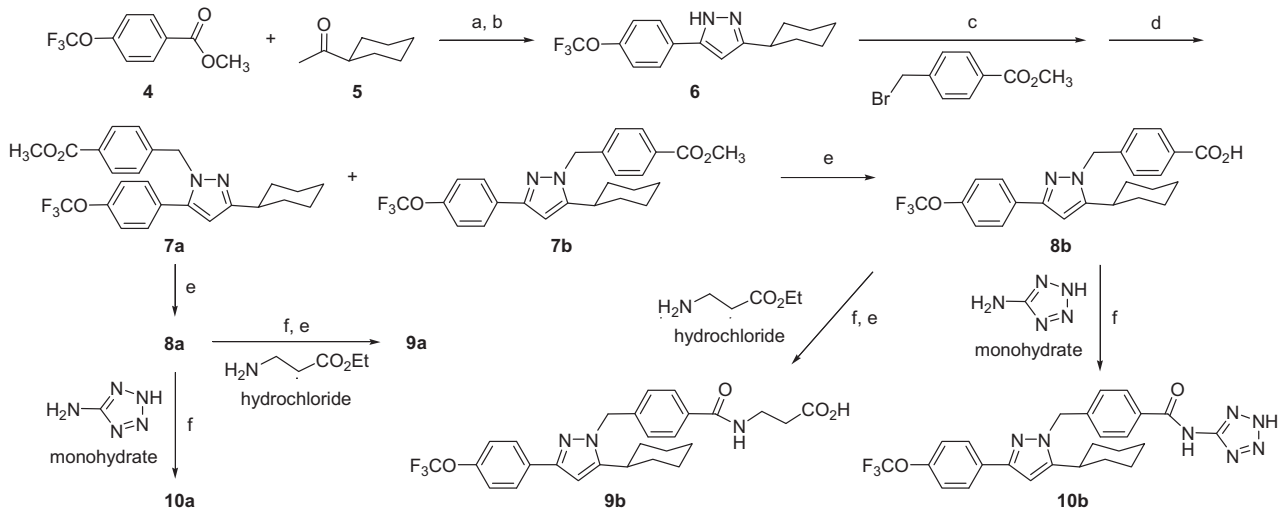


Figure 1. Overlay of truncated compound **1** (gray) with its 1,3,5-trisubstituted pyrazole replacement **3** (gold) from AM1 calculations (red = O, blue = N, others = C). Hydrogen atoms were omitted for clarity.

without the *t*-butyl group on the cyclohexane ring (Scheme 1). Claisen condensation of ester **4** and ketone **5** followed by cyclization with hydrazine afforded N–H pyrazole **6**. Alkylation of **6** with methyl 4-(bromomethyl)benzoate and separation of the resulting regioisomeric mixture on silica gel[11] provided **7a** and **7b** in about 1:5 ratio. The identities of the two isomers were established by NOE difference spectroscopy. Using chemistry described before[7] the first set of compounds **9** and **10** were prepared for biological testing.[12]

The in vitro biological activity of these compounds on the hGCGR was evaluated in binding and functional assays and compared with **1** and its 5-aminotetrazole amide analog **1a** (Table 1). The binding IC_{50} measurements were based on the inhibition of ^{125}I-glucagon to the hGCGR expressed in CHO cell membranes. For compounds showing good binding affinity, functional inhibition of glucagon induced cAMP accumulation in hGCGR transfected CHO cells was also measured (cAMP IC_{50}).[13] Functional activity against hGIP was measured as a proxy for selectivity against related family B GPCR's.

In our hands the known ureas **1** and **1a** were very potent binders to the hGCGR (3–21 nM), but exhibited somewhat diminished functional antagonism (Table 1). Considering the first group of four pyrazole analogs prepared lacked the *t*-butyl group on the cyclohexane, we were quite encouraged by the activity of these pyrazoles. The most potent of which was tetrazole **10b**, which was only about threefold off in its functional activity at the glucagon receptor compared to its urea analog **1a** and had much improved selectivity. Furthermore, **10b** had a promising pharmacokinetic



Scheme 1. Reagents and conditions: (a) NaH, 0 °C to rt, 67%; (b) hydrazine, MeOH, 99%; (c) NaH, DMF, rt; (d) silica gel, 1.5–4% MeCN in DCM, 14% **7a** and 68% **7b**; (e) NaOH, EtOH/H_2O; (f) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole hydrate (HOBt), diisopropylethylamine (DIEA), DMF.

Table 1
Binding and functional activity (IC_{50}) of human glucagon receptor antagonists at human glucagon receptor (hGCGR) and functional activity at hGIP[a]

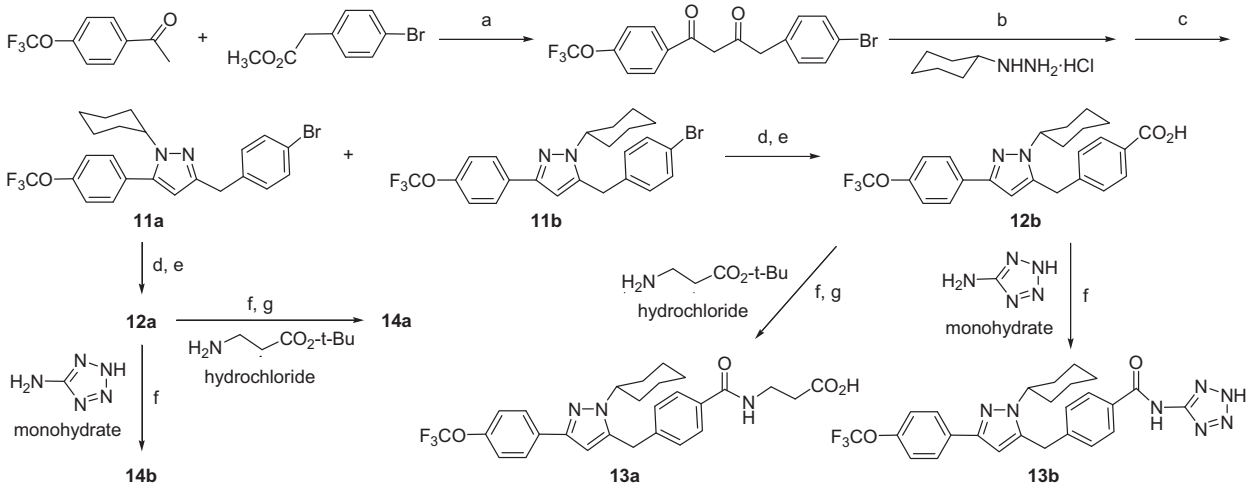
Compound	Type	hGCGR Binding IC_{50} (nM) (*n*)	hGCGR cAMP IC_{50} (nM) (*n*)[b]	hGIP cAMP IC_{50} (nM) (*n*)[b]
1	Acid	21 ± 7 (5)	140 ± 90 (4)	1300 ± 500 (5)
1a	Tetrazole	3.2 ± 1.7 (6)	46 ± 14 (5)	45 ± 14 (4)
9a	Acid	2000	ND	4100 ± 3400 (2)
9b	Acid	3800	ND	2800 ± 1700 (2)
10a	Tetrazole	810	ND	910 ± 270 (2)
10b	Tetrazole	120 ± 20 (2)	160	2100 ± 100 (2)
13a	Acid	2900	ND	39 ± 4% @ 10 μM
13b	Tetrazole	4200	ND	42 ± 3% @ 10 μM
14a	Acid	1700	ND	ND
14b	Tetrazole	2900	ND	50 ± 2% @ 10 μM
19a	Acid	1200	ND	7400
19b	Tetrazole	5300	ND	>10,000
20a	Acid	7200	ND	>25,000
20b	Tetrazole	16,000	ND	>20,000

[a] All IC_{50}s in this Letter were reported as averages rounded to two significant figures ± standard deviations when more than one measurement were made.
[b] Not determined.

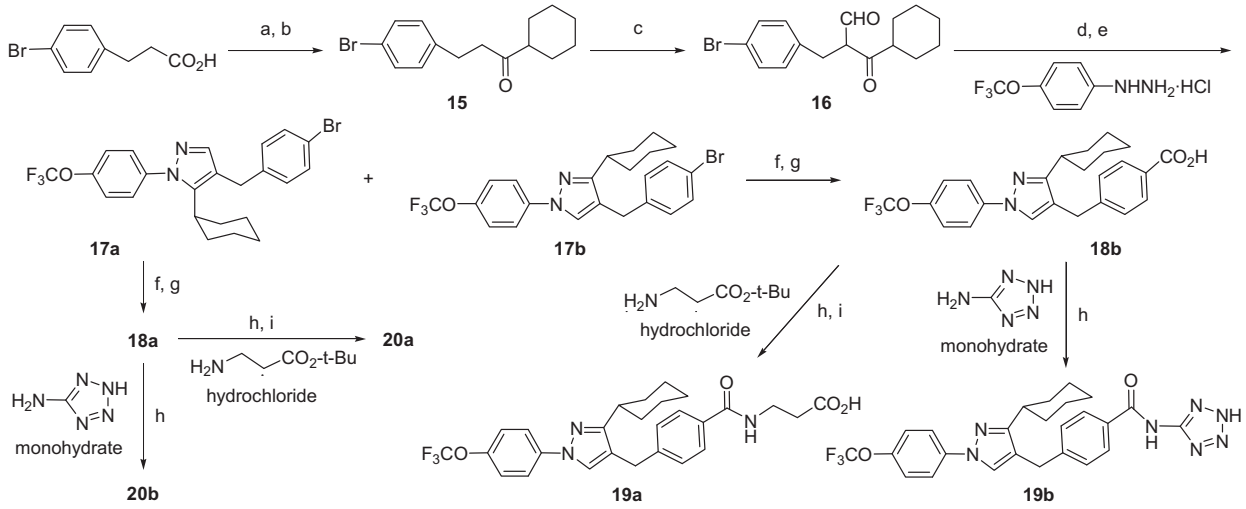
profile in the mice with blood clearance of 8.2 mL/min/kg, half-life of 2.7 h, and $AUCN_{po}$ of 1.5 μM h kg/mg. These results suggested the pyrazole core to be a promising novel scaffold for human glucagon receptor antagonists. The ethyl ester precursors to **9a**/**9b** and benzoic acid **8a**/**8b** exhibited no activity at the hGCGR with IC_{50} >100 μM and >10 μM in binding and functional assays, respectively. In addition, both racemic α and β methyl alanyl analogs were much less active than **9b** (data not shown). Removal of the cyclohexyl group in **9a,b** and **10a,b** and replacement with hydrogen also resulted in more than 10-fold loss in potency except in the case of **9b** where the differences were about 2–3 folds (data not shown).

Before embarking on further SAR of this 1,3,5-tri-substituted pyrazole series, we explored two of the other three possible isomeric pyrazole cores with the position of the nitrogens transposed in the pyrazole ring. The synthetic approaches to these compounds are shown in Schemes 2 and 3. Most of the transformations in Schemes 2 and 3 were straightforward. Except in Scheme 3, an attempt at introducing the carboxylic group before the formation of isomeric pyrazoles was not successful due to the low conversion of **15** during its cyanation, resulting in only a 15% yield of the cyano derivative. Therefore, the pyrazoles **17a,b** were prepared first followed by functional group manipulation to give the acid precursors **18a,b** as shown in Scheme 3.

The in vitro biological results for the two pyrazole isomers, **13** and **19**, showed that they were less active than the original isomer **10** (Table 1). Our modeling suggested that the placement of nitrogen could affect the rotation of the benzyl group relative to the pyrazole, which might have caused the difference in activity among pyrazole isomers. However, the level of theory used was not precise enough to draw conclusive conclusions on this matter. Nonetheless, these results prompted us to optimize the potency of the pyrazoles focusing on the pyrazole core in **10**. The initial target was to restore the *t*-butyl group on the cyclohexane to give **3** and its tetrazole analog **21**. The syntheses of **3** and **21** followed the procedure shown in Scheme 1, starting with 4-trifluoromethoxyacetophenone and methyl 4-(*t*-butyl)-cyclohexanecarboxylate. The latter was prepared from 4-(*t*-butyl)cyclohexanone using a

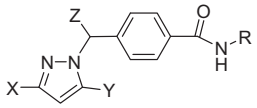


Scheme 2. Reagents and conditions: (a) NaH, 0 °C to rt, 97%; (b) MeOH, DIEA, 60 °C; (c) silica gel, 1% MeCN in DCM, 36% **a** and 45% **b**; (d) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, O/N, 96% from **11a**, 62% from **11b** (+27% recovered **11b**); (e) KOH, EtOH/H_2O, reflux, O/N, 100%; (f) EDC, HOBt, DIEA, DMF, 67% **13b**, 40% **14b**; (g) TFA, *i*-Pr_3SiH, DCM, rt, 3 h, 80% **13a** (80% two steps), 95% **14a** (66% two steps).



Scheme 3. Reagents and conditions: (a) CH_3NHOCH_3·HCl, EDC, HOBt, DIEA, DMF, 98%; (b) cyclohexylmagnesium chloride (2.5 equiv), 0 °C to rt, THF, 36% (the major product was the methyl amide derived from the Weinreb amide at 45%); (c) ethyl formate (20 equiv, in four portions over ~2 h), NaH, rt, 88%; (d) MeOH, 50 °C; (e) silica gel, 0–2% MeCN in DCM, 36% **17a** and 15% **17b**; (f) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C, O/N, 91% from **17a**, 95% from **17b**; (g) KOH, EtOH/H_2O, reflux, O/N, 100%; (h) EDC, HOBt, DIEA, DMF, 37% **19b**, 51% **20b**; (i) TFA, *i*-Pr_3SiH, DCM, rt, 3 h, **19a** (96% two steps), **20a** (66% two steps).

Table 2
Binding and functional activity (IC_{50}) of human glucagon receptor antagonists at human glucagon receptor (hGCGR) and functional activity at hGIP



Compound	X	Y	Z	R	hGCGR Binding IC_{50} (nM) (*n*)	hGCGR cAMP IC_{50} (nM) (*n*)[a]	hGIP cAMP IC_{50} (nM) (*n*)[a]
3	4-CF_3O-Ph	*t*-4-*t*-Bu-cyclohexyl	H	$-CH_2CH_2CO_2H$	480	ND	6% @ 10 μM
21	4-CF_3O-Ph	*t*-4-*t*-Bu-cyclohexyl	H	Tetrazol-5-yl	63 ± 18 (2)	170 ± 10 (2)	5700
22	4-CF_3O-Ph	4-CF_3O-Ph	H	$-CH_2CH_2CO_2H$	490	370	ND
23	4-CF_3O-Ph	4-CF_3O-Ph	H	Tetrazol-5-yl	43 ± 15 (4)	47 ± 20 (5)	46 ± 4% @ 10 μM (3)
24	4-CF_3O-Ph	3,5-Cl_2-Ph	H	$-CH_2CH_2CO_2H$	95 ± 29 (4)	230	21% @ 10 μM
25	4-CF_3O-Ph	3,5-Cl_2-Ph	H	Tetrazol-5-yl	36 ± 6 (2)	35 ± 15 (2)	3000
26	3,5-Cl_2-Ph	4-CF_3O-Ph	H	$-CH_2CH_2CO_2H$	84 ± 41 (4)	110 ± 40 (11)	7500 ± 2900 (3)
27	3,5-Cl_2-Ph	4-CF_3O-Ph	H	Tetrazol-5-yl	39 ± 17 (3)	44 ± 13 (11)	7100
28	3,5-Cl_2-Ph	4-CF_3O-Ph	H	$-(R)$-$CH_2CHOHCO_2H$	28 ± 15 (2)	330	>25,000
29	3,5-Cl_2-Ph	4-CF_3O-Ph	H	(Tetrazol-5-yl)methyl	120 ± 70 (2)	ND	>10,000
30	4-CF_3O-Ph	4-CF_3O-Ph	(±)-CH_3	$-CH_2CH_2CO_2H$	250 ± 100 (4)	650 ± 140 (3)	46% @ 10 μM
31	4-CF_3O-Ph	4-CF_3O-Ph	(±)-CH_3	Tetrazol-5-yl	130 ± 70 (2)	ND	6000
32	4-CF_3O-Ph	4-CF_3O-Ph	H	$-CH_2CO_2H$	3000	1300	−3% @ 10 μM
33	4-CF_3O-Ph	4-CF_3O-Ph	H	$-CH_2CH_2CH_2CO_2H$	1100	560	15% @ 10 μM
34	4-CF_3O-Ph	4-CF_3O-Ph	H	$-CH_3$, $-CH_2CH_2CO_2H$	7200 ± 4800 (2)	ND	9% @ 10 μM
35	4-CH_3SO_2-Ph	4-CF_3O-Ph	H	$-CH_2CH_2CO_2H$	2700	ND	43% @ 10 μM
36	4-CH_3SO_2-Ph	4-CF_3O-Ph	H	Tetrazol-5-yl	570	ND	45% @ 10 μM
37	4-CF_3O-Ph	4-CH_3SO_2-Ph	H	$-CH_2CH_2CO_2H$	1200	ND	31% @ 10 μM
38	4-CF_3O-Ph	4-CH_3SO_2-Ph	H	Tetrazol-5-yl	220 ± 80 (2)	ND	36% @ 10 μM
39	(±)-1-(4-Cl-Ph)ethyl	4-CF_3O-Ph	H	$-CH_2CH_2CO_2H$	2400	ND	0% @ 10 μM
40	(±)-1-(4-Cl-Ph)ethyl	4-CF_3O-Ph	H	Tetrazol-5-yl	430	ND	20% @ 10 μM
41	4-CF_3O-Ph	(±)-1-(4-Cl-Ph)ethyl	H	$-CH_2CH_2CO_2H$	730	ND	22% @ 10 μM
42	4-CF_3O-Ph	(±)-1-(4-Cl-Ph)ethyl	H	Tetrazol-5-yl	520	ND	21% @ 10 μM
43[b]	4-CF_3O-Ph	4-CF_3O-Ph	H	$-CH_2CH_2CO_2H$	330 ± 60 (2)	950	73% @ 10 μM
44[b]	4-CF_3O-Ph	4-CF_3O-Ph	H	Tetrazol-5-yl	1100	ND	2% @ 10 μM
45[b]	4-CF_3O-Ph	4-CF_3O-Ph	H	$-CH_2CO_2H$	2900	ND	34% @ 10 μM
46[b]	4-CF_3O-Ph	4-CF_3O-Ph	H	$-CH_2CH_2CH_2CO_2H$	1100	ND	49%@10 μM

[a] Not determined.
[b] These compounds contained a *meta*-benzoic acid unit instead of *para* for all the other compounds in this Table.

Wittig-type homologation with 2-lithio-2-trimethylsilyl-1,3-dithiane followed by mercuric chloride-mediated methanolysis.[14] The biological results are shown in Table 2. The other compounds in Table 2 were prepared using procedures similar to that depicted in Scheme 1.

The addition of the *t*-butyl group enhanced both binding and functional potency at the hGCGR and selectivity towards hGIP for the β-alanyl acid and the tetrazoles (compare **3** and **21** to **9b** and **10b**). The Claisen reaction during the preparation of **3** and **21** was carried out at a higher temperature[15] and this resulted in scrambling of 1,3-substituents in the product via a retro-Claisen/Claisen sequence which produced a symmetrical 1,3-diaryl-1,3-diketone as a side-product. Since many 1,3-di-aryl urea analogs of **1** were reported as glucagon receptor antagonists,[8] this 1,3-diaryl-1,3-ketone side-product was carried forward to afford **22** and **23**. Interestingly, **22** had similar potency at the glucagon receptor as **3** while **23** had slightly better activity and selectivity compared to **21**. Based on these results, considerations on ease of chemistry, and our belief that the diaryl pyrazoles should have better pharmacokinectic profiles than compounds containing a *t*-butylcyclohexyl group, we focused our subsequent SAR on the 3,5-diaryl pyrazoles. The first targets were compounds containing a 3,5-dichlorophenyl group based on our observations in the spiro-urea series.[7]

Replacing the 4-trifluomethoxyphenyl at the 5 position of the pyrazole in **22** and **23** with a 3,5-dichlorophenyl group afforded acid **24** and tetrazole **25**. Although the binding potency was improved in **24** compared to **22**, there was only modest improvement in functional activity. The activities of tetrazole **25** were similar to those of **23** at the glucagon receptor; however, there was some decrease in selectivity vs. hGIP. On the other hand, the same aryl replacement at the 3 position produced the acid **26** which was about six and threefold more potent as **22** in the binding and functional assays, respectively. The tetrazole **27** was similar to **25** in hGCGR potency with a slight improvement in selectivity. Compound **28**, a hydroxyl derivative[16] of **26**, showed very good binding activity; however, its functional activity was diminished compared to **26**. The tetrazolylmethyl analog **29** did not show advantage over **27** either.

Addition of a methyl group on the benzyl position of **22** and **23** did not improve their potency as hoped, especially in the functional assay (compounds **30** and **31**). The lower and higher homologs of **22**, compounds **32** and **33**, and the *N*-methyl secondary amide analog **34** all lost potency compared to **22**. As was observed in the spiro-urea series,[7] the introduction of a polar substituent such as 4-methylsulfonyl group in either of the 3 or 5-aryl group resulted in loss of potency (compare compounds **35**–**38** to **22** and **23**). Replacing one of the aryl groups with racemic 1-(4-chlorophenyl)ethyl[17] also gave similarly uninspiring results (compounds **39**–**42**). Finally, the *meta*-carboxamide isomers from

Table 3
Concentration of **26** in the blood of the hGCGR mouse during pharmacodynamic assay ($n = 3$ for each group)

Minutes post dose	10 mpk (μM)	30 mpk (μM)
45	3.55 ± 066	10.3 ± 0.7
120	3.35 ± 0.49	14.2 ± 1.8

Table 4
Pharmacokinetic profiles and glucagon receptor functional activity of human glucagon receptor antagonist **26** in additional animal species

Species	Cl_p (mL/min/kg)	V_{dss} (L/kg)	$t_{1/2}$ (h)	$AUCN_{po}$ (μM h kg/mg)	$C_{max\ po}$ (μM)	%F	GCGR cAMP IC_{50} (nM) (*n*)
Mouse [a]	6.1 ± 1.1 [b]	1.1 ± 0.3	2.3 ± 0.4	0.73 ± 0.09	0.23 ± 0.05	15 ± 2	2000
Rat [a]	1.6	0.75	6.5	11 ± 4	1.6 ± 0.3	57 ± 20	2600 ± 200 (3)
Dog [c]	0.49 ± 0.11	0.14 ± 0.03	3.6 ± 0.1	27 ± 9	7.7 ± 1.5	46 ± 16	400 ± 150 (2)
Rhesus Monkey [c]	0.68 ± 0.21	0.55 ± 0.05	10 ± 2	30.0 ± 0.5	4.2 ± 0.6	68 ± 1	140 ± 20 (2)

[a] Dosed at 1.0 mpk IV and 2.0 mpk PO formulated with a 5:10:85 mixture of DMSO, polysorbate 80, and water.
[b] Blood clearance.
[c] Dosed at 0.5 mpk IV in EtOH/PEG400/water (2:5:3) solution and 2.0 mpk PO as 0.5% methylcellulose/water suspension.

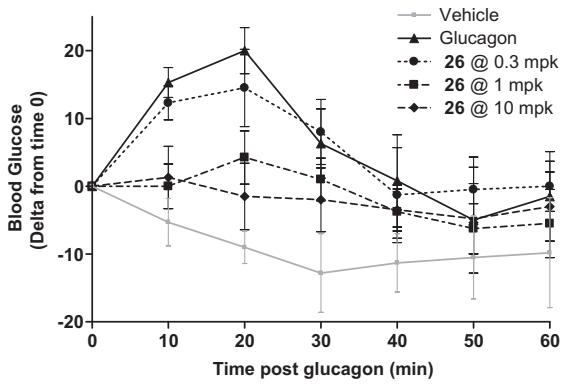


Figure 2. Titration of compound **26** in pharmacodynamic assay in the rhesus monkey.

the 1-benzyl group of the pyrazole in **22**, **23**, **32**, and **33** did not show improvements in binding or functional activity (compounds **43**–**46**).

Compounds **21**, **23**, **26**, **27**, and **29** were evaluated in vivo in a pharmacodynamic (PD) assay for their ability to block glucagon-induced glucose excursion at 30 and 10 mpk using transgenic mice expressing only a functional human glucagon receptor (hGCGR mice).[18] Oral administration of the antagonists (9 mice per group) was followed 60 min later by an IP injection of glucagon (15 μg/kg). Blood glucose levels were monitored 12, 24, 36, and 48 min later. Compounds **21, 23**, and **27** at 30 mpk and **26** at both doses fully ablated glucose excursion following the glucagon challenge. Compounds **21** and **23** at 10 mpk were partially active. Compound **27** at 10 mpk and **29** at both doses were not effective. The blood concentrations of compound **26** during the PD experiments are shown in Table 3. Compound **26** was also active in this assay when dosed orally at 30 mpk three hours prior to the glucagon challenge.

Based on its in vitro potency and PD activity, compound **26** was selected for further evaluation including off-target profile, evaluation in an in vivo receptor occupancy assay,[18b] measurement of pharmacokinetic properties and glucagon receptor functional activity in other species, and in vivo pharmacodynamic studies in other species. Among the related family B GPCR's, compound **26** showed good functional selectivity against hGIP, hGLP1, and PACAP (cAMP IC_{50} 7.5 ± 2.9, >10, and >7 μM, respectively). Compound **26** exhibited modest binding to the hERG K^+ channel (IC_{50} = 5.1 ± 0.1 μM) and did not bind to the human Na^+ and rabbit DLZ sensitive Ca^{2+} channels at concentrations up to 10 μM. In spite of its moderate hERG channel binding activity, when the sodium salt of **26** was infused iv at 1, 3, and 10 mpk (cumulative) over 30 min each in an anesthetized vagotomized cardiovascular dog model,[19] no changes in cardiovascular parameters were observed at plasma level of up to 96 ± 28 μM. Compound **26** also showed an acceptable CYP enzyme inhibition profile with IC_{50} of >100, 39, and 19 μM for 2D6, 3A4, and 2C8 isozymes, respectively. However, its selectivity at the 2C9 isozyme was moderate with IC_{50} of 5.8 μM. It also has no tendency to activate hPXR with EC_{50} > 25 μM.

In vivo receptor occupancy of **26** in the hGCGR mice at one hour post oral dose was measured to be 87% and 51% at 30 and 10 mpk, respectively.[19b] In another experiment, the receptor occupancy of **26** in the hGCGR mice at 10 mpk was determined to be 64%, 60%, and 51% at 1, 3, and 8 h post-dose, respectively. Consistent with these observations, compound **26** significantly reduced blood glucose AUC_{2-6h} by 27% and 37%, respectively, following 10 and 30 mpk oral dose in a diabetic mouse model using the hGCGR mice fed a high fat diet for eight weeks and treated with streptozotocin for the last four weeks.

Finally, the pharmacokinetic profile and glucagon receptor functional activity of **26** were determined in several preclinical species (Table 4). It showed good pharmacokinetic profiles in all species tested. The PK parameters were similar in the rat, dog, and monkey while the mouse appeared to be an outlier and gave somewhat higher clearance, shorter half-life, and lower bioavailability. The functional activity of **26** at the dog and monkey receptors were much closer to that of the human while **26** only showed μM functional potency in the rodents. Therefore, the rhesus monkey was chosen to evaluate the pharmacological effect of glucagon receptor inhibition in a higher species (Fig. 2).[20] Compound **26** inhibited the glucagon-induced glucose excursion 4 h post oral dose at 1 and 10 mpk. The plasma levels of **26** were 0.13 ± 0.04, 0.60 ± 0.18, and 8.8 ± 3.4 μM at the time of glucagon challenge (t = 0, n = 4) at 0.3, 1, and 10 mpk doses respectively and these levels were maintained during the course of the PD experiments.

In conclusion, a novel class of 1,3,5-pyrazoles has been discovered as potent human glucagon receptor antagonists. SAR studies have identified compound **26** as a potent human glucagon receptor antagonist with good pharmacokinetic profiles in four preclinical species. Pyrazole **26** showed excellent oral pharmacodynamic efficacy in rhesus monkeys and transgenic mice by blocking glucagon-induced hyperglycemia. Extensive profiling of **26** has demonstrated that the pyrazole class of human glucagon receptor antagonists holds great potential for the treatment of T2DM. Indeed, further modifications have led to the identification of two development candidates from this class, which will be the subject of future communications.

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References and notes

1. Ettaro, L.; Songer, T. J.; Zhang, P.; Engelgau, M. M. *Pharmacoeconomics* **2004**, *22*, 149.
2. (a) Sarabu, R.; Tiley, J. *Ann. Rep. Med. Chem.* **2005**, *40*, 167; (b) Nourparvar, A.; Bulotta, A.; Di-Mario, U.; Perfetti, R. *Trends Pharmacol. Sci.* **2004**, *25*, 86.
3. (a) Zhang, B. B.; Moller, D. B. *Curr. Opin. Chem. Biol.* **2000**, *4*, 461; (b) Jiang, G.; Zhang, B. B. *Am. J. Physiol.* **2003**, *284*, E671; (c) Sloop, K. W.; Michael, M. D. *Drugs Fut.* **2004**, *29*, 835.
4. (a) Brand, C. L.; Rolin, B.; Jorgensen, P. N.; Svendsen, I.; Kristensen, J. S.; Holst, J. J. *Diabetologia* **1994**, *37*, 985; (b) Brand, C. L.; Jorgensen, P. N.; Knigge, U.; Warberg, J.; Svendsen, I.; Kristensen, J. S.; Holst, J. J. *Am. J. Physiol.* **1995**, *269*, E469; (c) Brand, C. L.; Jorgensen, P. N.; Svendsen, I.; Holst, J. J. *Diabetes* **1996**, *45*, 1076; (d) Brand, C. L.; Hansen, B.; Groneman, S.; Boysen, M.; Holst, J. J. *Diabetes* **2000**, *49*, A81; (e) Liang, Y.; Osborne, M. C.; Monia, B. P.; Bhanot, S.; Gaarde, W. A.; Reed, C.; She, P.; Jetton, T. L.; Demarest, K. T. *Diabetes* **2004**, *53*, 410; (f) Sloop, K. W.; Cao, J. X.-C.; Siesky, A. M.; Zhang, H. Y.; Bodenmiller, D. M.; Cox, A. L.; Jacobs, S. J.; Moyers, J. S.; Owens, R. A.; Showalter, A. D.; Brenner, M. B.; Raap, A.; Gromada, J.; Berridge, B. R.; Monteith, D. K. B.; Porksen, N.; McKay, R. A.; Monia, B. P.; Bhanot, S.; Watts, L. M.; Michael, M. D. *J. Clin. Invest.* **2004**, *113*, 1571.
5. Petersen, K. F.; Sullivan, J. T. *Diabetologia* **2001**, *44*, 2018.
6. (a) DeMong, D. E.; Miller, M. W.; Lachowicz, J. E. *Ann. Rep. Med. Chem.* **2008**, *43*, 119; (b) Kurukulasuriya, R.; Link, J. T. *Expert Opin. Ther. Patents* **2005**, *15*(12), 1739.
7. Shen, D.-M.; Zhang, F.; Brady, E. J.; Candelore, M. R.; Dallas-Yang, Q.; Ding, V. D.-H.; Dragovic, J.; Feeney, W. P.; Jiang, G.; McCann, P. E.; Mock, S.; Qureshi, S. A.; Saperstein, R.; Shen, X.; Tamvakopoulos, C.; Tong, X.; Tota, L. M.; Wright, M. J.; Yang, X.; Zheng, S.; Chapman, K. T.; Zhang, B. B.; Tata, J. R.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4564.
8. (a) Ling, A.; Plewe, M. B.; Truesdale, L. K.; Lau, J.; Madsen, P.; Sams, C.; Behrens, C.; Vagner, J.; Christensen, I. T.; Lundt, B. F.; Sidelmann, U. G.; Thøgersen, H. U.S. Patent 6503,949, 2003; *Chem. Abstr.* **2001**, *134*, 4764.; (b) Lau, J.; Behrens, C.; Sidelmann, U. G.; Knudsen, L. B.; Lundt, B.; Sams, C.; Ynddal, L.; Brand, C. L.; Pridal, L.; Ling, A.; Kiel, D.; Plewe, M. B.; Shi, S.; Madsen, P. *J. Med. Chem.* **2007**, *50*, 113.
9. (a) After the completion of our studies described here, benzimidazoles, indoles, aminothiazoles, pyridazines, and several other cyclic structures as additional conformationally constrained replacements of ureas such **1** have appeared: Lau, J.; Christensen, I. T.; Madsen, P.; Behrens, C.; WO 2003 053938.; (b) Lau, J.; Christensen, I. T.; Madsen, P.; Block, P.; Behrens, C.; Kodra, J. T.; Nielsen, P. E. WO 2004 002480.; (c) Madsen, P.; Lau, J.; Kodra, J. T.; Christensen, I. T. WO2005 058845.; (d) Kodra, J. T.; Jørgensen, A. S.; Andersen, B.; Behrens, C.; Brand, C. L.; Christensen, I. T.; Guldbrandt, M.; Jeppesen, C. B.; Knudsen, L. B.; Madsen, P.; Nishimura, E.; Sams, C.; Sidelmann, U. G.; Pedersen, R. A.; Lynn, F. C.; Lau, J. *J. Med. Chem.* **2008**, *51*, 5387; (e) Madsen, P.; Kodra, J. T.; Behrens, C.; Nishimura, E.; Jeppesen, C. B.; Pridal, L.; Andersen, B.; Knudsen, L. B.; Valcarce-Aspegren, C.; Guldbrandt, M.; Christensen, I. T.; Jørgensen, A. S.; Ynddal, L.; Brand, C. L.; Bagger, M. A.; Lau, J. *J. Med. Chem.* **2009**, *52*, 2989.
10. (a) Shen, D-M.; Shu, M.; Chapman, K. T. *Org. Lett.* **2000**, *2*, 2789; (b) Shen, D.-M.; Shu, M.; Mills, S. G.; Chapman, K. T.; Malkowitz, L.; Springer, M. S.; Gould, S. L.; DeMartino, J. A.; Siciliano, S. J.; Kwei, G. Y.; Carella, A.; Carver, G.; Holmes, K.; Schlief, W. A.; Danzeisen, R.; Hazuda, D.; Kessler, J.; Lineberger, J.; Miller, M. D.; Emini, E. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 935. and two following Letters.
11. The system of MeCN/DCM appears uniquely effective in separating pyrazole isomers. It was used for separating pyrazole isomers in Ref. 10b also.
12. All intermediates described here showed expected ^{1}H NMR and LC–MS. All isomeric pyrazole intermediates were identified by NOE difference spectra. All final products showed expected LC–MS and some were characterized by ^{1}H NMR.
13. Qureshi, S. A.; Candelore, M. R.; Xie, D.; Yang, X.; Tota, L. M.; Ding, V. D.-H.; Li, Z.; Bansal, A.; Miller, C.; Cohen, S.; Jiang, G.; Brady, E.; Saperstein, R.; Duffy, J. L.; Tata, J. R.; Chapman, K. T.; Moller, D. E.; Zhang, B. B. *Diabetes* **2004**, *53*, 3267.
14. Jones, P. F.; Lappert, M. F. *J. Chem. Soc., Chem. Commun.* **1972**, 526.
15. The Claisen reaction mixture for **3** and **21** was heated under reflux in THF for 10 min followed by standing at room temperature overnight versus room temperature only for all other Claisen reactions.
16. Jorgensen, A. S.; Christensen, I. T. Kodra, J. T.; Madsen, P.; Behrens, C.; Sams, C.; Lau, J. WO 2002 000612.
17. Madsen, P.; Lau, J.; Ling, A. WO 2002 040444.
18. The full characterization of the hGCGR mice and their response to known antagonists has been reported: (a) Shiao, L.-L.; Cascieri, M. A.; Trumbauer, M.; Chen, H.; Sullivan, K. A. *Transgenic Res.* **1999**, *8*, 295; (b) Dallas-Yang, Q.; Shen, X.; Strowski, M.; Brady, E.; Saperstein, R.; Gibson, R. E.; Szalkowski, D.; Candelore, M. R.; Fenyk-Melody, J. E.; Parmee, E. R.; Zhang, B. B.; Jiang, G. *Eur. J. Pharmacol.* **2004**, *501*, 225.
19. Fish, L. R.; Gilligan, M. T.; Humphries, A. C.; Ivarsson, M.; Ladduwahetty, T.; Merchant, K. J.; O'Connor, D.; Patel, S.; Philipps, E.; Vargas, H. M.; Hutson, P. H.; MacLeod, A. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3665.
20. Pharmacodynamic experiments in rhesus monkeys: overnight fasted monkeys were dosed either with vehicle or test compound **26** 4 h prior to glucagon challenge. At time zero, the animals were treated with glucagon (15 μg/kg im) and plasma glucose was monitored every 10 min. The data were platted as the change of plasma glucose relative to time zero for each group.