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A novel series of glucagon receptor antagonists with reduced molecular weight and lipophilicity

Kevin J. Filipski *,†, Jianwei Bian, David C. Ebner, Esther C.Y. Lee, Jian-Cheng Li, Matthew F. Sammons, Stephen W. Wright, Benjamin D. Stevens, Mary T. Didiuk, Meihua Tu, Christian Perreault, Janice Brown, Karen Atkinson, Beijing Tan, Christopher T. Salatto, John Litchfield, Jeffrey A. Pfefferkorn, Angel Guzman-Perez

Pfizer Worldwide Research & Development, Eastern Point Rd, Groton, CT 06442, USA

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ABSTRACT

A novel series of glucagon receptor antagonists has been discovered. These pyrazole ethers and aminopyrazoles have lower molecular weight and increased polarity such that the molecules fall into better drug-like property space. This work has culminated in compounds **44** and **50** that were shown to have good pharmacokinetic attributes in dog, in contrast to rats, in which clearance was high; and compound **49**, which demonstrated a dose-dependent reduction in glucose excursion in a rat glucagon challenge experiment.

Type 2 Diabetes Mellitus (T2DM) is a growing worldwide epidemic currently affecting an estimated 285 million people.[1] Although several classes of diabetic therapies are routinely used clinically, there remains a significant unmet medical need for additional therapies.[2] T2DM is partially characterized by elevated and dysregulated hepatic glucose output. Glucagon is a 29-amino acid peptide that acts as the principle counter-regulatory hormone to insulin in the control of glucose homeostasis.[3] Through binding of glucagon to the G-protein coupled glucagon receptor, hepatic glycogenolysis and gluconeogenesis are stimulated resulting in enhanced hepatic glucose production. It is therefore postulated that blocking the action of the glucagon receptor would lead to improved glycemic control in T2DM patients.[4]

Over the last two decades, various research teams have brought forward both large and small molecules that act as glucagon receptor (GCGR) antagonists.[4,5] One notable early molecule is Bay 27-9955 (**1**) that clinically demonstrated a reduction in glucagon-induced glucose excursion by modulating hepatic glucose production in humans.[6] Researchers at Novo-Nordisk later disclosed a series containing a β-alanine side chain in structures such as NNC 25-0926 (**2**).[7] This motif has been incorporated into subsequent work by many groups including work leading to the discovery of Merck clinical candidate MK-0893 (**3**)[8] and compound **4**.[9]

Most of the β-alanine-containing GCGRA antagonists are relatively high molecular weight, lipophilic molecules (Figure 1) situated at the edge of optimal drug-like space as defined by Lipinski and others. This could theoretically result in sub-optimal biopharmaceutical properties.[10] As part of a discovery program, we sought to develop a series of small molecule glucagon receptor antagonists with reduced molecular weight and lipophilicity with the goal of improving pharmacokinetic (PK) and safety properties.[11] Specifically, we sought potent and selective antagonists with molecular weight <500 and log*D* <3.

We identified a pyrazole ether series of glucagon receptor antagonists through our discovery program. The syntheses of these analogs were accomplished according to Scheme 1. Dimethyl malonate (**5**) was condensed with ethyl diazoacetate to form dimethyl ester **6**.[12] Hot concentrated hydrochloric acid led to hydrolysis and decarboxylation of **6** affording hydroxypyrazole **7**. The pyrazole nitrogen was selectively trityl protected by initial *in situ* protection of the hydroxy with a trimethylsilyl group. The transient TMS group was then hydrolyzed under mild aqueous basic conditions to provide **8**. Separately, ethyl 4-iodobenzoate **9** was converted to alcohol **10** by formation of the Grignard species followed by addition into butyraldehyde.[13] Compound **10** was then converted to the methanesulfonate **11** under standard conditions.

* Corresponding author. Tel.: +1 617 551 3267; fax: +1 617 551 3082.
E-mail address: kevin.filipski@pfizer.com (K.J. Filipski).
† Present address: Pfizer Worldwide Research & Development, RTC 367, 620 Memorial Dr., Cambridge, MA 02139, USA.

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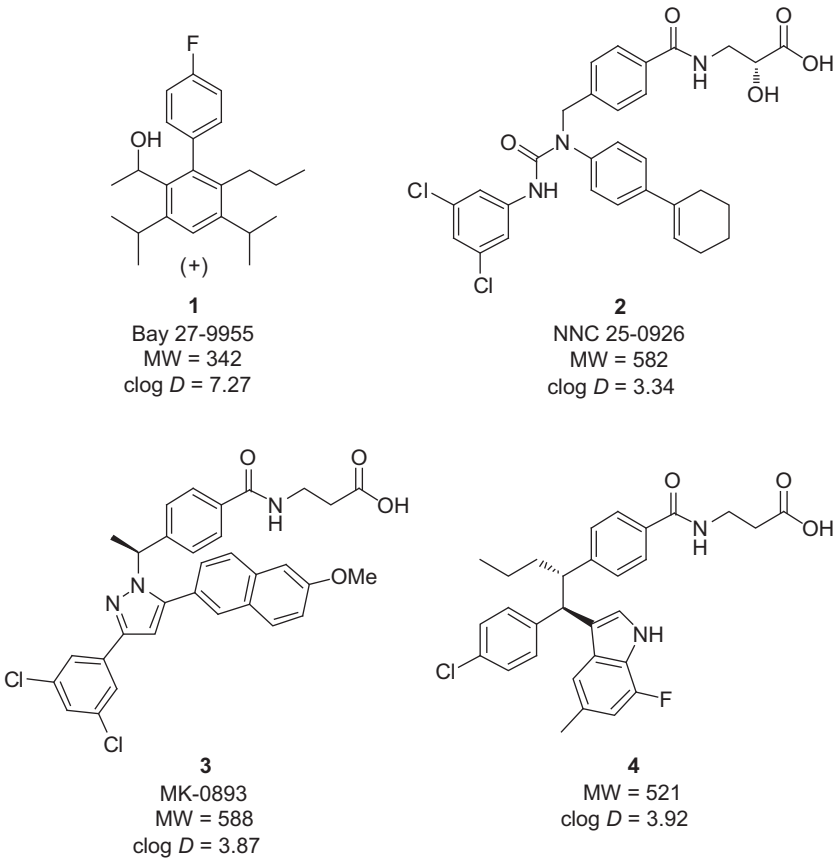


Figure 1. Structure, molecular weight, and clog *D* (calculated distribution coefficient) for key glucagon receptor antagonists.

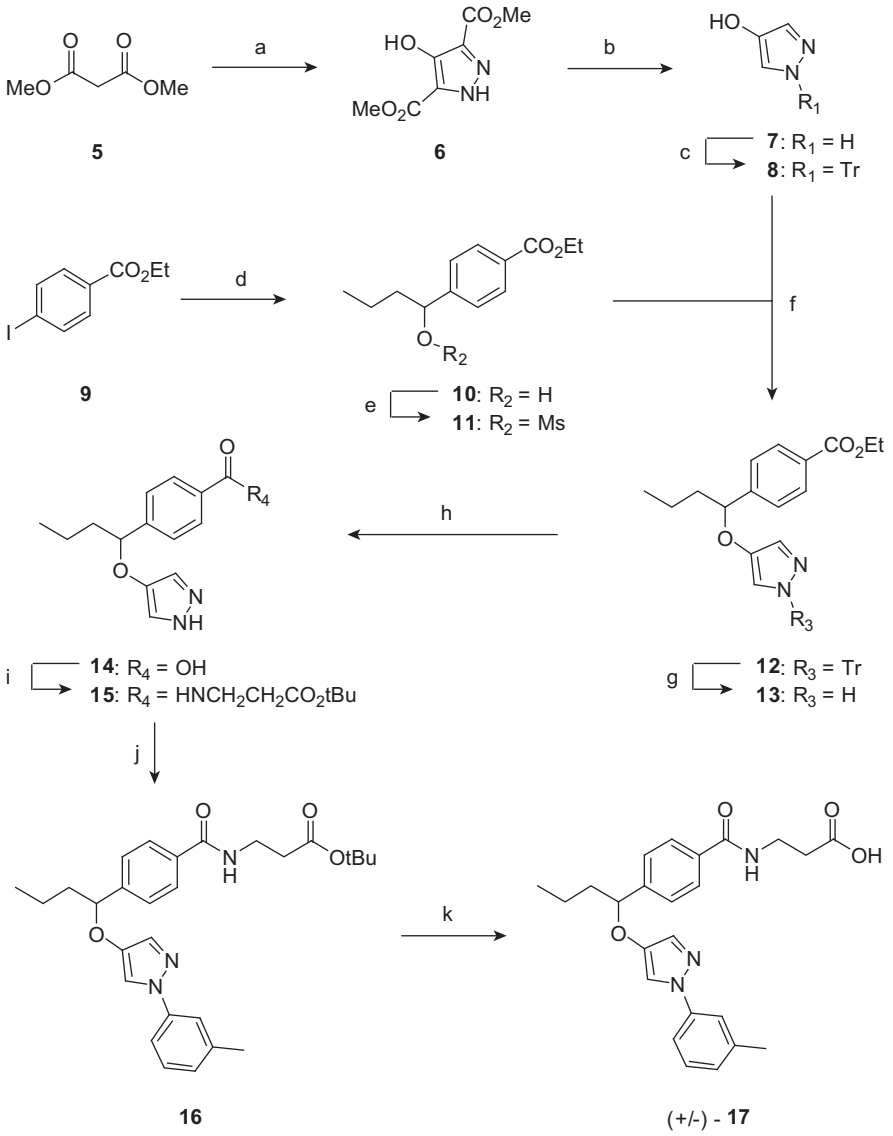
Ether **12** was formed by displacement of mesylate **11** with the anion of hydroxypyrazole **8**. The trityl group was removed with *in situ* generated HCl to give **13**, which was hydrolyzed under basic conditions to form benzoic acid **14**. Standard peptide coupling conditions were used to produce the key amide intermediate **15**, which was subsequently coupled with 3-iodotoluene and a variety of other aryl halides, using Buchwald's copper-catalyzed amination conditions to give esters such as **16**.[14] Compound **16** and similar intermediates were finally hydrolyzed under basic conditions to provide **17** as a racemic mixture.

Aminopyrazoles were synthesized according to the procedure shown in Scheme 2. Nitropyrazole **18** was coupled with 4-trifluoromethylphenylboronic acid under Chan-Lam conditions to form **19**.[15] The nitro group was reduced by catalytic hydrogenation to give amine **20**. Separately, 4-iodobenzoic acid **21** was first esterified to *tert*-butyl ester **22** using Wright's conditions,[16] and then converted to ketone **23** by formation of the cuprate and addition into butyryl chloride.[17] A decaborane-mediated reductive amination provided **24**,[18] which was hydrolyzed to acid **25**. A standard amide formation gave racemic **26**, which was separated into enantiomers **27** and **28** by SFC. The enantiomers were separately hydrolyzed to provide analogs **29** and **30**.

SAR of the pyrazole ether series is shown in Table 1. In each of these cases analogs were tested as racemates. The *in vitro* potency of test compounds was assessed using both binding and functional assay formats. The binding affinity of compounds for the human glucagon receptor was determined by their ability to displace [^{125}I] Glucagon-Cex from membranes containing the human glucagon receptor,[19] while functional activity was determined by the ability of these compounds to inhibit glucagon-induced cAMP production in a cell line expressing the human glucagon receptor. The binding assay was performed in the presence of low BSA concentrations (0.2%) while functional activity was determined in the presence of 4% BSA in order to provide an estimate of plasma bound total potency. As can be observed in the series **31–33**, a methyl group in the *ortho* position is not tolerated while in the *meta* or *para* position, modest binding potency is observed without measurable functional activity. Therefore, subsequent work was focused to the *meta* and *para* positions for substitution. Ethyl analogs **34** and **35** show improved potency both in binding and functional assays. 3-Chloro **36** and 4-chloro **37** are similar in potency to their methyl counterparts. Introducing polarity to the substituent, as with methoxy-substituted **38** and **39** decreases activity as compared to the ethyl-substituted analogs. 4-Trifluoromethyl-substituted **40** shows good potency in both assays. Finally, efforts to replace the phenyl ring of **40** with pyridine derivatives **41** and **42**, resulted in greatly reduced activity.

Based on the positive results with the 4-trifluoromethyl-substituted analog **40**, this structure was chosen for further exploration (Table 2). This analog was first resolved into its enantiomers by SFC to give **43** and **44**. The (−)-isomer (**44**) was determined to be the more potent enantiomer with a binding K_i of 91 nM and functional activity K_b of 520 nM. The molecular weight is a desirable 475 and the log*D* is 2.31, both values in our target zone. The passive permeability[20] is also good, solubility modest, and HLM stability excellent.

Replacing the ether linkage with an amine was also examined. To our delight this modification improved both binding potency and functional activity with the more active (+) enantiomer **45**. Even though the lipophilicity was decreased (log*D* = 1.73), permeability was conserved. Importantly, solubility was greatly increased and microsomal stability remained excellent. Changes to



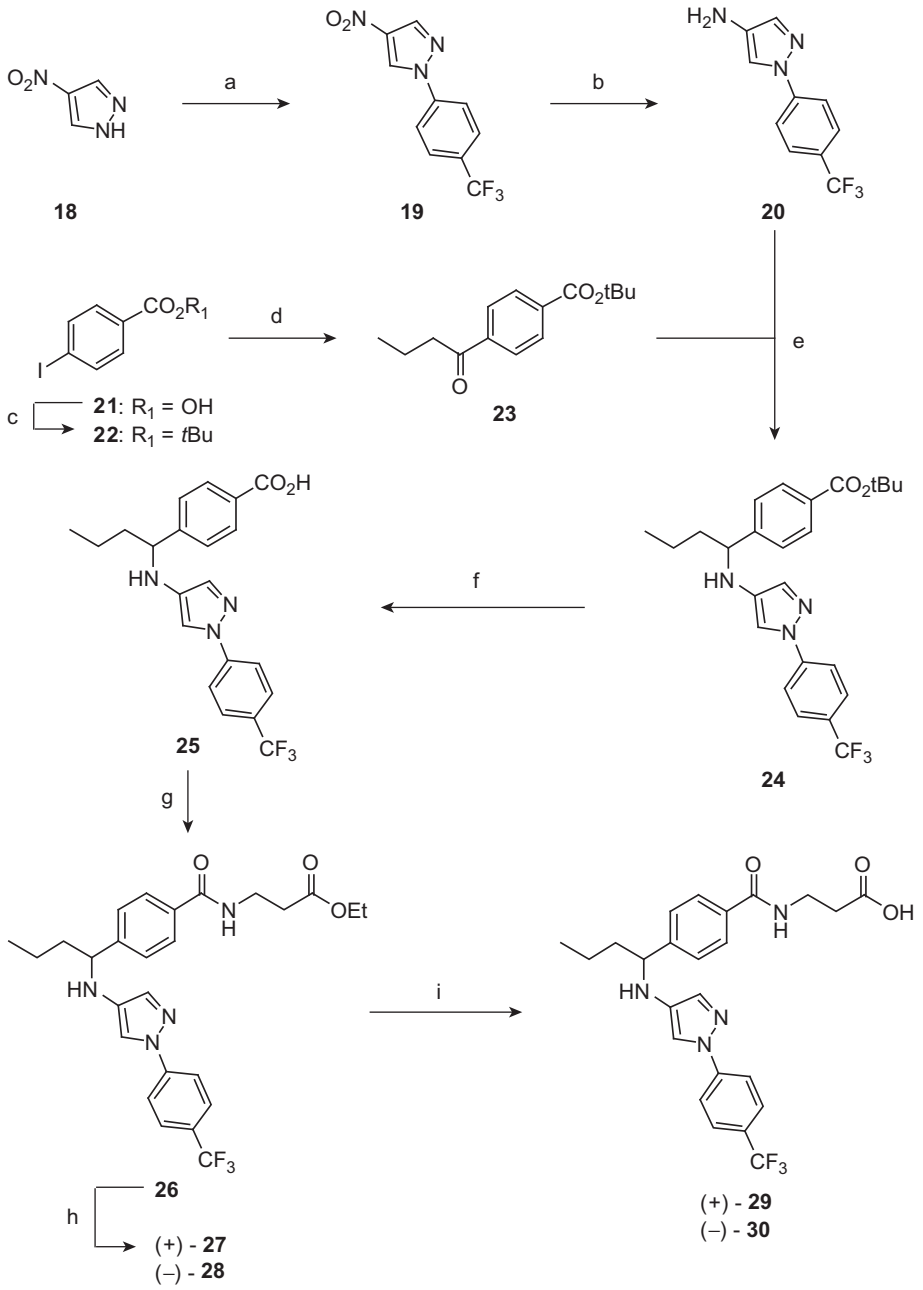
Scheme 1. Synthetic method for synthesis of pyrazole ethers. Reagents and conditions: (a) i. Na, MeOH, 0 °C, ii. ethyl diazoacetate, 23 °C, 3 d, iii. 5 N HCl, 23 °C; (b) conc. HCl, reflux, 16 h, 47% over two steps; (c) i. TMS-Cl, Et_3N, CH_3CN, 0 – 23 °C, 2 h, ii. TrCl, 23 °C – reflux, 17 h, iii. aq. sat. $NaHCO_3$, 23 °C, 1 h, 66%; (d) i. *i*PrMgCl-LiCl, THF, -40 °C, 1 h, ii. butyraldehyde, -40 – 23 °C, 1 h, 93%; (e) MsCl, Et_3N, DCM, 0 °C, 90 min, 100%; (f) NaH, DMF, 23 °C, 23 h, 61%; (g) AcCl, EtOH, 0 – 70 °C, 2 h; 92%; (h) 10% aq. NaOH, THF, 70 °C, 16 h, 100%; (i) β-alanine *t*-butyl ester hydrochloride, HATU, Et_3N, DCM, 30 °C, 16 h; 80%; (j) 3-iodotoluene, CuI, trans-*N*,*N*′-dimethylcyclohexane-1,2-diamine, K_2CO_3, toluene, 110 °C, 24 h; (k) TFA, DCM, 30 °C, 2.5 h.

the alkyl region were then explored with 3,3,3-trifluoropropyl and cyclopentyl replacing the propyl side chain. These analogs were still in the desired lipophilicity space and potency was improved. Permeability and solubility were retained, however HLM stability of **49** was poor. Interestingly, the less potent cyclopentyl enantiomer **50** had lower HLM clearance than the more potent enantiomer and still had acceptable potency. Across this class, solubility was high (310 – 550 μM) [compare to reference compound **4** (22 μM)]. Overall, many of the best compounds from this work compared well against comparator compounds **1** – **3** with respect to binding and functional potency (compare Table 1 entries 1–4 with Table 2). Compound **4** does offer greater potency, however.

To verify selectivity against other class B GPCR's, selected compounds were screened in both human GLP-1 and human GIP binding assays (Table 3). A compound's GLP-1 potency was determined by its ability to displace [^{125}I] GLP-1 from membranes expressing the hGLP-1 receptor. Tested analogs were determined to be highly selective for the glucagon receptor over the GLP-1 receptor with ratios falling between 50- and 500-fold selective. A compound's GIP potency was determined by its ability to displace [^{125}I] GIP from membranes expressing the hGIP receptor. Here, the margin of selectivity was smaller with compounds ranging from 10- to 80-fold selective.

Based on their favorable *in vitro* profiles, several analogs were chosen for *in vivo* pharmacokinetic characterization. Pyrazole ether **44**, propylamine **45**, 3,3,3-trifluoropropylamine **47**, and both cyclopentylamine enantiomers **49** and **50** were investigated in i.v. and oral rat PK studies. Results are shown in Table 4. In general these compounds showed moderate to high clearance. Based on their stability in rat liver microsomes, the major clearance mechanism is assumed to be non-oxidative, likely biliary. Due in part to their high *in vivo* clearances, these compounds also showed generally short half lives and low oral bioavailability.

Two compounds with more favorable rat PK profiles, **44** and **50**, were then taken into dog i.v. PK studies, with results shown in Table 5. Gratifyingly, clearance values in dog were much lower indicating a significant species difference in clearance. Half lives in dog were short to moderate and exposure was good. Investigations into



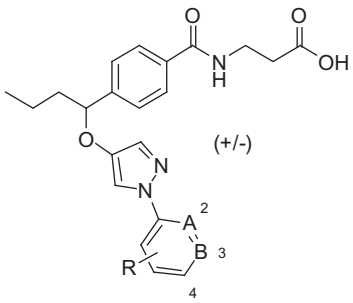
Scheme 2. Synthetic method for synthesis of aminopyrazoles. Reagents and conditions: (a) 4-trifluoromethylphenylboronic acid, $Cu(OAc)_2$, pyridine, DCM, 4 Å MS, 23 °C, 3 d, 95%; (b) H_2, 40 psi, 10% Pd/C, EtOH, 99%; (c) $MgSO_4$, H_2SO_4, *t*BuOH, DCM, 23 °C, 2 d, 15%; (d) i. *i*PrMgCl-LiCl, THF, -40 °C, 1 h, ii. CuI, -15 °C, 15 min, iii. butyryl chloride, -40 – 23 °C, 16 h, 83%; (e) decaborane, MeOH, 23 °C, 16 h, 30%; (f) TFA, DCM, 23 °C, 30 min, 100%; (g) β-alanine ethyl ester hydrochloride, HOAt, EDAC, Et_3N, DCM, 23 °C, 2.5 h, 83%; (h) chiral SFC: 75/25 CO_2/EtOH, Chiralcel OJ-H; (i) 1 N aq. NaOH, MeOH, 23 °C, 0.5 – 2 h, 89–100%.

the species most likely to be predictive of human pharmacokinetic behavior for this and related series are in progress.

To assess their potential safety liabilities, selected compounds from this series were screened in a transformed human liver epithelial (THLE) cell cytotoxicity assay.[21] This assay measures ATP depletion as a determinant of general cellular toxicity. Activity is reported as the dose predicted to kill 50% of cells following a 72 h exposure, with compounds **44**, **49**, and **50** having IC_{50}'s of 90, 235, and 166 μM, respectively. This compares favorably to reference compounds **3** (IC_{50} = 38 μM) and **4** (IC_{50} = 55 μM), lending some evidence to the improved safety profile achieved by reducing the lipophilicity.

To examine whether this series of analogs displayed efficacy *in vivo*, compound **49** was tested in an i.v. glucagon challenge model in rats.[7b] In Wistar-Han rats at time = 0, one of three doses of compound **49** or vehicle alone were dosed intravenously. At 15 min, a 3 μg/kg i.v. bolus of glucagon was administered to elicit a glucose excursion. Blood glucose levels were monitored at the following time points: 0, 10, 26, 32, 50, and 80 min. Results, shown in Figure 2, show a dose-dependent reduction in glucose excursion by compound **49**. At the 11 min time point, glucose levels, compared to vehicle, were reduced 12, 20, and 24% at doses of 1, 10, and 30 mg/kg, respectively. Higher doses showed an extended effect. Plasma levels of **49** at 11 min were 300 ± 37, 2285 ± 140, and 20950 ± 1374 ng/mL, respectively. In an *in vitro* rat GCGRA functional assay using similar conditions to the human assay, the K_b of **49** was 2.34 μM.

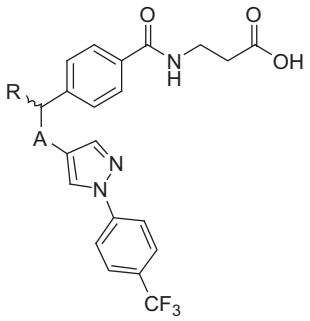
Table 1
Binding potency and functional activity of glucagon receptor antagonists **1–4**, **31–42**

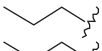
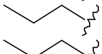
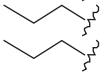
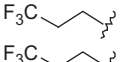
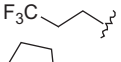
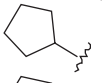
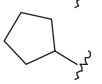


	R	A	B	hGCGR binding K_i (μM)[a]	hGCGR cAMP K_b (μM)[a]
1	—	—	—	0.356	0.078
2	—	—	—	0.085	0.430
3	—	—	—	0.070	0.266
4	—	—	—	0.004	0.018
31	2-Me	C	C	14.7[b]	>23.6
32	3-Me	C	C	1.11	>20.9
33	4-Me	C	C	0.968	>12.9
34	3-Et	C	C	0.556	4.53
35	4-Et	C	C	0.214	3.42
36	3-Cl	C	C	1.09	16.9
37	4-Cl	C	C	0.600	>7.52
38	3-OMe	C	C	2.16	>19.1
39	4-OMe	C	C	1.98	>21.3
40	4-CF_3	C	C	0.126	0.992
41	4-CF_3	N	C	1.19	16.4
42	4-CF_3	C	N	1.45	21.5

[a] Reported as the geometric mean of n ⩾ 2 determinations except where otherwise noted.
[b] Reported as $n = 1$.

Table 2
Compound properties of glucagon receptor antagonists **43–50**



	R	Optical Rotation Sign	A	MW (g/mol)	logD[a]	hGCGR Binding K_i (nM)[b]	hGCGR cAMP K_b (nM)[b]	MDCKII-LE Permeability ($\times 10^{-6}$ cm/s)[c]	Kinetic Solubility (μM)[d]	HLM $Cl_{int,\ app}$ (mL/min/kg)	RLM $Cl_{int,\ app}$ (mL/min/kg)
43		+	O	475	2.33	430	1230	7.74	53	34	<25
44		–	O	475	2.31	91	520	5.87	77	<8.0	<25
45		+	NH	474	1.73	19	310	8.81	550	<8.0	<25
46		–	NH	474	1.77	67	3300	2.76	500	<8.0	<25
47		+	NH	528	1.67	12	100	3.48	490	<8.0	<25
48		–	NH	528	1.76	48	520	3.80	490	<8.0	<25
49		+	NH	501	2.27	9	120	6.34	320	91	<25
50		–	NH	501	2.29	17	320	7.54	310	<8.0	30

[a] pH 7.4 octanol/buffer shake-flask method.
[b] Reported as the geometric mean of $n \geqslant 2$ determinations.
[c] This is a low transporter-containing MDCK cell line. See Ref. 20 for details.
[d] Solubility at pH 6.5.

Table 3
Selectivity data for glucagon receptor antagonists **43**–**50**

	hGCGR Binding K_i (μM)[a]	hGLP-1 Binding K_i (μM)[a]	hGLP-1 K_i/hGCGRA K_i	hGIP Binding K_i (μM)[a]	hGIP K_i/hGCGRA K_i
43	0.430	21.8	51	4.08	9.5
44	0.091	16.5	181	0.939	10
45	0.019	10.4[b]	547	NT[c]	—
46	0.067	9.08	136	1.95	29
47	0.012	0.816	68	0.300	25
48	0.048	4.22	88	3.84	80
49	0.009	3.34[b]	371	0.152	17
50	0.017	6.62	389	0.927	55

[a] Reported as the geometric mean of n ⩾ 2 determinations except where otherwise noted.
[b] Reported as n=1.
[c] NT = not tested.

Table 4
Rat pharmacokinetic parameters for compounds **44**, **45**, **47**, and **50**[a]

	$t_{1/2}$ (h)	V_{dss} (L/kg)	Cl (mL/min/kg)	F (%)
44	0.7	1.17	48	11
45	0.3	1.13	67	4
47	0.3	0.615	56	0.5
49	0.4	1.27	81	2
50	1.7	2.83	76	6

[a] Wistar-Han rats dosed at 1 mg/kg iv and 5 mg/kg po.

Table 5
Dog pharmacokinetic parameters for compounds **44** and **50**[a]

	$t_{1/2}$ (h)	V_{dss} (L/kg)	Cl (mL/min/kg)	AUC (ng h/mL)
44	2.3	1.04	9.6	828
50	1.8	0.623	4.6	1870

[a] Beagle dogs dosed at 0.5 mg/kg iv.

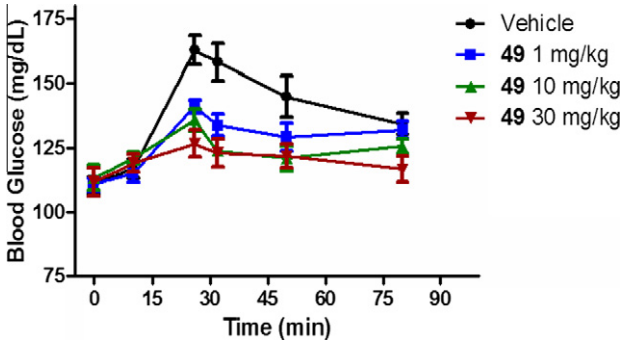


Figure 2. Blood glucose response following 3 μg/kg glucagon in Wistar-Han rats administered compound **49**. Antagonist administered i.v. at $t = 0$. Glucagon challenge administered i.v. at $t = 15$ min. $N = 6$ rats per dosing group.

In conclusion, we have discovered a series of glucagon receptor antagonists with reduced molecular weight and lipophilicity compared to many previously reported antagonists. Both pyrazole ethers and aminopyrazoles were found with desirable *in vitro* potency, permeability, and solubility profiles. These compounds showed good pharmacokinetic profiles in dog, while in rat high clearance was observed. One compound demonstrated a dose-dependent reduction in glucose excursion following a glucagon challenge in rats. Notably, these analogs have significantly reduced lipophilicity compared to previously reported series containing the β-alanine side chain. As already observed in *in vitro* systems, this hopefully will lead to better safety profiles *in vivo*.

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