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## Graphical Abstract

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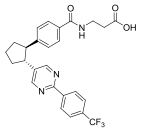

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# Identification of a Novel Conformationally Constrained Glucagon Receptor Antagonist

Esther C. Y. Lee,[1,§] Meihua Tu,[§] Benjamin D. Stevens,[§] Jianwei Bian,[¥] Gary Aspnes,[¥] Christian Perreault,[¥] Matthew F. Sammons,[§] Stephen W. Wright, [¥] John Litchfield,[§] Amit S. Kalgutkar, [§] Raman Sharma,[¥] Mary T. Didiuk,[¥] David C. Ebner,[§] Kevin J. Filipski,[§] Janice Brown,[¥] Karen Atkinson,[¥] Jeffrey A. Pfefferkorn,[§] and Angel Guzman-Perez[¥]

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ABSTRACT

Identification of orally active, small molecule antagonists of the glucagon receptor represents a novel treatment paradigm for the management of type 2 diabetes mellitus. The present work discloses novel glucagon receptor antagonists, identified *via* conformational constraint of current existing literature antagonists. Optimization of lipophilic ligand efficiency (LLE or LipE) culminated in enantiomers (+)-*trans*-**26** and (-)-*trans*-**27** which exhibit good physicochemical and *in vitro* drug metabolism profiles. *In vivo*, significant pharmacokinetic differences were noted with the two enantiomers, which were primarily driven through differences in clearance rates. Enantioselective oxidation by cytochrome P450 was ruled out as a causative factor for pharmacokinetic differences.



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Type 2 Diabetes Mellitus (T2DM), a chronic disease prevalent worldwide, is characterized by impaired insulin secretion and insulin resistance in muscle and liver, resulting in higher blood glucose levels. As insulin resistance and the resulting hyperinsulinemia worsen over time, patients eventually experience β-cell failure. Despite the existence of several therapies, there remains a need to identify novel mechanisms of restoring glucose homeostasis.[1] Glucagon is a 29-amino acid peptide secreted in the α-cells of the pancreas that binds to the glucagon receptor in the liver to modulate hepatic glycogenolysis and gluconeogenesis. Blockade of the glucagon receptor has been proposed as an avenue to reduce hepatic glucose output, thus enabling improved glycemic control.[2]

In the last two decades, various small molecule glucagon receptor antagonists have been identified.[3] Most of these candidates contain an acidic moiety with the exception of Bay 27-9955 (**1**)[4] (Figure 1), which demonstrated a clinical proof of mechanism *via* blockade of glucose excursion following a glucagon challenge in healthy subjects. Throughout several lead optimization programs, the β-alanine side-chain has emerged as an important motif for interaction with the glucagon receptor (see Figure 1, highlighted in red). Novo-Nordisk was the first to report this motif[5a] and subsequent optimizations culminated in NNC 25-0926 (**2**), which showed reduction in hepatic glucose production during a glucagon challenge in dogs.[5c] Merck and Lilly have also disclosed clinical candidates, MK-0893 (**3**)[6a] and LY2409021 (structure undisclosed),[6b] respectively, which demonstrated robust clinical HbA1c reductions in diabetic patients.

Another common theme from these glucagon antagonists are high molecular weight and high lipophilicity, which is similar to the endogenous ligands for class B GPCRs.[7] In continuation of our previous work to identify novel glucagon receptor antagonists (Figure 1, compounds **4**[8a] and **5**[8b]) that possess drug-like attributes (MW <500, logD <3), we pursued the incorporation of a cyclic core in our previously reported series[8b] as a tactic to further increase antagonist activity against the glucagon receptor.

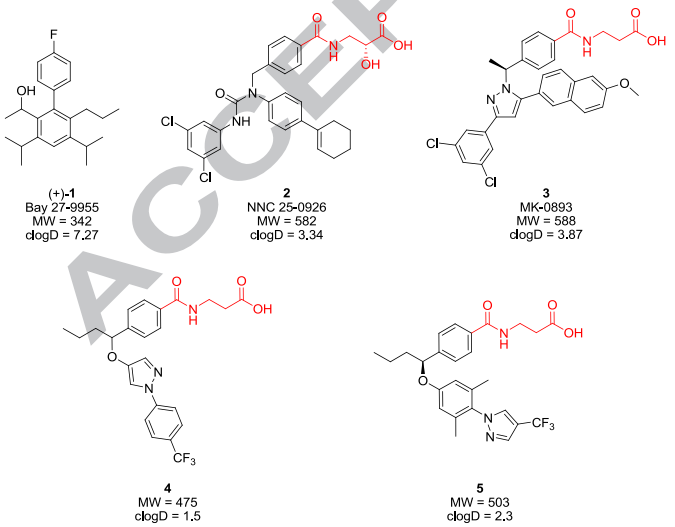



**Figure 1.** Literature glucagon receptor antagonists and their molecular weight and calculated logD (clogD).

Conformational restraint is an established strategy for improving potency and selectivity against pharmacologic targets,[9] including the glucagon receptor.[10] In the present case, the rationale for introducing a cyclic core was to improve potency without increasing logD, thereby increasing the lipophilic ligand efficiency, (LLE or LipE, as it will be presented from here on).[11]

In an effort to identify the conformational preference of glucagon receptor antagonists, a computational conformational search of literature glucagon antagonists **2**,[5] **3**,[6] and **5**[8b] was conducted in MacroModel (Version 9.0. Schrodinger Inc., New York). To simplify the analysis, the β-alanine amide (Figure 1: highlighted in red) was kept constrained during the calculation. The lowest energy conformer of compound **3** was used as the preferred binding conformation, because of its relative rigidity. The measured low energy conformers (within 3 Kcal/mol of relative conformational energy) of compounds **2** and **5** were then manually overlaid. The second lowest energy conformer of compound **2** and the lowest energy conformer of compound **5** provided the best overlay with the template (Figure 2), and were selected as the possible binding conformations for optimal glucagon antagonism. The proposed binding conformation of compound **5** was later proved to be identical to the X-ray structure of the small molecule alone.[8b]

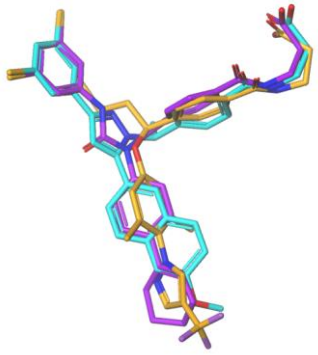


**Figure 2.** Overlay of low energy conformers of compound **2** (purple), **3** (blue) and **5** (orange).

The proposed binding conformation led to the hypothesis that the conformation of compound **5** might be reinforced by the introduction of appropriate cyclic constraints, for example, the pyrrolidine structure (**6**) in Figure 3, or other 5-member rings which will be discussed. Given synthetic considerations, we decided to initially test the hypothesis using the des-methyl pyrrolidine analogue ((+/-)-**11**) (Scheme 1).

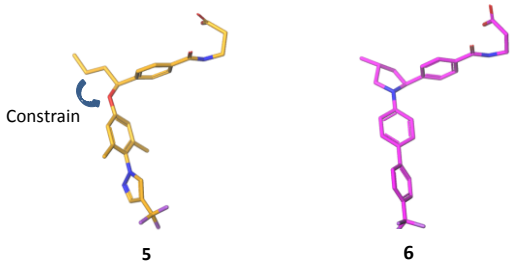
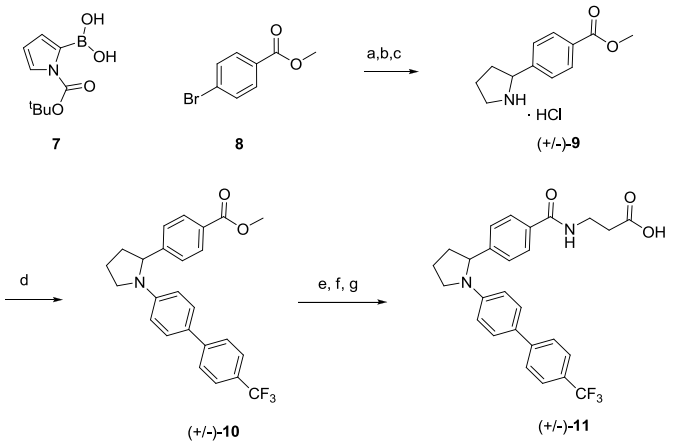



**Figure 3.** Introducing a cyclic constraint in compound **5** to provide compound **6**.

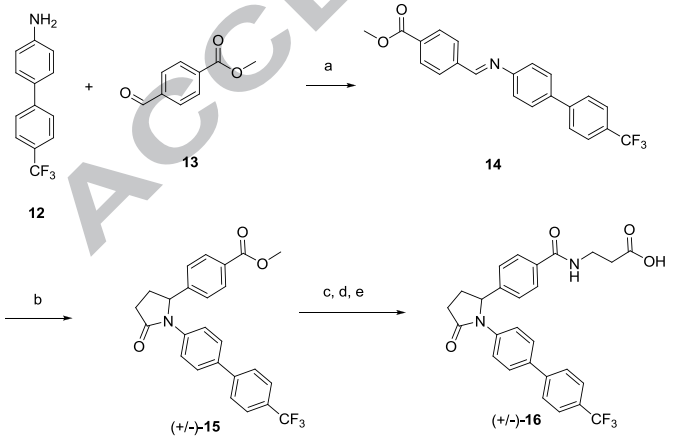
The pyrrolidine analogue ((+/-)-**11**) was synthesized according to the route shown in Scheme 1. Pyrrolidine intermediate (+/-)-**9** was prepared *via* a Suzuki reaction between pyrrole boronic acid **7** and 4-bromobenzoate **8** followed by hydrogenation of the pyrrole and removal of the

Boc group. *N*-arylation of pyrrolidine (+/-)-**9** with 4-bromo-4'-trifluoromethylbiphenyl provided the bis-substituted pyrroldine **10** as a racemic mixture. Base-mediated hydrolysis of racemic ester **10** afforded the corresponding carboxylic acid which was then subjected to a standard amide coupling with β-alanine methyl ester to provide the homologated ester, which was hydrolyzed to (+/-)-**11**.



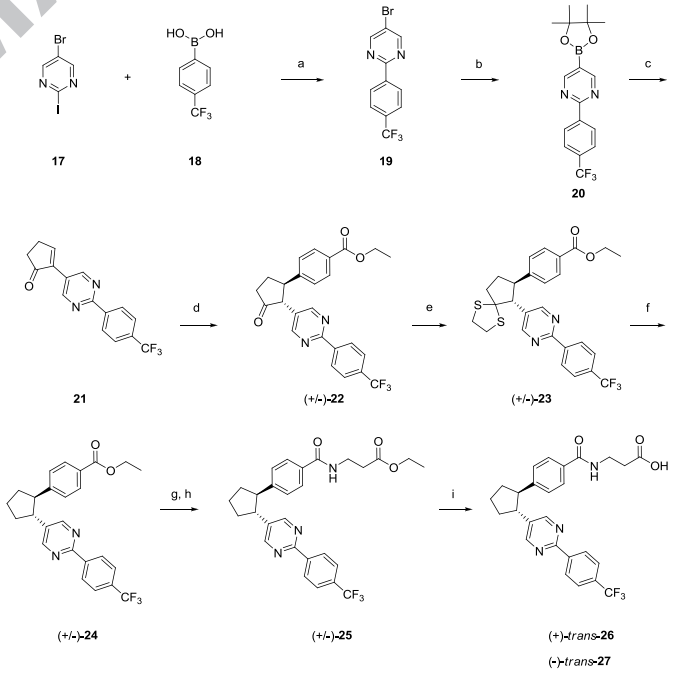

**Scheme 1.** Synthetic method for the synthesis of pyrrolidine analogue (a) $Pd(PPh_3)_4$, $Na_2CO_3$, DME/water, 3 h, reflux, 88%; (b) 30 psi $H_2$, 5% Pt/C, AcOH, 25 °C, 24 h, 84%; (c) 4 M HCl, dioxane/$CH_2Cl_2$, 25 °C, 2.5 h; (d) 4-bromo-4'-trifluoromethylbiphenyl, $Pd_2(dba)_3$, DavePhos, NaHMDS, THF, 65 °C, 20 h, 21%; (e) LiOH, THF/$H_2O$/MeOH, 60 °C, 20 h; (f) β-alanine methyl ester hydrochloride, EDC, $Et_3N$, HOAt, $CH_2Cl_2$, 25 °C, 17 h, 80%, 2 steps; (f) LiOH, THF/$H_2O$, 25 °C, 3 h, 62%.

The pyrrolidinone analogue ((+/-)-**16**) was prepared as shown in Scheme 2. Condensation between 4'-(trifluoromethyl)biphenyl-4-amine (**12**) and methyl 4-formylbenzoate (**13**) provided imine **14**. A nickel-catalyzed regioselective reductive coupling between imine **14** and methyl acrylate afforded an intermediate that cyclized to the bis-substituted pyrrolidinone (+/-)-**15** upon heating with *p*-TsOH.[12] Standard side chain elaborations as displayed in Scheme 1 were performed to produce analogue (+/-)-**16**.




**Scheme 2.** Synthetic method for the synthesis of pyrrolidinone analogue (a) MeOH, reflux, 1 h, 62%; (b) methyl acrylate, Zn, $NiBr_2$(phen), $CH_3CN/H_2O$, 80 °C, 18 h, then *p*-TsOH, *n*-BuOH, 120 °C, 18 h, 21%; (c) NaOH, THF/$H_2O$, 25 °C, 18 h, 97%; (d) β-alanine methyl ester hydrochloride, EDC, $Et_3N$, HOAt, $CH_2Cl_2$, 25 °C, 18 h; (e) LiOH, THF/$H_2O$, 25 °C, 30 min., 34% yield over 2 steps.

The synthesis of cyclopentanes (+)-*trans*-**26** and (-)-*trans*-**27** are illustrated in Scheme 3. Iodopyrimidine **17** was cross-coupled with 4-trifluoromethylphenyl boronic acid (**18**) to yield bromide **19**, which was subsequently converted to intermediate boronate **20** *via* a palladium-mediated coupling with bis(pinacolato)diboron. A Suzuki reaction with commercially available 2-iodocyclopent-2-enone afforded α-aryl cyclopentenone **21,** which represents the first appendage onto the cyclopentane core. A conjugate addition was performed using a cuprate generated from the Knochel-type Grignard reagent prepared from ethyl-4-iodobenzoate and copper(I) iodide under Lewis acid conditions to provide the bis-substituted cyclopentanone (+/-)-**22**[13] in the *trans* configuration as shown. In order to reduce the ketone, (+/-)-**22** was protected with ethane-1,2-dithiol and the dithiolane was subjected to hydrogenolysis with Raney nickel to provide the cyclopentane core (+/-)-**24** in 72% yield. Saponification generated the carboxylic acid that subsequently was treated with β-alanine ethyl ester to secure the fully elaborated side-chain (+/-)-**25**. Ethyl ester (+/-)-**25** was purified by chiral supercritical fluid chromatography to deliver the separated enantiomers. Each enantiomer was hydrolyzed separately to provide the final compounds (+)-*trans*-**26** and (-)-*trans*-**27**, each at 99% enantiomeric excess.



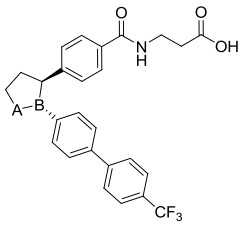

**Scheme 3.** Synthetic method for the synthesis of cyclopentyl analogs: (a) $Pd(dppf)Cl_2$, $Na_2CO_3$ (2 M), $CH_3CN$, 0 °C, 16 h, 90%; (b) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DMF/$H_2O$, 75 °C, 3 h, 84%; (c) 2-iodocyclopent-2-enone, $Pd(dppf)Cl_2$, $K_3PO_4$, DME/$H_2O$, 80 °C, 1 h, 70%; (d) ethyl-4-iodobenzoate, $^i$PrMgCl•LiCl, -45 °C, 1 h, then CuI, -20 °C , 30 min., then $BF_3$•$OEt_2$, THF, -40 to -20 °C, 1 h, 44%; (e) ethane-1,2-dithiol, *p*-TsOH, PhMe, 100 °C, 7 h, 69%; (f) Raney Ni, EtOH, 100 °C, 3 h, 72%; (g) 1 N NaOH, THF/MeOH, 25 °C, 18 h; (h) ethyl-3-aminopropanonate, EDC, HOAt, $Et_3N$, $CH_2Cl_2$, 25 °C, 75% over 2 steps; (i) 1 N NaOH, THF/MeOH, 25 °C, 18 h, (+)-*trans*-**26**, 91%, 99% ee and (-)-*trans*-**27**, 93%, 99% ee, respectively.

Binding and functional assays were utilized to assess *in vitro* pharmacology of the glucagon receptor antagonists prepared in these studies. The binding affinity of test compounds for the human glucagon receptor was assessed by their ability to displace [$^{125}$I] Glucagon-Cex from membranes containing the human glucagon receptor.[14] The functional activity was determined by the ability to inhibit glucagon-induced cAMP production in a cell line expressing the human glucagon receptor. Binding and functional assays were performed in the presence of 0.2% and 4% bovine serum albumin (BSA), respectively, to understand the impact of protein binding on compound potency.

As seen in Table 1, (+/-)-**11**, a close-in compound to the modeled analogue (**6**), displays moderate binding and functional activity for the human glucagon receptor compared to compound **5**, which provided satisfactory proof of the conformational restraint concept. However, apart from the modest potency, (+/-)-**11** also possesses unfavorable physicochemical properties. (+/-)-**11** is lipophilic (logD = 3.92) leading to a low LipE (1.1) and demonstrates poor passive absorptive permeability (0.48 x $10^{-6}$ cm/sec) in the Madin-Darby canine kidney-low efflux (MDCKII-LE) assay.[15] Furthermore, (+/-)-**11** displays oxidative metabolic turnover as judged from its intrinsic clearance ($Cl_{int}$) in human liver microsomes (HLM). The metabolic clearance is a possible manifestation of its high logD. Encouraged by the activity of compound (+/-)-**11**, we decided to try to increase LipE by adding polarity on the core ring. A change of constraint from pyrrolidine to pyrrolidin-2-one (+/-)-**16** retained binding affinity but not functional activity. The LipE gain (1.1 to 3.2) was solely from reducing logD and not by increasing potency. Compound (+/-)-**16** was hypothesized to be resistant to metabolic turnover in HLM due to low lipophilicity (logD < 3). From these two examples, the nitrogen-containing ring motif would have a low probability of delivery of a potent, high LipE compound.

Further modeling effort showed that the *trans* substitution of a cyclopentane core would provide a similar desired overlap to the pyrrolidine (structure overlay provided in the supporting material). Indeed, a change of the constraint to all-carbon resulted in improved binding and functional affinities as shown in racemic cyclopentyl variant (+/-)-**28** ($K_i$ = 0.022 μM, $K_b$ = 0.495 μM). Although, the logD is high (3.68), which may explain the higher $CL_{int}$ in HLM (25.5 mL/min/kg), the LipE is higher than (+/-)-**11** which has comparable lipophilicity and clearance. Therefore, from a lipophilic efficiency standpoint, the cyclopentane core of enantiomers **29** and **30** held higher promise as a lead in terms of its likelihood to retain potency when changes were made to reduce logD.

**Table 1**
Compound properties of *N*- and *C*-linked 5-membered cyclic analogs.

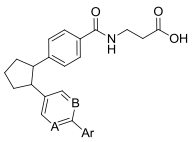



| | A | B | hGGR Binding $K_i$ (μM)[a] | hGGR cAMP $K_b$ (μM)[a] | logD[b] | MDCKII-LE Permeability (x$10^{-6}$ cm/sec) | HLM $Cl_{int,app}$ (mL/min/kg)[c] | LipE ($K_b$)[d] |
|---|---|---|---|---|---|---|---|---|
| **5** | - | - | 0.014 | 0.270 | 2.54 | 3.57 | <8.0 | 4.0 |
| (+/-)-**11** | $CH_2$ | N | 0.552 | 8.990 | 3.92 | 0.48 | 24.1 | 1.1 |
| (+/-)-**16** | CO | N | 0.757 | 29.90 | 1.31 | 1.23 | <8.0 | 3.2 |
| (+/-)-**28** | $CH_2$ | CH | 0.022 | 0.495 | 3.68 | ND[e] | 25.5 | 2.6 |
| **29**[f] | $CH_2$ | CH | 0.013 | 0.273 | ND[e] | 8.77 | 23.6 | 3.1 |
| **30**[g] | $CH_2$ | CH | 0.014 | 0.490 | 3.21 | ND[e] | <8.0 | 3.1 |

[a] Reported as a geometric mean of n ≥ 2 determinations.
[b] Measured shake flask LogD at pH 7.4
[c] Protocols for measuring half-lives in human liver microsomes (HLM) and subsequent scaling to blood clearance have been published (see Ref. 16)
[d] LipE = $pIC_{50}$ (or $pK_i$) - clogP (or logD or clogD)
[e] Not determined
[f] single enantiomer, not defined (opposite of compound **30**)
[g] single enantiomer, not defined (opposite of compound **29**)

The distal *para*-trifluoromethylbiphenyl and the central phenyl substituents in compound (+/-)-**28** served as starting points for incorporation of polarity. Replacement of the distal aryl ring with a trifluoromethylpyrazole group ((+/-)-*trans*-**31**) and placement of a nitrogen in the central phenyl ring of (+/-)-*trans*-**31** to yield compound (+/-)-*trans*-**32** decreased both lipophilicity and antagonist potency (Table 2). Since altering the distal ring seemed to have a smaller chance of success, we aimed for manipulation of the central ring. Previously, we have shown beneficial effects *via* addition of dimethyls *ortho* to the pyrazole. The dimethyls increase potency and have a minimal effect on lipophilicity presumably due to a change in the conformation of the terminal pyrazole.[8b] The logD of (+/-)-*trans*-**33** is similar to (+/-)-*trans*-**31** and the potency improved 3-fold leading to a LipE gain of 0.6. Unfortunately, the HLM turnover increased significantly to 132 mL/min/kg. Not surprisingly, the *cis* analogue, (+/-)-*cis*-**34**, was devoid of functional activity, as it did not overlay well with the binding conformation (structure overlay provided in the supporting material). In contrast, replacement of the central phenyl ring in **29** and **30** with a pyrimidine led to enantiomers (+)-*trans*-**26** and (-)-*trans*-**27** with a reduced logD value. Gratifyingly, they also retained the glucagon receptor antagonist potency observed with **29** and **30** with good LipE (3.5-3.6).

**Table 2**
Compound properties of *C*-linked 5-membered cyclic analogs with varied biphenyl substituents

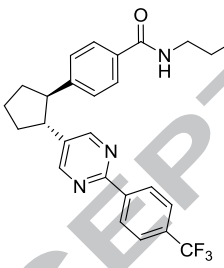


| | A | B | Ar | hGGR Binding $K_i$ (μM)[a] | hGGR cAMP $K_b$ (μM)[a] | logD[b] | MDCKII-LE Permeability (x$10^{-6}$ cm/sec) | HLM $Cl_{int,app}$ (mL/min/kg)[c] | LipE ($K_b$)[d] |
|---|---|---|---|---|---|---|---|---|---|
| (+/-)-*trans*-**31** | CH | CH | 4-$CF_3$-pyrazole | 0.301 | 3.40 | 2.33 | 5.94 | <8 | 3.1 |
| (+/-)-*trans*-**32** | CH | N | 4-$CF_3$-pyrazole | 0.620 | 6.83 | 1.70[e] | ND[f] | ND[f] | 3.5 |
| (+/-)-*trans*-**33** | CMe | CMe | 4-$CF_3$-pyrazole | 0.086 | 1.07 | 2.28 | 2.68 | 132 | 3.7 |
| (+/-)-*cis*-**34** | CMe | CMe | 4-$CF_3$-pyrazole | 0.319 | >8.72 | 2.17 | 7.48 | 187 | 2.9 |
| (+)-*trans*-**26**[g] | N | N | *p*-$CF_3$Ph | 0.014 | 1.21 | 2.34 | 6.41 | <8 | 3.6 |
| (-)-*trans*-**27**[h] | N | N | *p*-$CF_3$Ph | 0.042 | 1.46 | 2.34 | 4.85 | <8.4 | 3.5 |

[a] Reported as a geometric mean of n ≥ 2 determinations.
[b] Measured shake flask LogD at pH 7.4
[c] Protocols for measuring half-lives in human liver microsomes (HLM) and subsequent scaling to blood clearance have been published (see Ref. 16)
[d] LipE = $pIC_{50}$ (or $pK_i$) - clogP (or logD or clogD)
[e] calculated shake flask LogD
[f] Not determined
[g] $[\alpha]_D^{20}$ = +170.5 (*c* 0.2, MeOH); single enantiomer, not defined (opposite of compound **27**)
[h] $[\alpha]_D^{20}$ = -173.2 (*c* 0.4, MeOH); single enantiomer, not defined (opposite of compound **26**)

Both enantiomers (+)-*trans*-**26** and (-)-*trans*-**27** were resistant to oxidative metabolism in HLM, rat liver microsomes (RLM), and dog liver microsomes (DLM) (Table 3). Likewise, little to no metabolic turnover was observed upon incubation of (+)-*trans*-**26** and (-)-*trans*-**27** in cryopreserved hepatocytes from human, rat and dog.

**Table 3**
In vitro PK properties[a] for enamtiomers (+)-*trans*-**26** and (-)-*trans*-**27**



| | HhepG $Cl_{int,app}$ (μL/min/mill) | RLM $Cl_{int,app}$ (mL/min/kg) | RhepG $Cl_{int,app}$ μL/min/mill | DLM $Cl_{int,app}$ (mL/min/kg) | DhepG $Cl_{int,app}$ μL/min/mill |
|---|---|---|---|---|---|
| (+)-*trans*-**26** | 7.3 | <25.4 | 6.69 | <18.1 | <4.3 |
| (-)-*trans*-**27** | 7.2[b] | <25.4 | 7.39 | <18.1 | <4.3 |

[a] Reported as a geometric mean of n ≥ 2 determinations
[b] n = 1 determination

*In vivo* pharmacokinetics of (+)-*trans*-**26** and (-)-*trans*-**27** were also assessed in Wistar-Han rats following intravenous administration at 1 mg/kg (Table 4). Unfortunately, a disconnect was noted between the low predicted clearance from RLM and rat hepatocytes and the observed high plasma clearance for these compounds (in particular, compound (+)-*trans*-**26**). Upon reexamination of the pharmacokinetics in bile-duct cannulated rats, ~ 40-45% of unchanged dosed parent compounds were measured in rat bile, which implies that biliary excretion is a major contributor to the elimination of (+)-*trans*-**26** and (-)-*trans*-**27**. Interestingly, examination of the dog pharmacokinetics of (+)-*trans*-**26** and (-)-*trans*-**27** revealed significant differences in clearance for the individual enantiomers; compound (+)-*trans*-**26** showed higher clearance in dogs compared to (-)-*trans*-**27** in two experiments. Both enantiomers demonstrate little to no metabolic turnover in DLM and dog hepatocytes suggesting a lack of chiral bias in metabolic elimination. Likewise, metabolic profiling revealed only trace amounts of cyclopentyl ring oxidation (possibly mediated by cytochrome P450 enzymes) as a metabolic fate for both compounds. No metabolites derived from glucuronidation were observed in these studies. Considering that biliary excretion appears to be a major pathway for the clearance of (+)-*trans*-**26** and (-)-*trans*-**27** in rats, we speculate that the clearance differences in the dog possibly arise through an enantiospecific interaction of (+)-*trans*-**26** (relative to (-)-*trans*-**27**) with a transporter(s) responsible for biliary efflux in the dog.

**Table 4**
Rat and dog pharmacokinetic properties for enamtiomers (+)-*trans*-**26** and (-)-*trans*-**27**

| | (+)-*trans*-**26** | (-)-*trans*-**27** |
|---|---|---|
| **Rat PK[a]** | | |
| $t_{1/2}$ (h) | 0.424 | 1.23 |
| $V_{dss}$ (L/kg) | 0.899 | 0.885 |
| Cl (mL/min/kg) | 56.7 | 23.5 |
| **Rat BDC[b] PK[c]** | | |
| $t_{1/2}$ (h) | 3.21 | 3.33 |
| $V_{dss}$ (L/kg) | 2.84 | 0.973 |
| Cl (mL/min/kg) | 14.3 | 10.6 |
| % in bile | 41 | 46 |
| **Dog PK[d]** | | |
| $t_{1/2}$ (h) | 2.11 | 5.8 |
| $V_{dss}$ (L/kg) | 2.59 | 0.829 |
| Cl (mL/min/kg) | 25.8[e] | 2.7[f] |

[a] Wistar-Han rats dosed at 1 mg/kg iv.
[b] BDC (bile duct canulation)
[c] Wistar-Han rats dosed at 5 mg/kg iv; n = 3.
[d] Beagle dogs dosed at 0.5 mg/kg iv; n =2.
[e] Individual Cl values for each dog are 33.6 and 18.0.
[f] Individual Cl values for each dog are 4.1 and 1.3.

In conclusion, a pharmacophore model of potent glucagon antagonists guided the development of a new structural series with good physicochemical and pharmacokinetic properties. Through ring constraint, we hypothesized that we could lock the desired side chains to provide optimal interactions with the receptor. As a result, we found that both good properties and potency can be obtained in the cyclopentane core motif. These changes point to the possibility of further improving both potency and physicochemical properties for the glucagon receptor antagonist, yielding higher LipE and potentially better PK profiles *in vivo*. Finally, we have also identified an interesting disconnect between the *in vivo* PK of two enantiomers in dog that is worth further investigation.

**Supporting information**
See attached files.

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