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Design and synthesis of conformationally constrained tri-substituted ureas as potent antagonists of the human glucagon receptor

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Abstract—A series of conformationally constrained tri-substituted ureas were synthesized, and their potential as glucagon receptor antagonists was evaluated. This effort resulted in the identification of compound **4a**, which had a binding IC_{50} of 4.0 nM and was shown to reduce blood glucose levels at 3 mg/kg in glucagon-challenged mice containing a humanized glucagon receptor. Compound **4a** was efficacious in correcting hyperglycemia induced by a high fat diet in transgenic mice at an oral dose as low as 3 mg/kg.

Glucagon is a 29-amino acid peptide hormone secreted by the α-cells of the pancreatic islets. In the liver, glucagon binding to its G protein coupled receptor (GPCR) stimulates gluconeogenesis and glycogenolysis that result in increased plasma glucose levels. Together with insulin, that acts to decrease plasma glucose levels, glucagon plays a pivotal role in maintaining blood glucose homeostasis.[1] Type 2 diabetics are insulin resistant and show elevated postprandial glucagon levels resulting in impaired glucose homeostasis.[2] It has been demonstrated that intravenous administration of potent peptide glucagon antagonists, anti-glucagon antibodies, and more recently, antisense oligonucleotides against the glucagon receptor significantly decreases blood glucose levels in diabetic animal models.[3] These studies suggest that a glucagon receptor antagonist could decrease hepatic glucose output and improve glucose control in diabetic patients. It has also been shown that a small molecule glucagon receptor antagonist can effectively block the glucose response to a glucagon challenge in healthy humans.[4] Therefore, glucagon receptor antagonism is being pursued as a promising approach to treat type 2 diabetes.

Several classes of small molecule antagonists of the human glucagon receptor have been disclosed.[5,6] Among them, a class of tri-substituted ureas was reported as small molecule antagonists of the glucagon receptor.[6] These are exemplified by compounds **1a** and **1b** that showed a potent binding IC_{50} and moderate functional activity under our assay conditions (Fig. 1).[7] These antagonists have a characteristic acid moiety, such as β-alanine or amino tetrazole, attached to the benzyl group in the *para* position. As part of our investigation of conformationally constrained analogues of **1**,[8] the benzylic carbon was cyclized to either the carbonyl oxygen or the phenyl ring (Fig. 1). Analogues in which the benzylic position was attached to the carbonyl oxygen (**2** and **3**) were moderately potent antagonists of glucagon receptor (binding $IC_{50} \sim 130$–630 nM).[9] In contrast,

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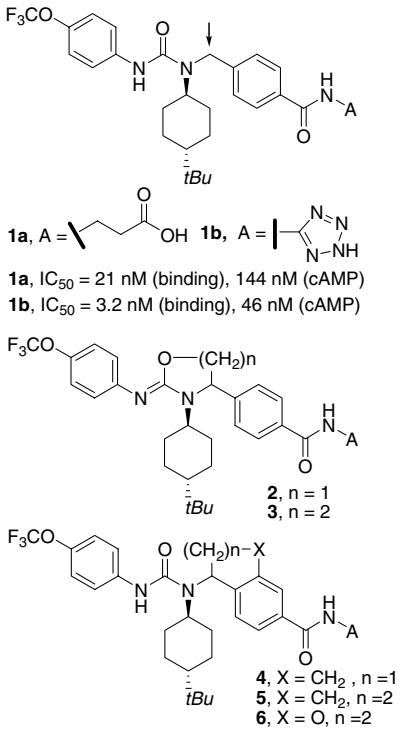
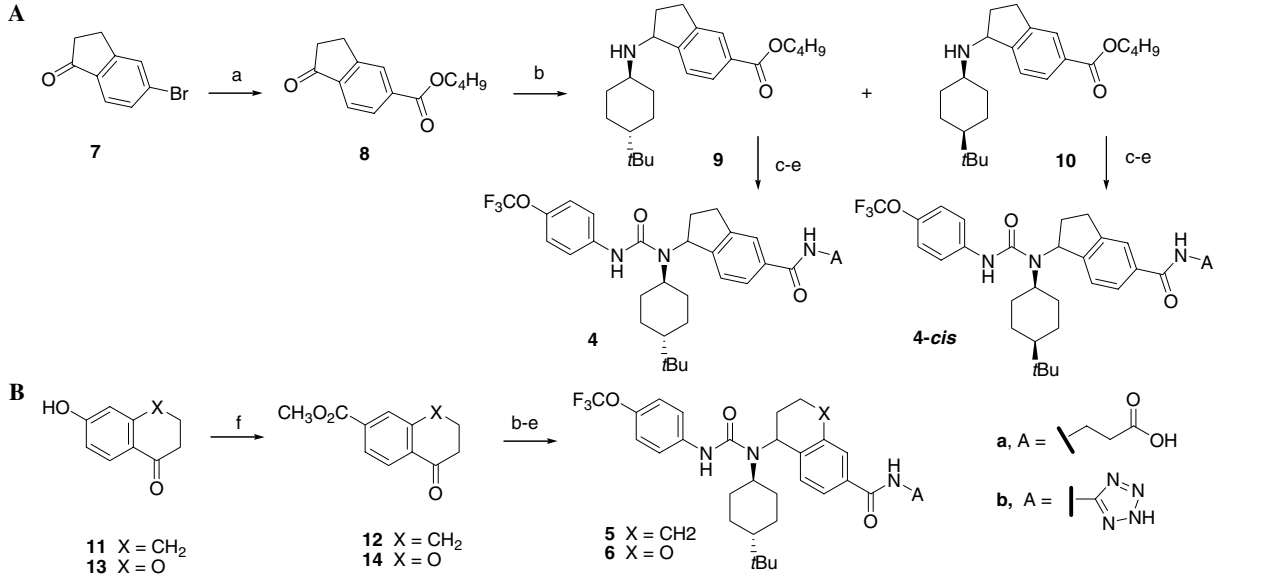


Figure 1. Tri-substituted ureas (**1a** and **1b**) are glucagon receptor antagonists. Arrow indicates the position for conformation constraint by tethering to the carbonyl oxygen or the adjacent phenyl ring.

significantly improved in vitro results were seen for analogues in which the benzylic position was attached to the phenyl ring (**4**–**6**, binding IC_{50}'s $\sim$ 0.5–5 nM). Here we report the synthesis of analogues in this series and their biological activity as glucagon receptor antagonists.

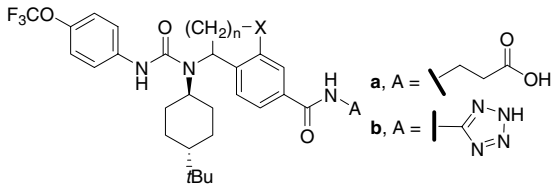
Scheme 1 describes the synthesis of antagonists **4**–**6**. Ketone esters **8**, **12**, and **14** were prepared by carbonylation of either 5-bromo-1-indanone **7** or the corresponding triflates of phenols **11** and **13**.[10] Reductive amination of ketones **8**, **12**, and **14** with 4-*tert*-butylcyclohexylamine using $NaBH_4$ and catalytic $Ti(O\text{-}iPr)_4$ gave 4:1 *trans*/*cis* mixtures of racemic secondary amines, which can be easily separated by silica gel chromatography. The *trans* amines were further treated with 4-trifluoromethoxyphenyl isocyanate to give tri-substituted ureas. The compounds were resolved at this stage on chiral HPLC to give optically pure enantiomers. Saponification, followed by amide coupling with the β-alanine derivatives or 5-amino tetrazole and if necessary deprotection, gave the final compounds **4**–**6**. For comparison of the effect of stereochemistry on biological activity, amine **10** was derivatized to give the final urea **4**-*cis* using analogous conditions.

Biological activity of compounds **4**–**6** was initially measured by the inhibition of binding of [^{125}I]glucagon to the human glucagon receptor expressed in CHO cell membranes. Functional activity was measured by the inhibition of glucagon-induced cAMP accumulation in hGCGR transfected CHO cells (cAMP IC_{50}, Table 1).[7] Significant differentiation in activities was observed between enantiomers, and the potent enantiomers were comparable to or showed improvement in activity over the parent compounds **1a** and **1b**. The binding activity of enantiomers E1 (prepared from the first-eluting urea enantiomers) ranged from 0.5 to 5 nM and was up to 800-fold more potent than that of enantiomers E2. Enantiomers E1 were also more potent functional antagonists. Consistent with data on compounds **1a** and **1b**, amino tetrazole derivatives **4b**, **5b**, and **6b** were more potent than the corresponding β-alanine deriva-



Scheme 1. Reagents and conditions: (a) CO, $PdCl_2(PPh_3)_2$, DIEA, *n*-BuOH, 115 °C (79%); (b) 4-*tert*-butylcyclohexylamine, $Ti(O\text{-}iPr)_4$, $NaBH_4$, EtOH, rt (65% *trans*, 16% *cis*); (c) 4-(trifluoromethoxy)phenyl isocyanate, THF, 0 °C (91%); (d) chiral HPLC (ChiralPak AD column, 10–15% IPA in *n*-Heptane); (e) 1—LiOH, H_2O/THF/MeOH; 2—ANH_2, HOBt, EDC, DIEA, DMF, rt; 3—1:1 TFA/CH_2Cl_2, rt (60–84%); (f) 1—Tf_2O, Et_3N, DMAP, CH_2Cl_2, −78 °C to rt (61–93%); 2—CO, $Pd(OAc)_2$, dppf, Et_3N, DMF, MeOH, 50 °C (85–98%).

Table 1. Binding and functional activity (IC_{50}) of the ring constrained urea antagonists **4–6** of the human glucagon receptor and the related human GIP receptor



Compound	*n*	X	Binding IC_{50}, nM (*n*) E1/E2	cAMP IC_{50}, nM (*n*) E1/E2	hGIP cAMP IC_{50}, nM (*n*) E1
4a	1	CH_2	4.1 ± 1.1 (2)/1085	33 ± 21 (8)/489	132 ± 38 (4)
4b	1	CH_2	2.31 ± 0.03 (2)/359	15/2888	296
4a-*cis*	1	CH_2	3.8 ± 1.1 (2)/3384	46/ND[a]	603
4b-*cis*	1	CH_2	1.01 ± 0.01 (2)/216	18 ± 9 (3)/795	435
5a	2	CH_2	3.4 ± 1.3 (2)/340	54 ± 23 (2)/2173	171
5b	2	CH_2	0.5/148	11 ± 6 (2)/1052	178 ± 123 (2)
6a	2	O	4.8/218	44 ± 17 (3)/1013	217 ± 80 (2)
6b	2	O	0.5/76	39 ± 22 (4)/1454	117 ± 54 (2)

All compounds were tested as pure enantiomers. E1, compounds **4–6** prepared from the first to elute urea enantiomers; E2, compounds **4–6** prepared from the second to elute urea enantiomers.
[a] Not determined.

tives **4a**, **5a**, and **6a**. The most potent analogue, E1 of **5b**, exhibited a binding IC_{50} of 0.5 nM and a functional IC_{50} of 11 nM, which was ca. 4-fold more potent than the parent compound **1b**.

Activity of compounds **4–6** toward the related human GIP and GLP-1 receptors was also examined.[11] As the primary action of both GIP and GLP-1 is the stimulation of glucose-dependent insulin secretion, antagonism of these receptors should be minimized. Compounds **4–6** showed little activity against the hGLP-1 receptor, with functional IC_{50}'s > 10 μM in the cAMP accumulation assay. However, they all had potent hGIP activity (cAMP IC_{50}'s = 0.1–0.6 μM). Compounds (**5** and **6**) from the 6-membered ring series showed equally potent activity against hGIP. In the 5-membered ring series, compound **4a** with a β-alanine side chain has potent hGIP activity, while compound **4b** with a tetrazole was marginally less potent against hGIP. The stereochemistry of the *tert*-butylcyclohexyl ring has some influence on the selectivity against hGIP receptor. Compound **4a**-*cis* was equally potent against glucagon receptor as the *trans* compound **4a**, but it displayed slightly better selectivity against hGIP than **4a**. Since hGIP activity of all these compounds was similar, further SAR studies focused on the more easily available *trans* isomer of the 5-membered ring series.

Analogues **15–18** (Fig. 2) which have the carboxylic acid or amino tetrazole at either *meta*-position to the benzylic urea moiety were also evaluated. They were considerably less active (binding IC_{50}'s of 200–1200 nM) as compared to the compounds **4** which have the acid moieties at the *para*-position to the benzylic urea.

We then attempted to improve the potency of compounds **4a** and **4b** by varying the hydrophobic substituents on the internal nitrogen of the urea (Table 2).[12] Replacement of the *tert*-butyl-cyclohexyl with 4-CF_3O-

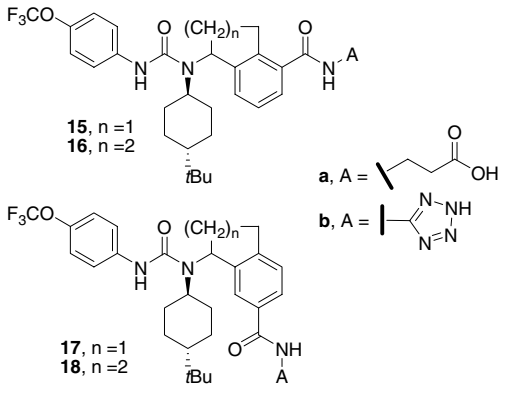
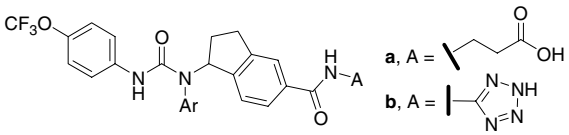


Figure 2. Analogues with the acid side chain at different position on the phenyl ring.

phenyl (**19**) or 4-cyclohexyl-phenyl group (**20**) on the tertiary nitrogen of the urea both led to significant decreases in both binding and cAMP activity. This suggested that *tert*-butyl-cyclohexyl group was preferred on the internal nitrogen over the substituted aromatic rings in the ring-constrained urea.

Some exploration of factors influencing the selectivity over hGIP receptor of compound **4** was investigated through removal of the external nitrogen of the urea (Fig. 3 and Table 3).[13] Replacement of the external nitrogen with a methylene group (**21a–21b**) resulted in loss of activity against the glucagon receptor, while the selectivity over hGIP did not improve. Directly attaching the phenyl moiety to the central carbonyl group as in **22a–22b** also led to substantial loss of activity against glucagon receptor. Further exploration through replacement of the 4-trifluorophenyl group with 3,5-di-substituted phenyl groups (**23–24**) improved selectivity over hGIP. 3,5-Dichlorophenyl derivatives **23a** and **23b** not only retained good in vitro potency but also had

Table 2. Binding and functional activity (IC_{50}) of the ring constrained urea antagonists **19**–**20** of the human glucagon receptor



Compound	Ar	Binding IC_{50}, nM (*n*)	cAMP IC_{50}, nM (*n*)
19a	4-CF_3O-Ph	820	Inactive
19b	4-CF_3O-Ph	62	3434
20a	4-Cyclohexyl-Ph	68 ± 32 (2)	189 ± 28 (2)
20b	4-Cyclohexyl-Ph	22 ± 8 (2)	85 ± 35 (3)

All compounds were tested as pure enantiomers. Data shown are from the more potent enantiomers.

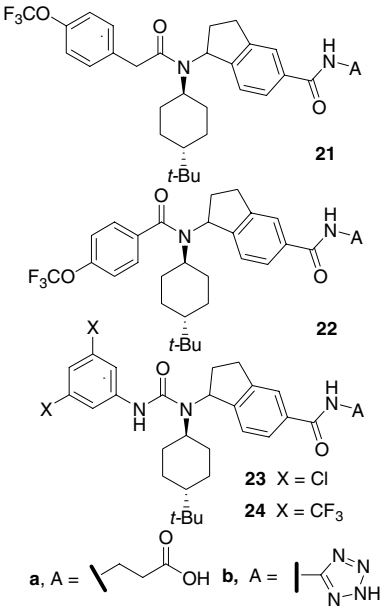


Figure 3. Analogues in which one of the urea nitrogens was either replaced by a methylene group (**21**) or deleted (**22**).

excellent functional selectivity (>100- and >500-fold, respectively) over hGIP.

Having identified potent and selective glucagon receptor antagonists, we wanted to evaluate the pharmacokinetic profiles of these ring constrained compounds **4**–**6** (Table 4).[14] Carboxylic acids **4a**, **5a**, and **6a** displayed similar pharmacokinetic properties in mice, showing low clearance in vivo, half-life of 2–6 h, and moderate bioavailability. Interestingly, the tetrazole derivative **5b** was markedly different. It had a very low AUC and poor oral bioavailability.

The antagonists **4**–**6a**, **4**–**6b**, and **23a** were tested for their ability to block glucagon-induced hyperglycemic response in vivo using transgenic mice that exclusively express a functional human glucagon receptor.[15] Oral administration of the antagonists was followed 60 min later by an intraperitoneal injection of glucagon. All compounds inhibited the hyperglycemic response at 30.0 mg/kg in 24 min except compound **5b**, most likely due to its poor pharmacokinetic properties. Compound **4a** was selected for a dose titration in the assay, and a

Table 3. Binding and functional activity (IC_{50}) of the ring constrained urea antagonists **21**–**24** of the human glucagon receptor

Compound	Binding IC_{50}, nM (*n*)	cAMP IC_{50}, nM (*n*)	GIP cAMP IC_{50}, nM (*n*)
21a	18 ± 10 (3)	500 ± 278 (3)	286
21b	4.8 ± 3.1 (3)	67 ± 12 (2)	118
22a	130 ± 21 (2)	2778 ± 897 (2)	ND[a]
22b	49 ± 31 (2)	1467 ± 275 (2)	610
23a	10 ± 2 (2)	11	1279
23b	4.9 ± 0.2 (2)	5.9	3525
24a	28 ± 13 (2)	46	2023
24b	7.6 ± 3.0 (2)	61	4256

All compounds were tested as pure enantiomers. Data shown are from the more potent enantiomers.
[a] Not determined.

Table 4. Pharmacokinetic profiles of selected human glucagon receptor antagonists dosed in mice (*n* = 3 mice/route of administration)[a]

Compound	CLp (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	AUC_N (PO) (μM h/dose)	C_{max} (μM)	*F*%
4a	9	1.6	5.6	0.56	0.13	17
5a	9	0.9	2.5	0.35	0.09	10
5b	6.4	1.4	8.0	0.09	0.04	1.4
6a	13	1.4	3.4	0.34	0.06	15

[a] Compounds were dosed at 1.0 mpk IV and 2.0 mpk PO formulated with a 5:10:85 mixture of DMSO, Tween 80, and water.

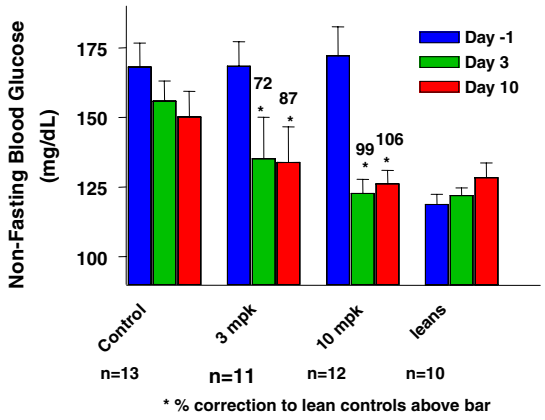


Figure 4. Effect of administration of compound **4a** on non-fasting blood glucose in the hGCGR mice which have been fed a high fat diet to induce moderate hyperglycemia.

significant response was observed at doses as low as 3 mg/kg. The glucose corrections to vehicle controls were 51%, 59%, and 122% at 3, 10, and 30 mg/kg, respectively.

Finally, compound **4a** was evaluated for its ability to lower non-fasting blood glucose in transgenic mice which have been placed on a high fat diet for 13 weeks to induce moderate hyperglycemia.[16] The initial glucose levels were about 170 mg/dL in the diabetic mice and about 110 mg/dL in the lean mice. Administration of compound **4a** as an admixture in the chow to the diabetic mice gave significant correction of the glucose levels to lean mouse control levels at 3 mg/kg by day 3 (Fig. 4). Most notable, full glucose correction to lean levels was achieved with 10 mg/kg by day 3 and maintained out to day 10.

In summary, we have discovered a series of structurally novel compounds, which are potent and selective glucagon receptor antagonists. For example, one of the most potent compounds **23b** has an IC_{50} of 5.9 nM in glucagon-stimulated cAMP accumulation assay with >500-fold selectivity over hGIP. Significantly, one representative compound **4a** from this series has an acceptable pharmacokinetic profile in the mice and suppressed a glucagon-stimulated increase of plasma glucose levels in transgenic mice. In the same transgenic mice compound **4a** was also efficacious in correcting hyperglycemia induced by a high fat diet at doses as low as 3.0 mg/kg.

These data reaffirm the findings that small molecule antagonists of the human glucagon receptor may have the potential to control hepatic glucose production which is exacerbated in type II diabetics. Further work on related series of compounds from these laboratories will be reported in the near future.

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12. Representative analogues **19**–**22** were prepared using similar conditions described for compounds **4**–**6** except that reductive amination between ketone **8** and 4-CF_3O-aniline or 4-cyclohexyl-aniline was affected by decaborane.
13. Compounds **25**–**27** were prepared through acylation of intermediate **9**, which was resolved on chiral HPLC as the *t*-BOC-protected carbamate.
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