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Discovery of novel, potent, and orally active spiro-urea human glucagon receptor antagonists

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Dedicated to the loving memory of a pioneering chemist, inspiring mentor, and generous friend, Prof. Orville L. Chapman.

Abstract—A novel class of spiro-ureas has been discovered as potent human glucagon receptor antagonists in both binding and functional assays. Preliminary studies have revealed that compound **15** is an orally active human glucagon receptor antagonist in a transgenic murine pharmacodynamic model at 10 and 30 mpk. Compound **15** is orally bioavailable in several preclinical species and shows selectivity toward cardiac ion channels and other family B receptors, such as hGIP1 and hGLP.

Type 2 diabetes mellitus (T2DM) is a complex disease[1] with increasing prevalence and cost, especially in developed countries.[2] Although numerous therapies are available for the treatment of diabetes, all of them are associated with strong side effects. Therefore, many new drug targets are still being actively pursued.[3,4] Glucagon is a 29 AA peptide hormone, which acts through the glucagon receptor (GCGR) to stimulate gluconeogenesis and glycogenolysis, thereby counteracting the role of insulin in the regulation of glucose homeostasis. Since hyperglycemia and, paradoxically, increased glucagon levels are both characteristic of T2DM, antagonism of the hGCGR is a logical target for the treatment of T2DM.[5–7] A recent report on the blockade of glucagon-induced hyperglycemia in human subjects with a small molecule hGCGR antagonist has undoubtedly stimulated more interest in this area.[8]

Recently, trisubstituted ureas, such as **1**, were reported to be human glucagon receptor antagonists.[9] We wished to explore spiro-cyclic variants of these trisubstituted ureas as potential novel human glucagon receptor antagonists.[10,11]

AM1 computer modeling of compound **1** with the three peripheral substituents truncated for ease of computation showed conformers A and B to have very similar energies.[12] A quick visual inspection revealed that a slightly lower energy conformer B was well-suited for the formation of a spiro-urea moiety, which appeared to preserve the orientation of the three substituents connected to the original acyclic urea core. AM1 modeling confirmed this conjecture (Fig. 1). It showed that a [6.5] spiro-urea overlapped very well with the acyclic urea having the three carbon atoms marked with arrows

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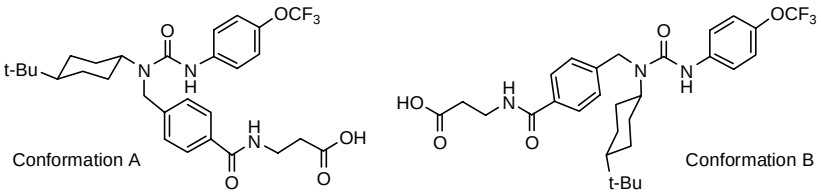

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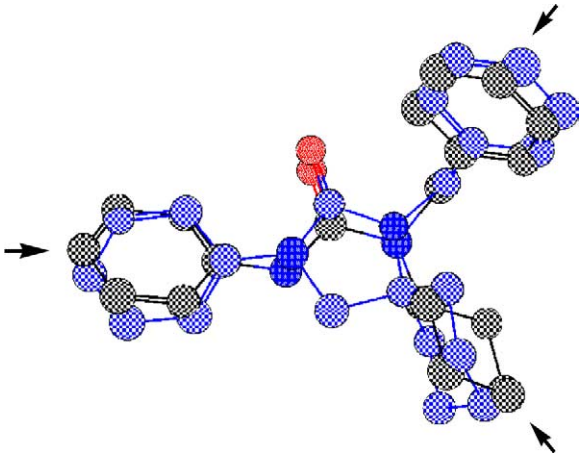


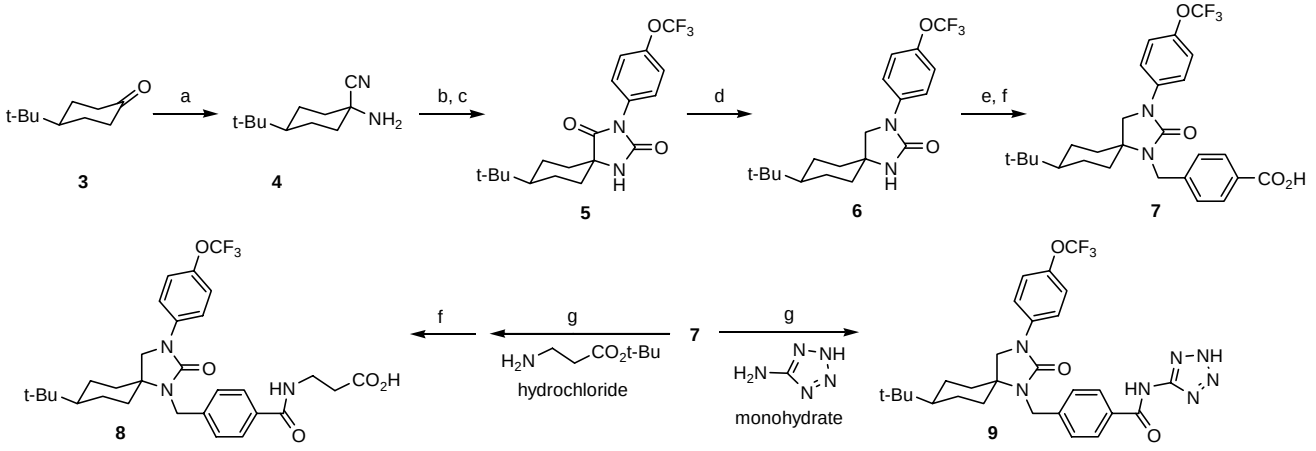
Figure 1. Overlay of truncated compound **1** (gray) with [6.5] spiro-urea replacement (blue) from AM1 calculations (red = O, dark blue = N, others = C). Hydrogen atoms were omitted in this drawing for clarity.

in **1** placed within 0.6 Å of the corresponding atoms in the spiro-urea replacement. The carbonyl oxygen atoms in the two compounds were also only 0.5 Å apart. Similar results were obtained with a [6.6] spiro-urea replacement having an ethylene moiety bridging the urea NH and the cyclohexane in **1**.

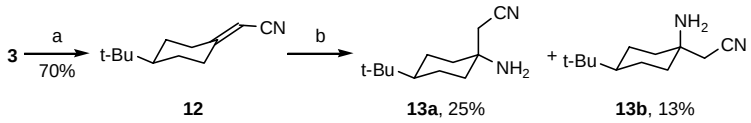
These computational results encouraged us to prepare and test these spiro-ureas as potential novel glucagon receptor antagonists. The synthesis of the [6.5] spiro-urea is shown in Scheme 1 with the preparation of compounds **8** and **9**. The cis isomer of the aminonitrile **4** from a Strecker reaction[13] of ketone **3** was added to an isocyanate, cyclized in situ under basic conditions to give a 5-imino-imidazolidin-2-one intermediate, and hydrolyzed to give the hydantoin **5**.[14] The 4-carbonyl group in **5** was selectively reduced to a methylene (**6**) using $LiAlH_4$–$AlCl_3$.[15] Acid **7** was then obtained from **6** by alkylation of the urea NH and deprotection of the *tert*-butyl ester. Finally, standard coupling conditions were used to yield amino tetrazole **9** or, following deprotection, β-alanine **8**. Hydantoin analogs **10** and **11** were prepared from intermediate **5** in a similar manner.[16]

The preparation of the corresponding [6.6] spiro compounds **14**–**17** was also performed, as outlined in Scheme 1, except in this case the required aminonitrile **13a** was prepared by a Horner–Emmons reaction[17], followed by 1,4-addition of ammonia[18] (Scheme 2). The two isomers of **13** were separated on silica gel and their identities were established by NMR using homonuclear decoupling and NOE spectroscopy.

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 **2** (Table 1). The binding IC_{50} values were measured from 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

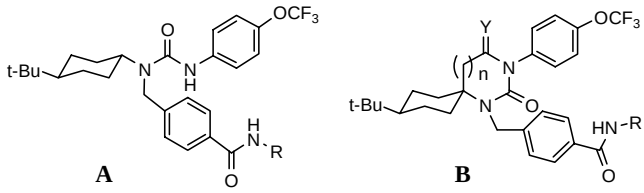


Scheme 1. Reagents and conditions: (a) KCN, NH_4Cl, 1:1 MeOH and H_2O, rt, 83%; (b) (i) 4-CF_3O–C_6H_4–NCO, benzene, rt, 8 h; (ii) NaH, 30 min, rt, 81%; (c) 1:2 1.5 M aq HCl and ethanol, reflux, 3 h, 94%; (d) $LiAlH_4$, $AlCl_3$, ether, rt, 85%; (e) *tert*-butyl 4-(bromomethyl)benzoate, NaH, DMF, rt, 56%; (f) 2:5 (v/v) TFA and DCM, rt, 20 min, 66%; (g) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole hydrate (HOBt), diisopropylethylamine (DIEA), DMF.



Scheme 2. Reagents and conditions: (a) diethyl (cyanomethyl)phosphonate, *n*-BuLi in hexanes, THF, −78 °C 3 h, warm to rt; (b) 29% aq NH_3, MeOH, 100 °C, autoclave, 25 h.

Table 1. Binding and functional activity (IC_{50}) of human glucagon receptor antagonists



Compound	Class	R	Y	*n*	Binding IC_{50}, nM (*n*)	cAMP IC_{50}, nM (*n*)
1	A	$-CH_2CH_2CO_2H$	—	—	21 ± 7 (5)	144 ± 88 (4)
2	A	Tetrazol-5-yl	—	—	3.2 ± 1.7 (6)	46 ± 14 (5)
8	B	$-CH_2CH_2CO_2H$	H_2	0	336 ± 282 (5)	459
9	B	Tetrazol-5-yl	H_2	0	105 ± 56 (5)	193 ± 145 (3)
10	B	$-CH_2CH_2CO_2H$	=O	0	856	ND[a]
11	B	Tetrazol-5-yl	=O	0	631	ND[a]
14	B	$-CH_2CH_2CO_2H$	H_2	1	182 ± 129 (6)	282 ± 85 (3)
15	B	Tetrazol-5-yl	H_2	1	34 ± 32 (10)	92 ± 29 (13)
16	B	$-CH_2CH_2CO_2H$	=O	1	1691	ND[a]
17	B	Tetrazol-5-yl	=O	1	1965	ND[a]
18	B	$-CH_2CH_2CO_2Et$	H_2	0	23% inh. at 100 μM	ND[a]
19	B	$-CH_2CH_2CONH_2$	H_2	0	13% inh. at 100 μM	ND[a]
20	B	$-(R)$-$CH_2CHOHCO_2H$	H_2	1	303 ± 352 (2)	ND[a]
21	B	(Tetrazol-5-yl) methyl	H_2	1	92 ± 36 (2)	1975 ± 673 (2)
7	—	—	—	—	68% inh. at 100 μM	ND[a]

[a] Not determined.

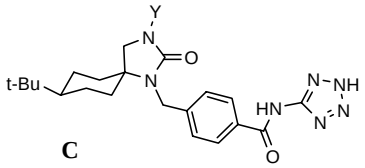
hGCGR-transfected CHO cells was also measured (cAMP IC_{50}).[19]

In our hands, the acyclic ureas **1** and **2** were very potent binders to the hGCGR (3–21 nM), but exhibited a somewhat diminished functional antagonism (Table 1). The most potent of the spiro-cyclic analogs prepared were the [6.6] spiro-ureas, **14** and **15**, while being up to 10-fold less potent than **1** and **2** in the binding assay, only showed a slightly diminished functional inhibition of the receptor (~2-fold). The [6.5] analogs **8** and **9** were about 4-fold less potent than their acyclic analogs in the cAMP assay. Containing an extra carbonyl group, the hydantoins, **10** and **11**, and dihydropyrimidine-2,4-diones, **16** and **17**, were only weak glucagon receptor antagonists (binding IC_{50} >600 nM). An acidic residue seems to be required for binding activity since ester **18** and amide **19** were both inactive up to 100 μM. Even minor modifications to β-alanine or amino tetrazole side chains of **8** and **9** were not well-tolerated. For example, hydroxy-acid **20** lost a further 2-fold in binding over **14**. Aminomethyl tetrazole **21**, while being potent in the binding assay, only showed weak functional antagonism (cAMP IC_{50} ~2000 nM). The truncated acid **7** also showed only a very weak binding activity.

Compound **15** was also tested for activity at the GCGRs of the rhesus monkey, dog, rat, and mouse. Using comparable functional assays as that used for hGCGR, **15** was found to be only 1.5- to 4-fold less potent at these receptors than at the hGCGR (cAMP IC_{50} = 159 ± 46 (2), 203 ± 66 (3), 307 ± 65 (4), and 398 nM, respectively).

These observations prompted us to focus further SAR studies on the spiro-urea series (Table 2). Initial efforts centered on the less potent [6.5] spiro-urea series since its chemistry was more accessible than the [6.6] counterparts. A variety of changes to the aryl group were explored with a tetrazole or β-alanine side chain. The parent phenyl compound **22** was much less active than **9**. On the other hand, cyclohexyl is a reasonable replacement for phenyl itself (**22** and **23**). Smaller aliphatic groups, such as *tert*-butyl and *i*-propyl, were also not well-tolerated (**24** and **25**). Among the three chlorophenyl isomers (**26**, **27**, and **29**), the *meta* isomer **27** was the most active and was comparable to the 4-trifluoromethoxy derivative **9**. Addition of a second *meta* substituent to give 3,5-dichlorophenyl derivative **31** resulted in a 3-fold boost in activity compared to **27**. While the β-alanine derivatives were usually less active than the tetrazole analogs, in the 3,5-dichloro series the acid derivative **32** was a potent binder to the hGCGR (IC_{50} = 43 nM), although it shifted 10-fold in the cAMP assay. Other replacements for the 4-trifluoromethoxyphenyl group, which showed similar in vitro activities,

Table 2. Binding and functional activity (IC_{50}) of the human glucagon receptor antagonists



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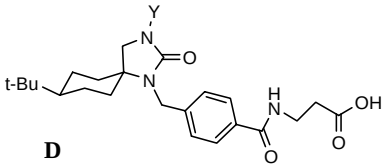

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Compound	Class	Y	Binding IC_{50}, nM (*n*)	cAMP IC_{50}, nM (*n*)
22	C	Ph	894	ND[a]
23	C	Cyclohexyl	700	ND[a]
24	C	*t*-Bu	1663	ND[a]
25	C	*i*-Pr	2556	ND[a]
26	C	2-ClPh	739	ND[a]
27	C	3-ClPh	94 ± 42 (2)	353 ± 153 (2)
28	D	3-ClPh	351	601
29	C	4-ClPh	116 ± 55 (2)	446 ± 52 (2)
30	C	2,4-Cl_2Ph	286 ± 209 (2)	1416 ± 188 (2)
31	C	3,5-Cl_2Ph	36 ± 26 (2)	116 ± 51 (2)
32	D	3,5-Cl_2Ph	43 ± 23 (3)	423 ± 41 (2)
33	C	4-FPh	178 ± 6 (2)	611 ± 405 (2)
34	C	4-CF_3Ph	135 ± 111 (2)	113 ± 72 (2)
35	C	4-CH_3Ph	427	ND[a]
36	C	4-BrPh	156 ± 39 (2)	655 ± 122 (2)
37	C	(±)-1-(4-BrPh)Et	30 ± 5 (2)	164 ± 89 (2)
38	C	4-CH_3SO_2Ph	407	ND[a]
39	C	3-CF_3OPh	33 ± 15 (2)	234 ± 89 (2)
40	D	3-CF_3OPh	60 ± 22 (2)	515 ± 69 (2)
41	C	3,4-F_2Ph	65 ± 7 (2)	227 ± 25 (2)

[a] Not determined.

Table 3. Pharmacokinetic profiles of human glucagon receptor antagonists in the mouse[a]

Compound	Cl_p (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	$AUCN_{po}$ (μM × h/dose)	C_{max} (μM)	%F
9	8.1	2.6	5.5	0.99	0.20	27
15	24	4.3	3.5	0.25	0.07	20
21	40	7.3	4.7	ND[b]	0.01	ND[b]
32	34	3.6	2.3	0.25	0.11	27

[a] Compounds were 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] Not determined due to plasma concentration below LOQ of 0.0083 μM at most time points in 3/3 animals.

include 4-trifluoromethylphenyl (**34**), (±)-1-(4-bromophenyl)ethyl (**37**), 3-trifluoromethoxyphenyl (**39**), and 3,4-difluorophenyl (**41**). More polar substituents, such as methylsulfonyl (**38**), reduced potency significantly. Replacing the 4-*tert*-butylcyclohex-1-yl in **9** with 1-benzylpiperidin-4-yl moiety afforded **42**, which exhibited a similar binding affinity to **9** (IC_{50} = 97 ± 7 nM), but was less potent in the functional assay having an IC_{50} of 892 nM.

Pharmacokinetic profiles of selected compounds were obtained in the mouse (Table 3). Tetrazole analogues **9** and **15** and β-alanine analogue **32** exhibited reasonable 2.3–5.5 h half-lives and >20% oral bioavailability. The oral exposure of aminomethyl tetrazole **21** was found to be much lower.

Compounds **8**, **9**, **14**, **15**, **21**, **32**, and **34** were evaluated in vivo in a pharmacodynamic (PD) assay for their ability to block glucagon-induced glucose excursion at 30 mpk using transgenic mice expressing only a functional human glucagon receptor (hGCGR mice). The full characterization of these mice and their response to known antagonists have been reported elsewhere.[20] Oral administration of the antagonists (nine 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 **9**, **14**, **15**, and **32** fully suppressed glucose excursion following the glucagon challenge at 30 mpk. Compounds **8**, **21**, and **34** were not effective.

Based on its in vitro potency and PD activity, compound **15** was selected for further evaluation including off-target profile, titration in the pharmacodynamic assay, evaluation in an in vivo receptor occupancy assay,[20b] and measurement of pharmacokinetic properties in other species. Among the related family B GPCRs, compound **15** showed modest functional selectivity against hGIP and hGLP1 (cAMP IC_{50} = 0.43 ± 0.60 μM and 4.6 ± 1.9 μM, respectively). Compound **15** exhibited only mediocre binding to the hERG K^+ channel (IC_{50} = 5.4 ± 1.5 μM) and did not bind to the human Na^+ and rabbit DLZ sensitive Ca^{2+}

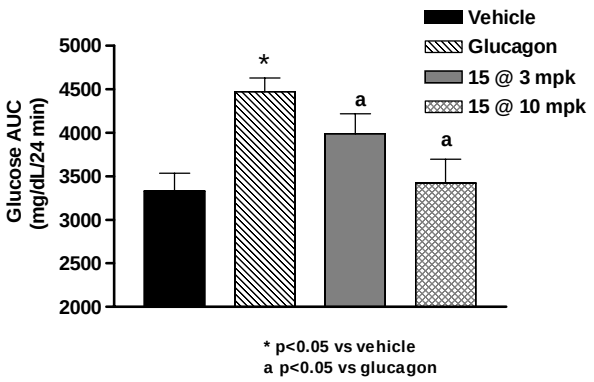

Figure 2. Titration of compound **15** in pharmacodynamic assay in the hGCGR mouse.

Table 4. Concentration of **15** in the plasma of the hGCGR mouse during pharmacodynamic assay ($n = 3$ for each group)

Minutes post dose	3 mpk dose (μM)	10 mpk dose (μM)
45	0.180 ± 0.074	0.272 ± 0.139
120	0.123 ± 0.066	0.394 ± 0.211

channels at concentrations up to 10 μM. The results from dose titration of **15** in the PD assay using an identical protocol are shown in Figure 2. It was fully active in suppressing hyperglycemia following a glucagon challenge at 10 mpk and partially active at 3 mpk. The plasma concentrations of **15** during the PD experiment are given in Table 4. This compound also showed good duration of action, as it was fully active in this assay when dosed orally at 30 mpk 5 h prior to the glucagon challenge (data not shown). Drug levels in plasma at the time of glucagon administration were 0.503 ± 0.198 μM.

In vivo receptor occupancy of **15** in the hGCGR mice was measured at 1 h post oral dose to be 52.2 ± 2.9, 39.4 ± 4.8, and 25.7 ± 4.0 % at 10, 3, and 1 mpk, respectively.[20b] Therefore, receptor occupancy of 40–50% correlates with observed inhibition of glucagon-induced hyperglycemia in the PD model. Finally, the pharmacokinetic profile of **15** was evaluated in additional preclinical species (Table 5). In the rat and dog, the half-life was somewhat shorter than in the mouse and oral bioavailability ranged from 13% to 38 %.

Thus, a novel class of spiro-ureas been discovered as potent human glucagon receptor antagonists. SAR studies have identified compound **15** as a potent human glucagon receptor antagonist with an acceptable pharmacokinetic profile in several preclinical species. Spiro-urea **15** showed good oral activity in a pharmacodynamic assay using transgenic mice by blocking the effect of glucagon-induced hyperglycemia at doses which correlated with partial blockade of the receptor in an in vivo receptor occupancy assay. Further studies in this and related series will be reported in due course.

Acknowledgments

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Table 5. Pharmacokinetic profiles of human glucagon receptor antagonist **15** in additional animal species

Species	Cl_p (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	$AUCN_{po}$ (μM × h/dose)	C_{max} (μM)	%F
Rat[a]	15	1.3	1.8	0.26	0.15	13
hGCGR mouse[a]	16	4.2	4.6	0.47	0.13	24
Dog[b]	6.8	0.48	2.4	1.6	0.66	38

[a] Dosed at 1.0 mpk IV and 2.0 mpk PO.
[b] Dosed at 0.5 mpk IV and 1.0 mpk PO.

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16. All intermediates and final products described here showed expected ^{1}H NMR and LC-MS. In addition, ^{1}H, COSY, and NOESY spectra of the alkylation product of **5** with 4-bromobenzyl bromide showed it had the desired stereochemistry on the cyclohexane ring. Only one isomer of **5** was observed when cyclization was conducted at room temperature. At higher temperatures, a minor amount of another isomer of **5** was isolated in some cases. For example, detailed NMR studies of both isomers of 2-chlorophenyl analogues of **6** confirmed our structure assignments. Finally, a single crystal X-ray structure of **9** confirmed its identity. We thank Ms. Nancy Tsou for the X-ray structure determination.
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