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Biaryl amide glucagon receptor antagonists

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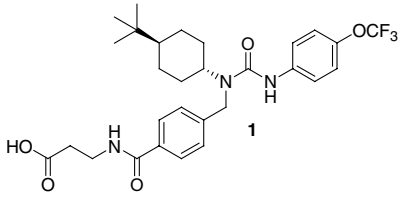
Abstract—Biaryl amides derived from a reported series of ureas **1** were evaluated and found to be potent human glucagon receptor antagonists. The benzofuran analogue **6i** was administered in Sprague–Dawley rats and blocked the effects of an exogenous glucagon challenge.

As the incidence of type 2 diabetes reaches epidemic proportions the search for new potential treatments has intensified. The discovery of agents that moderate hepatic glucose production (HGP) for the treatment of the disease has developed into an active research area.[1] Glucagon, a twenty-nine amino acid peptide, is the primary counter regulatory hormone to insulin in glucose homeostasis. Glucagon acts primarily in the liver where it binds to the G-protein coupled glucagon receptor (GlucR) to initiate gluconeogenesis and glycogenolysis via the adenylate cyclase mediated activation of protein kinase A. Protein kinase A also suppresses glycogen synthesis and glycolysis and promotes ketogenesis. There is substantial evidence to suggest GlucR antagonists will be useful in the treatment of diabetes. In type 2 diabetics, glucagon mediated HGP is elevated and does not decrease appropriately in the postprandial state giving rise to hyperglycemia.[2] The target has also been the focus of validation studies in rodent models of diabetes. Immunoneutralization of endogenous glucagon with a monoclonal antibody significantly lowered blood glucose levels of STZ rats and *ob*/*ob* mice.[3] Furthermore, in GlucR−/− knockout mice, glucose levels fall within the normal range in both the fed and fasted states.[4] Therefore glucagon receptor antagonism has been pursued as a promising approach to treat hyperglycemia.

Multiple classes of small molecule glucagon receptor antagonists have been reported.[5] Bayer has advanced one of their biaryl antagonists into evaluation in healthy human volunteers and has demonstrated its ability to block the effect of a glucagon challenge.[6] Recently Novo Nordisk patented a novel series of urea based glucagon receptor antagonists.[7] One example is urea **1**, with a human glucagon receptor binding (h-GlucR$_{bind}$) K_i of 63 nM and a moderate glucagon induced adenylate cyclase inhibition (h-GlucR$_{cyclase}$) K_i of 254 nM under our assay conditions (Fig 1).[8] Structure–activity studies indicated that all three groups attached to the urea were critical to the binding and functional activity of the molecule. The template was evaluated for further modification to increase binding and functional activity, and potentially to improve the pharmacokinetic profile. Initially we replaced the tertiary nitrogen of the urea with a carbon and investigated the resulting amides.[9]



1

Figure 1. Urea glucagon receptor antagonist.

Keywords: Glucagon receptor antagonist; Hepatic glucose production inhibitors.
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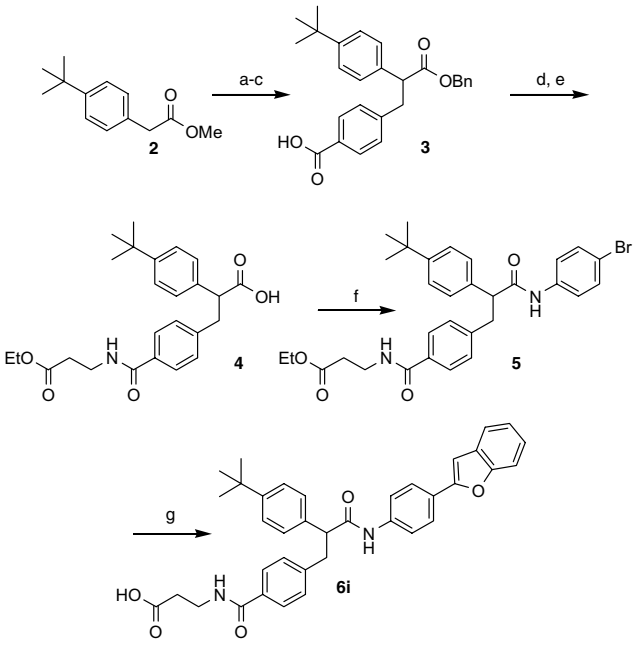
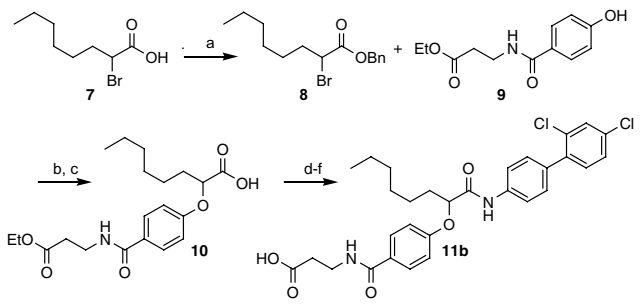


Scheme 1. Reagents and conditions: (a) NaOH, MeOH, water; (b) BnBr, Cs_2CO_3, DMF; (c) LiHMDS, THF, −78 °C then *p*-Li(O)BnBr, THF, −78 °C → 25 °C, 80%; (d) $H_2N(CH_2)_2COOEt \cdot HCl$, TBTU, *i*-$Pr_2NEt$, DMF; (e) H_2, Pd/C, MeOH, 95%; (f) *p*-bromoaniline, DCC, DMAP, Tol, 100 °C, 80%; (g) 2-benzofuranboronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DME, EtOH, water, microwave, 125 °C then NaOH, MeOH, water, 70%.

Antagonists were synthesized from ester **3**, prepared by hydrolysis and esterification of methyl *tert*-butylphenyl acetate **2** followed by alkylation with the lithium salt of α-bromo-*p*-toluic acid using LiHMDS at −78 °C.[10] The β-alanine ethyl ester was then attached by a TBTU[11] mediated coupling reaction. Subsequent deprotection of the benzyl ester by hydrogenation afforded the acid **4**, for further derivatization to amides with a variety of amines. For the preparation of biaryl amides, a DCC coupling[12] of *p*-bromoaniline with acid **4** that yielded aryl bromide **5** was utilized. Suzuki reaction[13] and ester hydrolysis gave the final product biaryl amide **6i**. Related amides were synthesized by similar procedures (Scheme 1).

The amide template was further modified by introduction of a heteroatom to replace the benzylic carbon connecting the urea to the β-alanine substituted phenyl ring. This was achieved by preparing the benzyl ester of the α-bromo acid **7**, followed by reaction with phenol **9**. Deprotection of the resultant ester gave the acid core **10** that was derivatized to amide analogues with different amines. Biaryl amide was formed by DCC coupling[12] of *p*-bromoaniline followed by a Suzuki reaction[13] and ester hydrolysis to give the desired product **11b**. Related amides were prepared similarly (Scheme 2).

Table 1 summarizes the h-GlucR antagonist activity of the amides in binding and cyclase assays.[8] Although the binding assay was consistent across a range of potencies, the cyclase assay showed some variability for less potent



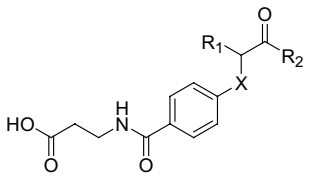
Scheme 2. Reagents and conditions: (a) BnBr, Cs_2CO_3, DMF, 87%; (b) NaH, DMF; (c) H_2, 10% Pd/C, MeOH, 53%; (d) 4-bromoaniline, DCC, DMAP, Tol, 100 °C; (e) 2,4-dichlorophenylboronic acid, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, K_3PO_4, THF, water, 70 °C; (f) NaOH, MeOH, water, 50%.

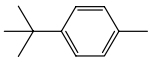
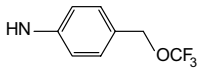
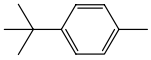
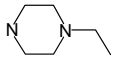
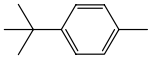
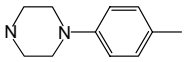
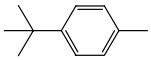
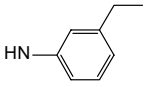
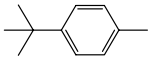
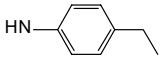
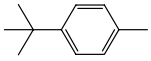
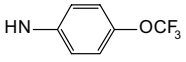
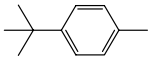
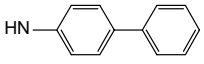
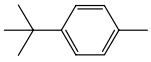
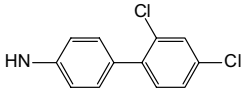
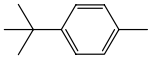
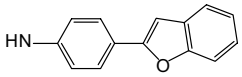
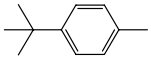
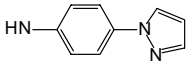
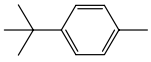
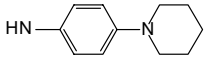
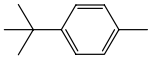
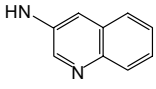
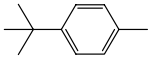
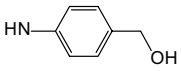
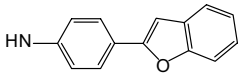
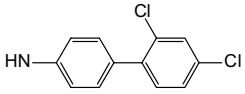
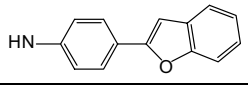
compounds (h-GlucR$_{cyclase}$ $K_i > 150$ nM). As illustrated by **6a** and **6b**, not all amides were tolerated. In general aromatic, nonpolar groups were superior to polar and/or alkyl amides (data not shown). Secondary amines generally performed poorly except for phenyl piperazines like **6c** that showed modest binding activity. However, amides derived from anilines, and particularly biphenyl anilines, provided a series of potent antagonists. Substitution of the *para* position of an aniline enhanced binding activity compared to the *meta* position as in **6d** and **6e**, although both were inactive in the functional assay. However *p*-trifluoromethoxy aniline **6f** showed a h-GlucR$_{bind}$ K_i of 51 nM and a h-GlucR$_{cyclase}$ K_i of 110 nM, which was more potent in the functional assay than the parent urea **1**. Biphenyl amides derived from nonpolar aromatic units on the 4-position of the aniline were the most potent binders and dramatically improved the functional activity as well. When the 4-position of the anilino-amide was substituted with a phenyl group as in **6g**, the h-GlucR$_{cyclase}$ $K_i = 300$ nM. A 2,4-dichlorophenyl **6h**, or a benzofuran substitution **6i** decreased the functional K_i substantially (h-GlucR$_{cyclase}$ $K_i = 33$ nM and 29 nM). However, heterocyclic substitutions **6j**, **6k**, and **6l**, or polar phenyl moieties **6m** on the 4-position of the aniline remained potent binders but significantly decreased functional potency. Binding and functional potencies of the compounds did not change when the carbon linker was altered to an ether as in **11a** compared to **12**. A trend towards increasing functional potency was observed when amides were derived from changing the side chain from a *tert*-butylphenyl to a *n*-hexyl as illustrated by potent h-GlucR antagonists (**11a**, **11b**, and **12**).

Changes to the β-alanine drastically altered the functional activity profile of the compounds (Table 2). Substitution by an amino tetrazole as in **14** decreased binding and functional activity. Surprisingly, subtle changes to the β-alanine moiety such as introduction of a OH or F substitution in compounds **15** and **16** dramatically weakens the functional potency while preserving most of the binding activity.

By altering the urea scaffold **1**, we have discovered potent glucagon receptor antagonists. However none of

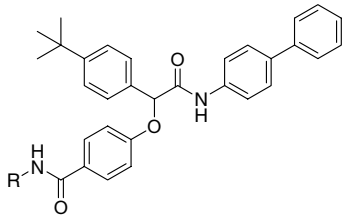
Table 1. Binding and functional activity K_i of amide h-GlucR antagonists

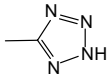


Compound	R_1	R_2	X	Binding K_i (nM)[a]	Cyclase K_i (nM)[a]
6a			CH_2	5000	>2000
6b			CH_2	>5000	>2000
6c			CH_2	400	>2000
6d			CH_2	80	>2000
6e			CH_2	26	>2000
6f			CH_2	51	110
6g			CH_2	11	300
6h			CH_2	6	33
6i			CH_2	4	29
6j			CH_2	70	>2000
6k			CH_2	73	>2000
6l			CH_2	11	610
6m			CH_2	29	1000
11a	*n*-Hexyl		O	3	17
11b	*n*-Hexyl		O	6	14
12	*n*-Hexyl		CH_2	6	20

[a] Values are geometric means of two experiments.

Table 2. Binding and functional activity (K_i, nM) of selected h-GlucR antagonists



Compound	R	Binding K_i (nM)[a]	Cyclase K_i (nM)[a]
13	$-CH_2CH_2COOH$	9	144
14		66	317
15	$-CH_2CHFCOOH$	26	>2000
16	$-CH_2CH(OH)COOH$	112	>2000

[a] Values are geometric means of two experiments.

the representative analogues that were selected for further evaluation showed good pharmacokinetic profiles in rat. Compounds were also affected by protein binding. Bovine serum albumin (BSA) was found to decrease the in vitro functional activity of the compounds. For example **6i** had less than 5% oral bioavailability. Also its h-GlucR$_{cyclase}$ K_i decreased from 29 to 140 nM in the presence of 10% BSA. The functional activity of **6i** was similar for human, murine (mu-GlucR$_{cyclase}$ $K_i = 29$ nM) and monkey (m-GlucR$_{cyclase}$ $K_i = 15$ nM) glucagon receptors but showed a decrease for the rat receptor (r-GlucR$_{cyclase}$ $K_i = 69$ nM). To test the efficiency of glucagon receptor blockade in vivo, **6i** was examined in Sprague–Dawley rats against a exogenous glucagon challenge. A 50 mg/kg iv dose of **6i** reduced the glucagon (3 nmol/kg−) induced glucose excursion by 50% within 30 min of dosing when compared to vehicle.

In summary a series of potent biaryl amide glucagon receptor antagonists was discovered. One of these antagonists was demonstrated to blunt the effects of a glucagon challenge in vivo when dosed intravenously. Of future interest is the discovery of antagonists for use in oral dosing long-term studies.

Acknowledgements

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References and notes

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