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Discovery of potent, orally active benzimidazole glucagon receptor antagonists

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ABSTRACT

The discovery and optimization of potent and selective aminobenzimidazole glucagon receptor antagonists are described. One compound possessing moderate pharmacokinetic properties in multiple preclinical species was orally efficacious at inhibiting glucagon-mediated glucose excursion in transgenic mice expressing the human glucagon receptor, and in rhesus monkeys. The compound also significantly lowered glucose levels in a murine model of diabetes.

Type 2 diabetes is characterized by chronically elevated plasma glucose levels. A major contributor to this condition is excessive hepatic glucose production resulting from inappropriately high levels of the hormone glucagon, which signals via the glucagon receptor to promote hepatic gluconeogenesis and glycogenolysis.[1] Disruption of glucagon signaling may therefore provide a means to alleviate hyperglycemia in type 2 diabetic patients.[2] Indeed, biological agents that do so, including glucagon-neutralizing antibodies,[3] antisense oligonucleotides,[4] and peptide glucagon receptor antagonists[5] have been shown to reduce glucose levels in animal models of diabetes. Several small molecule glucagon receptor antagonists have also been reported to block glucagon-induced glucose production in animals[6] and in man,[7] and more recently, to decrease glucose levels in animal models of diabetes.[8,9e] Continuing our efforts to develop small molecule glucagon receptor antagonists,[9] we describe the discovery and development of potent and selective 2-aminobenzimidazoles that are orally efficacious in blocking glucagon-mediated glucose excursion in vivo, and lower glucose levels in a diabetic animal model.

Screening efforts against the human glucagon receptor (hGCGR) led to the identification of moderately active acylurea antagonists including **1** (Fig. 1). Poor physical properties of the compounds, however, led us to replace the acylurea moiety with aminoheterocycles, providing analogs such as **2a**. While the aminoheterocycle derivatives showed significantly decreased activity against the glucagon receptor, molecular modeling comparisons with cyclic urea **3**[9d] and other previously reported glucagon receptor antagonists[8] prompted us to append benzyl-linked acidic groups to the amino moiety to provide compounds such as **2b**. Indeed, **2b** showed improved in vitro potency, and was orally efficacious in inhibiting glucagon-induced glucose excursion in transgenic mice expressing the human glucagon receptor (hGCGR mice) at 30 mg/kg (data not shown).

Further evaluation of the aminoheterocycle platform led to the preparation of aminobenzimidazole glucagon receptor antagonist **4**. Listed in Table 1, the compound showed high receptor affinity as measured by inhibition of ^{125}I-glucagon binding to hGCGR expressed in CHO cell membranes (Bnd IC_{50}), though functional antagonism of glucagon-induced cAMP accumulation in hGCGR-transfected CHO cells (cAMP IC_{50}) was weak.[10] Replacement of the diaryl ether moiety with a *tert*-butylcyclohexyl group provided compound **5**, which showed similar binding affinity to **4**, and also poor functional activity. N-Methylation of the benzimidazole ring of **5** provided compound **6**, which maintained high receptor affinity, and now showed significantly improved functional activity.

Benzimidazole **6** was synthesized as depicted in Scheme 1, which typifies the methodology used to obtain the majority of analogs. A one-pot reaction involving reaction of methyl-4-(4-*trans*-*tert*-butylcyclohexylaminomethyl) benzoate[10] with one equivalent of thiophosgene, followed by addition of phenylenediamine **13**,

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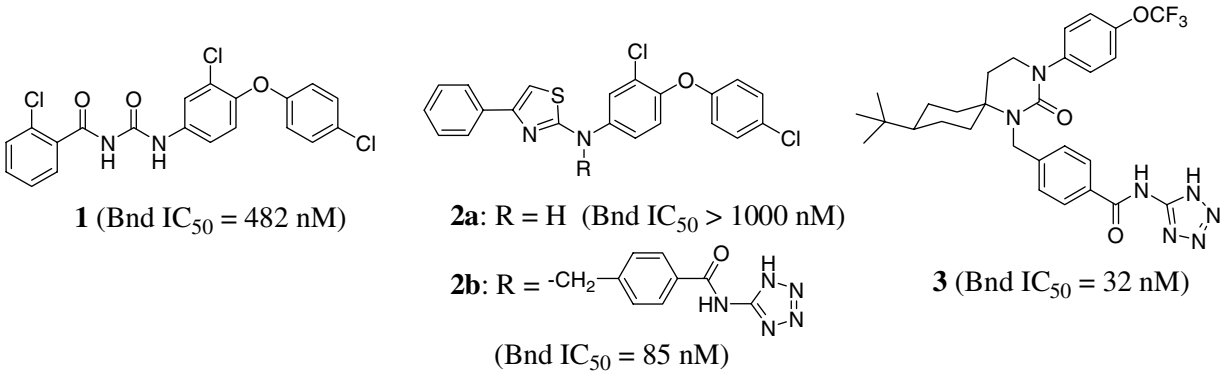
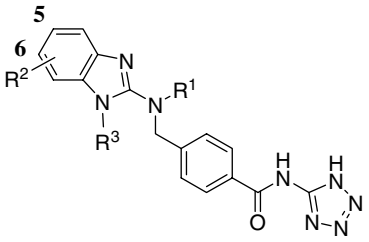
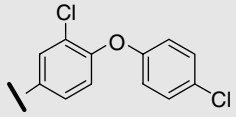
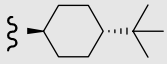
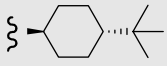
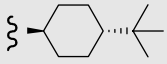
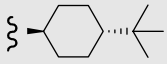
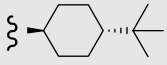
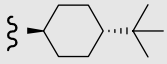
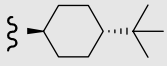
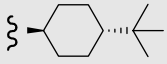


Figure 1. Small molecule glucagon receptor antagonists.

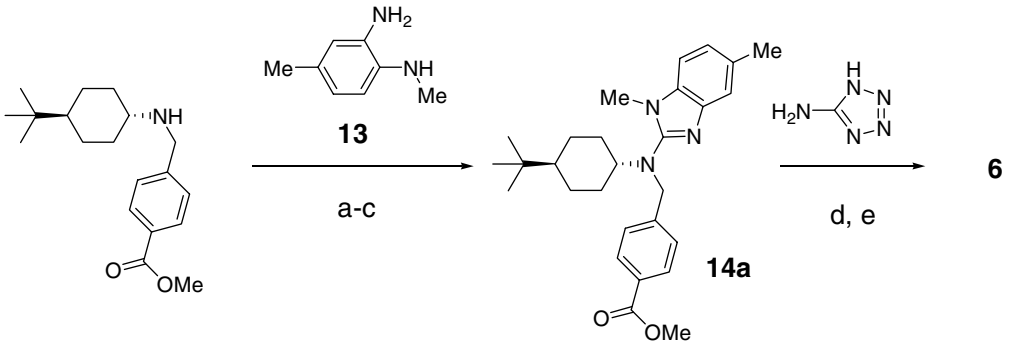
Table 1
Binding and functional activity of benzimidazole glucagon receptor antagonists



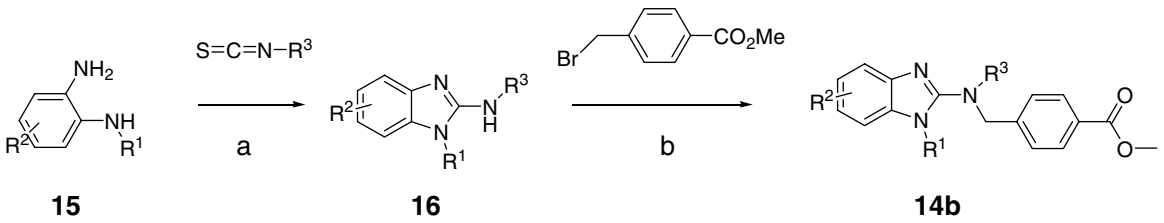
Entry	R^1	R^2	R^3	Bnd IC_{50} (nM)	cAMP IC_{50} (nM)
4		5-Me	H	13	>1000
5		5-Me	H	10	>1000
6		5-Me	Me	15	71
7		H	Me	4	55
8		H	Et	16	ND[a]
9		H	Pr	24	ND[a]
10		H	cPent	120	ND[a]
11		H	Ph	950	ND[a]
12		H	Bn	16	ND[a]

[a] ND, not determined.

then cyclization with mercuric trifluoroacetate, provided the aminobenzimidazole **14a**. Saponification of the ester and coupling of the aminotetrazole provided the desired product **6**. Outlined in Scheme 2, compounds could also be prepared by an alternative route, involving one-pot reaction of phenylenediamine **15** with the desired isothiocyanate, followed by mercury-mediated cyclization to afford the aminobenzimidazole **16**. Benzylation in the presence of sodium hydride strongly favored alkylation of the 2-amino group to provide ester **14b**, which could then be processed to the tetrazole product, as described in Scheme 1.



Scheme 1. Synthesis of compound **6**. Reagents and conditions: (a) $C(S)Cl_2$ (1.0 equiv), DIEA, CH_2Cl_2, 0 °C, 30 min; (b) phenylenediamine (1.2 equiv), 1 h, ambient temperature; (c) $Hg(O_2CCF_3)_2$ (1.0 equiv) added as a suspension in DMF, 1 h; (d) LiOH, 2:1 dioxane:H_2O; (e) EDC, HOBt, DIEA, DMF.



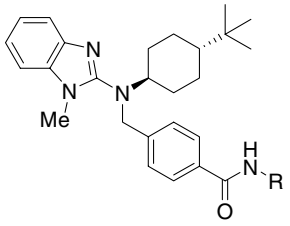
Scheme 2. Alternate synthesis of aminobenzimidazoles. Reagents and condition: (a) DMF, 1 h, ambient temperature, then $Hg(O_2CCF_3)_2$ (1.0 equiv), 1 h; (b) NaH, DMF.

Parent *N*-methylbenzimidazole **7** showed slightly enhanced potency compared to **6**. Surveying of *N*-alkyl substituents revealed that replacement of the methyl group with larger and more sterically bulky substituents generally resulted in decreased activity (entries **8**–**11**), though *N*-benzyl derivative **12** showed good receptor affinity.

Shown in Table 2, antagonist activity was highly dependent on the acid moiety, with replacement of the aminotetrazole with other acidic groups resulting in significantly diminished activity (entries **17**–**19**). β-Hydroxyacid **19** showed good binding affinity, though functional activity in the cell-based assay was greatly decreased. Shown in Table 3, antagonist activity was also highly sensitive to the 2-amino substituent, with large hydrophobic groups being required for high affinity and functional activity. Underscoring the sensitivity of this position is the significantly greater activity of *trans*-*tert*-butylcyclohexyl derivative **7** compared to the corresponding *cis*-isomer **20** and *tert*-butylphenyl analog **22**.

Extensive investigation of SAR on the benzimidazole ring was also undertaken, with representative examples listed in Table 4. Because oxidative metabolism of benzimidazoles can occur at C-5,[11] introduction of substituents at this position to block this potential site of metabolism was of particular interest. C-5 alkoxy derivatives **25**–**31** were especially potent, with benzyloxy and small alkoxy groups providing optimal binding and functional

Table 2
Activity of acid analogs



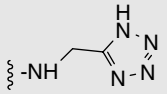
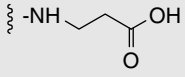
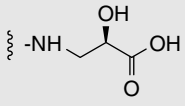
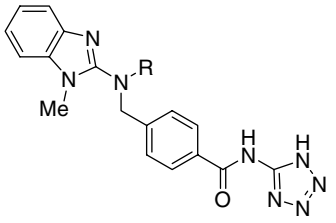
Entry	R	Bnd IC_{50} (nM)	cAMP IC_{50} (nM)
17		160	ND
18		230	ND
19		14	>1000

Table 3
2-Amino substituent SAR



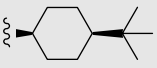
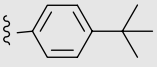
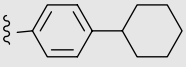
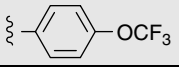
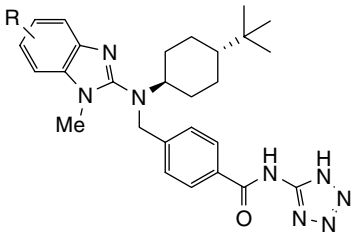
Entry	R	Bnd IC_{50} (nM)	cAMP IC_{50} (nM)
20		170	430
21	Me	>1000	ND
22		31	810
23		9	28
24		90	>1000

Table 4
Benzimidazole SAR



Entry	R	Bnd IC_{50} (nM)	cAMP IC_{50} (nM)	hGIP cAMP IC_{50} (nM)	hGLP-1 cAMP IC_{50} (nM)
25	5-OH	2	>1000	>1000	ND
26	5-OMe	4	38	2500	5900
27	5-OEt	2	22	2800	7600
28	5-OPr	2	4	3200	ND
29	5-OcPent	1	9	2000	5000
30	5-OCH_2-cPent	2	19	>1000	ND
31	5-OBn	1	7	1000	ND
32	6-OMe	4	213	>1000	ND
33	6-OPr	1	8	4900	5000
34	5-F	6	220	>1000	ND
35	5-Br	7	62	ND	ND
36	5-CF_3	11	62	6000	6800
37	5,6-DiMe	6	18	>10000	ND
38	5,6-DiCl	9	23	3000	3700

activity. C-6 alkoxy derivatives (e.g. compounds **32** and **33**) were also highly active, though slightly less so than their C-5 counterparts. Hydrophobic substituents were generally well tolerated on the benzimidazole ring (entries **34**–**38**). Selected compounds were screened against hGIP and hGLP-1 due to the role of these related receptors in glucose-dependent insulin secretion; selectivity over both receptors was typically greater than 50-fold (Table 4).

The benzimidazoles generally showed low oral bioavailability in rodents. The benzimidazole ethers in particular showed poor oral bioavailability, as exemplified by compound **29** (Table 5). Trifluoromethyl derivative **36**, however, displayed moderate pharmacokinetic properties in multiple species (Table 5). The compound was also highly selective against the hERG K^+ channel (IC_{50} > 10,000 nM) and cytochrome P450 isozymes 3A4, 2C9, and 2D6 (IC_{50} > 10,000 nM), and it was therefore further evaluated for glucagon receptor antagonist activity in vivo.

In a pharmacodynamic assay, oral administration of **36** to transgenic mice expressing the human glucagon receptor (hGCGR mice)[12] 1 h prior to an IP injection of glucagon (15 μg/kg) significantly inhibited glucagon-dependent glucose excursion at doses of 30, 10, and 3 mg/kg (Fig. 2). Compound **36**, which showed similar in vitro activity against the rhesus and human glucagon receptors (rhesus cAMP IC_{50} = 111 nM), also inhibited a glucagon challenge in rhesus monkeys at oral doses of 10 and 3 mg/kg (Fig. 3).

Table 5
Pharmacokinetic properties of compounds **29** and **36**

Entry	Species	Clearance (mL/min/kg)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	AUC_N (po) (μM h/dose)	F (%)
29	Mouse	10	0.5	3.2	0.07	2
29	Rat	15	0.60	2.9	0.10	5
36	Mouse	4	0.33	1.9	0.40	5
36	Rat	12	0.74	1.6	0.40	15
36	Dog	3.8	0.5	2.1	2.1	27
36	Rhesus	16	0.8	2.4	0.45	23

Mice and rats were dosed at 1.0 mg/kg iv in DMSO:Tween80:water (5:10:85) and 2.0 mg/kg po in 0.5% methylcellulose. Dogs were dosed at 0.25 mg/kg iv in EtOH:PEG:water (2:5:3), and at 1 mg/kg po in 0.5% methylcellulose. Rhesus monkeys were dosed at 0.5 mg/kg iv in EtOH:PEG200:water (2:5:3) and at 2 mg/kg po in Imwitor:Tween80 (1:1).

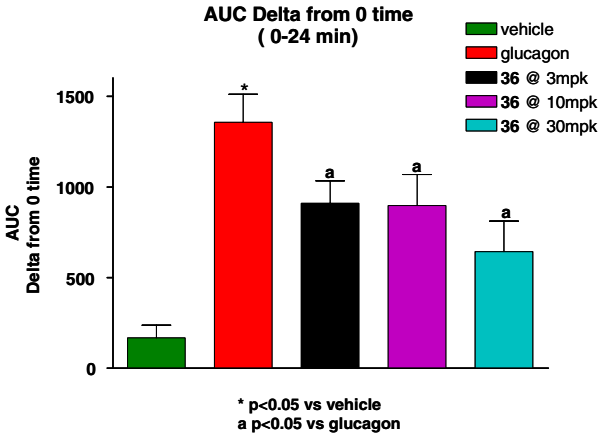


Figure 2. Effect of compound **36** on glucagon-induced glucose excursion in hGCGR mice. Compound was administered orally in 0.25% methylcellulose (n = 3/group).

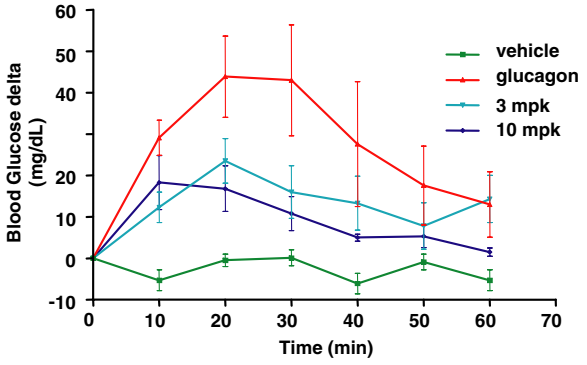


Figure 3. Effect of compound **36** on glucagon-induced glucose production in rhesus monkeys 1 h post-dose. Compound was administered orally in Imwitor:Tween80 (1:1); n = 4/group.

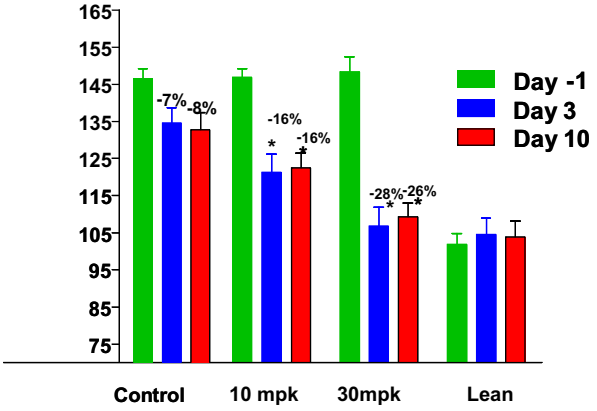


Figure 4. Effect of compound **36** on blood glucose levels in hGCGR mice on a high-fat diet. Compound was administered as an admixture in the feed (n = 12 animals per group). $^*p < 0.05$ versus control.

The ability of compound **36** to reduce glucose levels in a diabetic animal model was also determined using hGCGR mice placed on a high-fat diet to induce moderate hyperglycemia.[13] Shown in Figure 4, oral administration of **36** in the feed at 10 and 30 mg/kg/day over 10 days provided a dose-dependent reduction in glucose levels, with nearly complete correction compared to lean controls at the high dose.

The 2-aminobenzimidazoles are potent glucagon receptor antagonists, which show good selectivity over related receptors hGIP and hGLP-1. Compound **36**, which displayed moderate pharmacokinetic properties in multiple preclinical species and a favorable off-target profile, was further evaluated in vivo. The compound was orally efficacious in blocking glucagon-mediated glucose production in hGCGR mice and in rhesus monkeys at an oral dose of 3 mg/kg. Furthermore, chronic oral administration of **36** to hGCGR mice with high-fat diet-induced hyperglycemia afforded significant reductions in blood glucose levels, providing nearly complete correction compared to lean controls at a dose of 30 mg/kg/day. These results provide further preclinical validation for small molecule glucagon receptor antagonists as a potentially effective treatment of type 2 diabetes.

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