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Discovery and investigation of a novel class of thiophene-derived antagonists of the human glucagon receptor

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Abstract—A novel class of antagonists of the human glucagon receptor (hGCGR) has been discovered. Systematic modification of the lead compound identified substituents that were essential for activity and those that were amenable to further optimization. This SAR exploration resulted in the synthesis of **13**, which exhibited good potency as an hGCGR functional antagonist (IC_{50} = 34 nM) and moderate bioavailability (36% in mice).

Diabetes mellitus is a condition characterized by chronically elevated levels of blood glucose. This condition arises from inappropriate secretion and activity of the two major hormones that control glucose homeostasis, insulin, and glucagon. Diabetic insulin resistance and insulin deficiency impair the body's ability to utilize glucose from the bloodstream. This condition is commonly aggravated by chronically elevated levels of the hormone glucagon, or hyperglucagonemia.[1] The peptide glucagon is normally secreted from pancreatic α cells in response to depleted plasma glucose levels. This hormone activates the hepatic glucagon receptor (GCGR) and triggers the synthesis of glucose (gluconeogenesis) and processing and release of hepatic glycogen stores (glycogenolysis) to elevate and restore plasma glucose levels. In diabetes mellitus the inappropriately high hepatic glucose production from glucagon and the inappropriately low glucose utilization from insulin both contribute to chronic hyperglycemia. Many therapeutic approaches to the treatment of diabetes have focused on the normalization and utilization of plasma insulin.[1c,2] An alternative or additional therapy may be realized by blocking hepatic glucose production using glucagon antagonists.[3] Peptidyl antagonists such as **1** (Table 1) are known,[4] and several small molecule antagonists are under investigation as well.[1a,5]

Recently we completed a high throughput screening program directed toward discovering compounds that competitively inhibit the binding of ^{125}I-glucagon to the human glucagon receptor (hGCGR). Among the compounds identified for further investigation were a series of acylated aminothiophene nitriles exemplified by **2**.[6]

The syntheses of the bicyclic thiophene derivatives **2**–**9** were accomplished using the Gewald cyclization as illustrated in Scheme 1.[7] The cyclohexanone derivatives **I** were condensed with malononitrile, followed by addition of elemental sulfur, affording the bicyclic aminothiophenes **II**. Acylation of these intermediates afforded the desired amides **III**. The amine-substituted derivatives **5**–**9** were prepared from the corresponding dioxolane derivative **IV**. Removal of the ketal protecting group with aqueous acid followed by successive reductive aminations afforded the final products **5**–**9**.

Keywords: Glucagon; Diabetes; Thiophene; Gewald.
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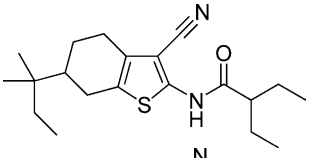
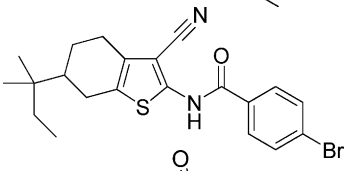
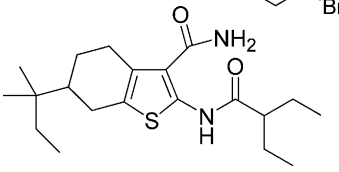
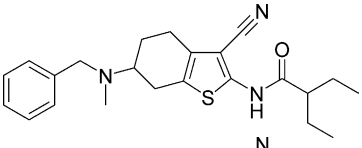
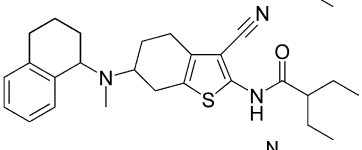
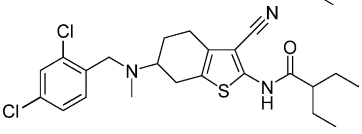
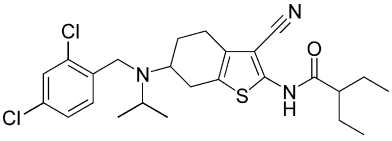
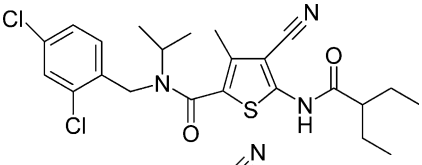
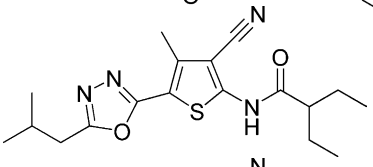
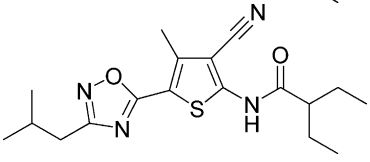
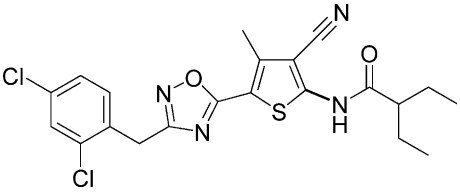
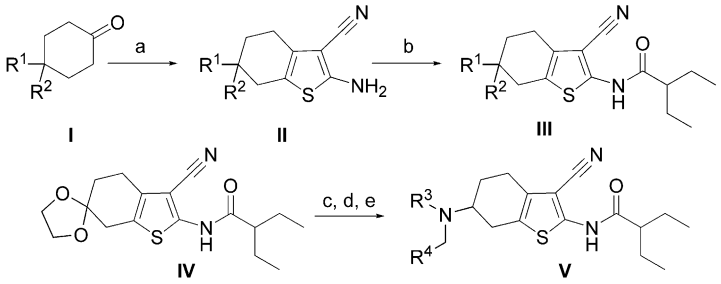
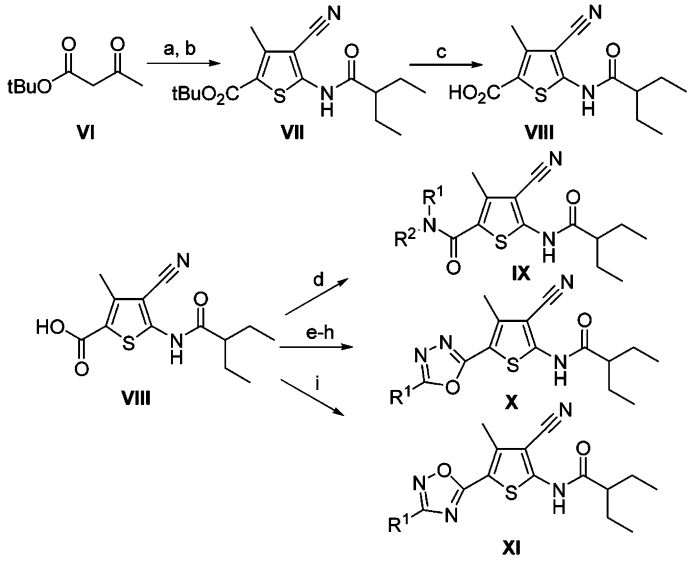
Table 1. Binding and functional activity of antagonists of the human glucagon receptor

Compound		Diastereomer[a]	Binding IC_{50} (nM)[b]	cAMP IC_{50} (nM)[b]
1	Des-His[1][Glu[9]]glucagon-NH_2		18	18
2			181	129
3			>20,000	—
4			870	38%[c]
5			3820	—
6		**D1**	139	158
7		**D2**	118	59
8			92	63
9			129	59
10			153	41
11			3210	—
12			368	185
13			89	34

[a] All compounds containing one or more chiral centers were tested as racemic mixtures.
[b] Values reported are the mean of a minimum of two experiments with the standard deviation <50% of the mean.
[c] Percent inhibition of glucagon cAMP accumulation at 10 μM compound concentration.



Scheme 1. Reagents and conditions: (a) malononitrile, S_8, morpholine, EtOH, 70 °C; (b) 2-ethylbutyryl chloride, DIEA, CH_2Cl_2; (c) 1.0 N HCl (aq), THF; (d) R^3NH_2, $NaBH(OAc)_3$, 1% AcOH in CH_2Cl_2; (e) R^4CHO, $NaBH(OAc)_3$, 1% AcOH in CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) malononitrile, S_8, morpholine, EtOH, 70 °C; (b) 2-ethylbutyryl chloride, DIEA, CH_2Cl_2 (49%—two steps); (c) TFA, CH_2Cl_2 (92%); (d) R^1R^2NH, EDC, HOBT, DIEA, DMF; (e) EDC, MeOH; (f) NH_2NH_2, MeOH, 65 °C; (g) R^1CO_2H, EDC, HOBT, DMF; (h) SO_2Cl, pyridine, Et_2O, 0 °C, then 100 °C in toluene; (i) $R^1C{=}NHOH(NH_2)$, PyBop (2 equiv), DMF.

These compounds were assayed by measurement of inhibition of binding of ^{125}I-glucagon to the hGCGR expressed in CHO cell membrane, affording the binding IC_{50} values in Table 1. The active compounds were further examined for functional antagonist potency, as measured by the inhibition of glucagon induced cAMP accumulation in hGCGR transfected CHO cells (cAMP IC_{50}, Table 1).[6]

The thiophene derivative **2** displayed moderate activity as an antagonist of the human glucagon receptor with a binding IC_{50} of 181 nM and similar potency (IC_{50} = 129 nM) in the cAMP accumulation assay. Aliphatic amides were tolerated as substituents on the amino moiety, whereas aromatic amides such as **3** were inactive. The nitrile substituent was also found to be important for activity, although the related terminal amide derivative **4** retained measurable potency in the binding and functional assays. The thiophene heterocycle was found to be crucial for activity, as direct analogues of active thiophene derivatives featuring a suitably substituted furan, pyrrole, or phenyl ring were inactive in the binding assay (data not shown). While we have no information on specific interactions between these compounds and the glucagon receptor, we conclude empirically that the 3-cyanothiophene amide represents an optimized pharmacophore.

The cyclohexyl moiety on **2** was more amenable to SAR optimization. We sought to improve the physical properties of **2** ($\log D$ = 6.6) by incorporating a more hydrophilic amine substituent. Although the *N*-methyl benzylamine **5** was substantially less active in the binding assay, the activity was restored by constraining the benzyl substituent with a fused cyclohexyl ring as in the diastereomers **6** and **7**. The diastereomers also exhibited enhanced hydrophilicity ($\log D$ = 4.8). Alternatively, gains in the potency of **5** were achieved with the addition of substituents on the phenyl ring. Extensive investigation lead to the identification of the 2,4-dichlorobenzylamine substituent in **8** and **9**, which retained the antagonist potency of **7** but yielded the gains in hydrophilicity (**8** $\log D$ = 6.6).

The Gewald cyclization is generally applicable to both cyclic and acyclic ketones, and may employ a variety of malonyl electrophiles. Thus, using an extension of this procedure as exemplified in Scheme 2, additional positions on the aminothiophene ring were independently modified to explore the SAR of this class. We

Table 2. Pharmacokinetic parameters of glucagon antagonists dosed in mice ($n = 3$ mice/route of administration)

Compound	Species	Dose PO/IV (mg/kg)	CLp (mL/min/kg)	AUC_N (PO) (μM h/dose)	Vd_{ss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	F %
8	Mouse	2.0/1.0	50	0.07	3.4	1.9	0.08	9.2
12	Mouse	10.0/5.0	84	0.21	16	2.7	0.80	39
13	Mouse	10.0/5.0	23	0.62	8.7	5.1	0.80	36

investigated the replacement of the lipophilic cyclohexyl moiety with alternative functionality that would similarly constrain the conformational freedom of the distal substituents relative to the thiophene, including the use of amides and heterocycles as in **10**–**13** (Table 1). The syntheses of these derivatives are illustrated in Scheme 2. The Gewald cyclization was performed on β-ketoesters such as **VI**, followed by acylation of the aminothiophene product, to afford **VII** in moderate yield. Deprotection of the carboxyl substituent afforded the carboxy thiophene **VIII** for derivatization. This intermediate was coupled with secondary amines under standard conditions to afford amides **IX**. The carboxyl intermediate **VIII** was otherwise derivatized to the corresponding oxadiazoles[8] **X** or isoxadiazoles[9] **XI** using published methods.

The intrinsic potency of the thiophene-derived antagonists was maintained with the removal of the cyclohexyl ring. The *N*-isopropyl benzyl amide **10** afforded potency similar to the cyclohexylamine derivative **9**, with an $IC_{50} = 41$ nM in the cAMP assay. In order to reduce the peptidic nature of the compounds, we sought to replace the secondary amide with heterocyclic isosteres. Replacement of the amide substituent with the isobutyl substituted oxadiazole afforded **11**, which lost over an order of magnitude in potency in the binding assay. However, the corresponding isoxadiazole **12** was substantially more potent in the binding assay, and afforded moderate activity in the functional assay as well, with an IC_{50} of 185 nM. Finally, substitution of the isoxadiazole with the 2,4-dichlorobenzyl substituent, which was found to be optimal in the amine and amide series, afforded **13** with gains in potency demonstrated in both the binding assay ($IC_{50} = 89$ nM) and the functional cAMP assay ($IC_{50} = 34$ nM).

The activity of both the initial lead **2** and the more potent derivative **13** toward the related human GIP and GLP-1 receptors was also examined.[10] As these two receptors represent insulinotropic mechanisms, antagonism of these targets should be minimized. The bicyclic thiophene **2** was indeed inactive against both the hGLP-1 and hGIP receptors, with a functional $IC_{50} > 20$ μM in the respective cAMP accumulation assays. The more potent isoxadiazole derivative **13** retained over 30-fold selectivity over the hGLP-1 receptor, with an $IC_{50} = 1.18$ μM in the cAMP assay, and exhibited similarly modest activity ($IC_{50} = 973$ nM) against the hGIP receptor.

The pharmacokinetic parameters of several of the active compounds were measured by dosing both IV and PO in mice, and the results are shown in Table 2. The racemic cyclohexylamine derivative **8** was poorly bioavailable (9.2%), affording rapid clearance (50 mL/min/kg) and a very low maximal blood concentration of only 80 nM. While **12** did afford greater overall bioavailability at a higher dose (39%), the compound was rapidly metabolized in vivo, affording a clearance of 84 mL/min/kg. The corresponding dichlorobenzyl analogue **13** afforded substantially lower clearance in vivo (23 mL/min/kg), a longer half life (5.1 h) and a 3-fold greater oral AUC than the isobutyl analogue **12**.

Finally, the antagonist **13** was tested for the ability to block glucagon-induced glycogenolysis in vivo using transgenic mice that exclusively express a functional human glucagon receptor. The full characterization of these mice and their response to known antagonists have been reported previously.[11] Oral administration of **13** (100 mpk, $n = 9$ mice) was followed 45 min later by an IP injection of glucagon (15 μg/kg). In this experiment **13** did not afford a statistically significant reduction in glucagon-induced glucose excursion AUC as compared to administration of glucagon alone. One possible reason for this lack of in vivo efficacy may be the shift toward lower intrinsic potency in the presence of plasma. Addition of 5% mouse plasma to the functional assay afforded a 15-fold decrease in the cAMP IC_{50} of **13**.[12]

A novel class of thiophene-derived antagonists of the hGCGR has been discovered. An investigation of this class has resulted in the identification of the substituted isoxadiazole **13** as a lead for further investigation. Improvements were made in both the intrinsic potency and in vivo exposure of this class of compounds. The further optimization of oral exposure and minimization of plasma interference in activity remain as challenges to achieving pharmacodynamic efficacy with this class of antagonists.

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12. Antagonist **2** was similarly inactive in this experiment when dosed orally (100 mpk). However, **2** afforded a robust decrease in glucagon-induced glucose excursion in this model following 50 mpk IP administration of the compound (Ref. 6a).