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Discovery of furan-2-carbohydrazides as orally active glucagon receptor antagonists

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ABSTRACT

Furan-2-carbohydrazides were found as orally active glucagon receptor antagonists. Starting from the hit compound **5**, we successfully determined the structure activity relationships of a series of derivatives obtained by modifying the acidity of the phenol. We identified the *ortho*-nitrophenol as a good scaffold for glucagon receptor inhibitory activity. Our efforts have led to the discovery of compound **7l** as a potent glucagon receptor antagonist with good bioavailability and satisfactory long half-life.

Type 2 diabetes is characterized not only by insulin resistance and β-cell dysfunction but also by hyperglucagonemia in the fasting state and lack of glucagon suppression following meal ingestion.[1,2] It is therefore necessary for a complete treatment of type 2 diabetes to include agents that reverse hyperglucagonemia.

Glucagon, a peptide hormone consisting of 29 amino acid residues and produced in the α-cells of the pancreas, acts in the liver where it binds to the glucagon receptor (GCGR) to initiate gluconeogenesis and glycogenolysis.[3] It has been reported that plasma glucagon levels are abnormally high throughout the day in type 2 diabetic patients.[2] This led to the idea that GCGR antagonists may reduce hepatic glucose output and lower abnormal plasma glucose levels.[3b,4] In fact, Bayer reported that the GCGR antagonist, Bay 27-9955 (**1**, Fig. 1), suppresses excess glucagon-induced high plasma glucose levels in humans.[5] These findings indicate that GCGR antagonists may be useful in the treatment of type 2 diabetes.

To date, a number of non-peptidic GCGR antagonists with various acidic moieties including, β-alanine (NNC 25-0926[6] and MK-0893, **2**[7]), tetrazole (**3**[8]), or *ortho*-cyanophenol (**4**[9]) have been reported (Fig. 1). Although some of these compounds proceeded to clinical trials,[7,10] none is clinically available. In our search for new chemotypes of GCGR antagonists, we screened our chemical library and found compound **5**, 3,4-diphenylfuran-2-carbohydrazide derivative (Fig. 1), as a hit compound with moderate binding affinity for GCGR (50% inhibition at 10 μM in rat hepatocyte).[11] Our strategy for hit to lead generation focused on introducing the acidic moiety (Fig. 2).

Initially we replaced the furyl group in **5** with various groups as shown in Table 1. Since the phenyl compound **6a** exhibited a GCGR binding affinity similar to that of **5**, we next introduced a hydroxy group, as acidic moiety, at the phenyl group of **6a**. The obtained *para*-hydroxyphenyl compound **6b** showed a slight improvement in GCGR affinity, whereas the *meta*-hydroxyphenyl compound **6c** gave a loss in GCGR affinity. When the *para*-hydroxy group was masked with a methyl group, the resulting compound **6d** showed a complete loss of GCGR affinity. Regarding the other acidic group, benzoic acid **6e** showed a slight loss in GCGR affinity compared to the phenol **6b**. Introduction of two hydroxy groups (**6f**) resulted in no improvement in GCGR affinity. Remarkably, further improvement was seen with the hydroxypyridine **6g**, which showed a 10-fold IC_{50} value improvement compared to the hit compound **5**. Based on these results, it became clear that the pK_a values and GCGR affinity of compounds **6b**,**c**,**f**,**g** had similar variation. These findings suggested that an acidic proton at the *para*-position is needed for high GCGR affinity and that the acidity of the phenol group relates to GCGR affinity. However, a too strong acid such as benzoic acid **6e** would not exceptionally be well tolerated.

To confirm the relationship between the pK_a values and the affinity for GCGR, we screened substituents at the *meta*-position of the phenol shown as R^2 in Table 2 and calculated the pK_a values

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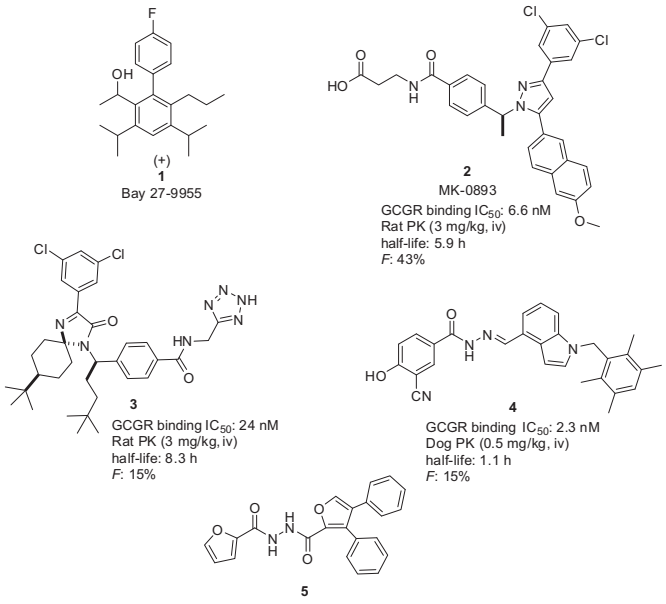


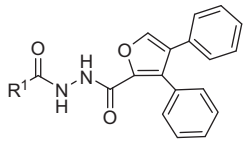
Figure 1. Representative chemotypes of GCGR antagonists and hit compound **5**.

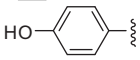
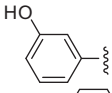
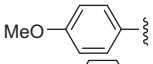
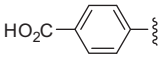
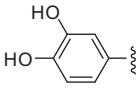
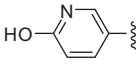
of the obtained compounds **6h–n**. The use of a fluoride (**6h**) resulted in a remarkable improvement in GCGR affinity with a pK_a value much lower than that of compound **6b**. Similarly, a chloride (**6i**) or a bromide (**6j**) led to improved GCGR affinity. Compounds possessing a strong electron withdrawing group, such as a trifluoromethyl group (**6k**) or a nitro group (**6l**) showed dramatically improved GCGR affinity, especially compound **6l** exhibited more than 100-fold improved affinity compared to the hit compound **5**. On the other hand, compounds with electron-donating groups, a methoxy (**6m**) or a phenyl group (**6n**) showed no improvement in GCGR affinity compared to **6b**. These findings confirmed our hypothesis as good correlation was observed between IC_{50} values and pK_a values (correlation coefficient: $r = 0.96$, from **6b** to **6n** in Table 2).

Next, the effects of a phenyl group at the 3- and 4-positions of the furan on GCGR affinity were investigated (Fig. 3). Surprisingly, despite the lack of a 4-phenyl group at the furan, compound **7a** exhibited almost the same GCGR affinity as compound **6l**. On the other hand, when the phenyl group at the 3-position of the furan was removed, GCGR affinity diminished (**8**, and **9**). Therefore, only the phenyl group at the 4-position of furan was removed from **6l** to decrease its molecular weight and lipophilicity.

Finally, we optimized the substituents at the *ortho*-, *meta*- or *para*-position of the phenyl ring (shown as R^3, R^4, R^5 in Table 3). The *para*-methyl compound **7d** exhibited high affinity compared to the *ortho*-isomer **7b** or the *meta*-isomer **7c**. As the *para*-substituent was critical for good affinity, we fixed a mono substituent at the *para*-position of the phenyl ring. The *para*-methoxy compound **7e** had weak GCGR affinity compared to the methyl compound **7d**.

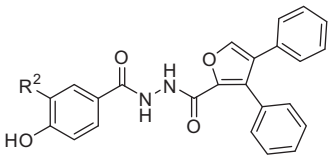
Table 1
SARs following modification of the furyl ring in **5**



Compound no.	R^1	GCGR binding[a,b] (%)	pK_a[c]
5		50	
6a		42	
6b		65	8.04
6c		21	8.52
6d		No inhibition	
6e		61	3.14
6f		57	7.96
6g		IC_{50} = 0.97 μM	7.83

[a] Activities are shown as the percent inhibition at 10 μM in rat hepatocytes.
[b] The assay was performed in duplicate ($n = 2$).
[c] Predicted using ADMET Predictor (SimulationsPlus, Lancaster, CA, USA).

Table 2
SARs following *meta*-substitution at the phenyl ring



Compound no.	R^2	GCGR binding IC_{50}[a] (μM)	pK_a[b]
6b	H	9.5	8.04
6h	F	1.4	6.41
6i	Cl	0.43	6.71
6j	Br	0.27	6.78
6k	CF_3	0.16	6.11
6l	NO_2	0.087	6.21
6m	MeO	9.5	8.02
6n	Ph	>10	8.08

[a] The assay was performed in duplicate using rat hepatocytes ($n = 2$).
[b] Predicted using ADMET Predictor (SimulationsPlus, Lancaster, CA, USA).

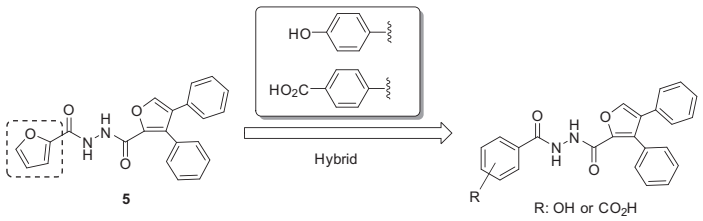


Figure 2. Strategy for hit to lead generation.

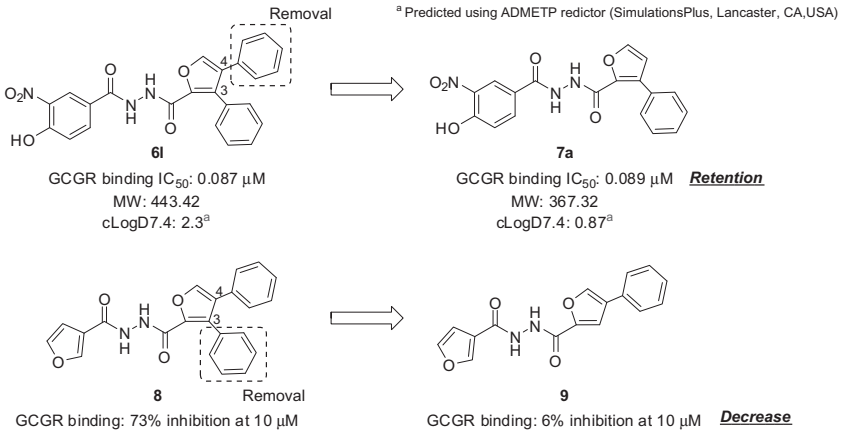
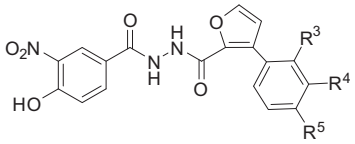


Figure 3. Effects of the furan phenyl group on GCGR affinity.

Table 3
SARs following substitution at the phenyl ring



Compound no.	R^3	R^4	R^5	GCGR binding IC_{50}[a] (nM)
7a	H	H	H	89
7b	Me	H	H	180
7c	H	Me	H	100
7d	H	H	Me	27
7e	H	H	MeO	93
7f	H	H	F	69
7g	H	H	Cl	270
7h	H	H	Et	44
7i	H	H	*n*-Pr	2.9
7j	H	H	*i*-Pr	24
7k	H	H	*n*-Bu	5.5
7l	H	H	*n*-Pen	9.6

[a] The assay was performed in duplicate using rat hepatocytes ($n = 2$).

Similarly, the fluoride compound **7f** and the chloride compound **7g** showed lower affinity than the methyl compound **7d**. Since an alkyl group seemed to be preferred to a halogen group, we extended the alkyl chain at the methyl group. As a result, the linear alkyl-chain derivatives **7i**,**k**,**l** showed more potent GCGR binding affinity than **7d**.

Three potent compounds that is, **7i**,**k**,**l** were selected and evaluated for their inhibition of cAMP production using rat, dog and human primary hepatocytes.[12] As cAMP is known as an intracellular second messenger for downstream signals of GCGR stimulation by glucagon,[2a,13] GCGR antagonists are believed to inhibit cAMP production in hepatocytes. Among the three selected compounds, compound **7l**[14] showed the most potent inhibitory activity of cAMP accumulation (Table 4). In addition, compound **7l** (1 μM) showed no noteworthy binding affinity for over 100 off-targets, including enzymes, receptors, ion channels and GLP-1R (Table 4),[15] indicating that compound **7l** has more than 100-fold selectivity for GCGR. To evaluate the in vivo efficacy of compound **7l**, a rat pharmacokinetic study of this compound was performed. As shown in Table 4, compound **7l** showed good bioavailability with satisfactory long half-life.

Figure 4 shows the results of glucagon challenge test of compound **7l** in normal rats ($n = 6$). Compound **7l** was orally administered 30 min before intravenous administration of glucagon (3 μg/kg). Compound **7l** at 30 mg/kg significantly inhibited glucagon-induced glucose elevation. Compound **7l** suppressed glucose elevation for up to 6 h after administration (data not shown).

We further investigated the glucose lowering effect of compound **7l** using Goto-Kakizaki (GK) rats, which are non-obese type

Table 4
Profiles of compound **7l**

Pharmacological profiles	
GCGR binding IC_{50} (nM)	9.6
cAMP IC_{50} rat/dog/human (nM)	5.3/183/292
GLP-1R IC_{50} (nM)	>1000
Safety profiles	
hERG IC_{50} (nM)	>1000
Sodium channel, site 2 IC_{50} (nM)	>1000
Pharmacokinetic profiles in rats	
1 mg/kg, iv	
Clearance (min/mL/kg)	0.28
$T_{1/2}$ (h)	5.9
V_{dss} (L/kg)	0.59
1 mg/kg, po	
C_{max} (ng/mL)	411
$T_{1/2}$ (h)	6.1
BA (%)	37

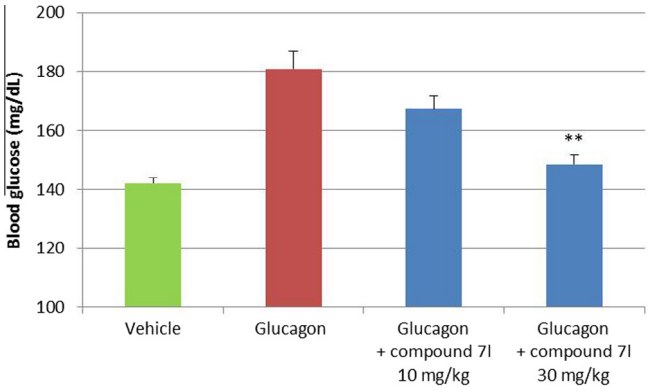


Figure 4. Compound **7l** suppression of glucagon-induced glucose elevation in normal rats. Compound **7l** was orally administered 30 min before intravenous administration of glucagon (3 μg/kg). Blood samples were collected 6 h after glucagon administration. Data are given as the mean values ± SEM ($n = 6$/group). $^{**}P < 0.01$ (Dunnett multiple comparison test).

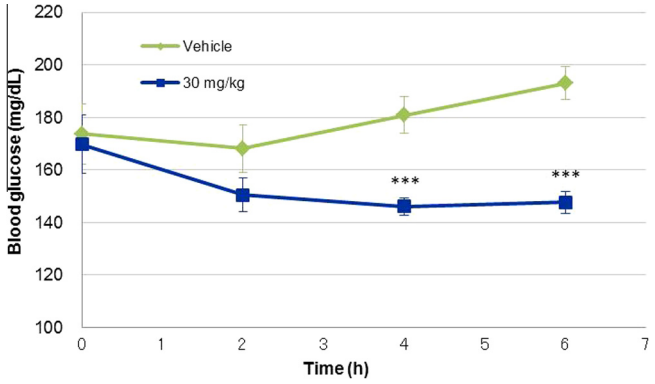
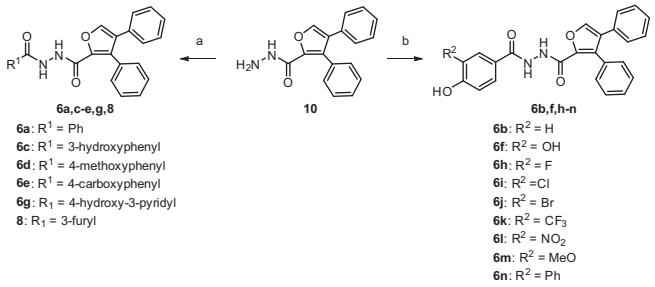


Figure 5. Glucose lowering effect of compound **7l** in GK rats. Compound **7l** was orally administered at 30 mg/kg. Blood samples were collected at 2, 4 and 6 h after compound **7l** administration. Data are given as the mean values ± SEM (n = 8–9/group). ***P <0.001 (Student's t-test)



Scheme 1. Reagents and conditions: (a) carboxylic acid, BOP, Et_3N, DMF, rt, 17–95%; (b) (i) benzoic acid, BOP, Et_3N, DMF, rt, 18–100%; (ii) BBr_3, CH_2Cl_2, rt, 13–96%.

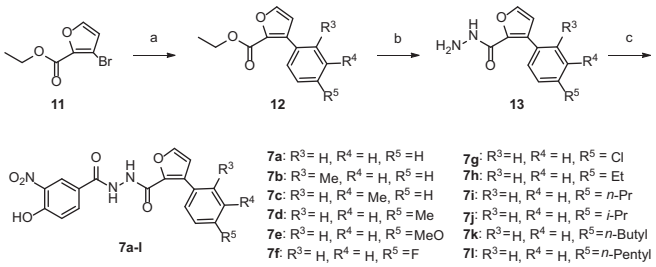
2 diabetic rats (Fig. 5).[16] Compound **7l** (30 mg/kg) significantly decreased glucose levels in GK rats. This decreasing effect was sustained for up to 6 h after administration. These results indicate that compound **7l** acts as a long-term GCGR antagonist not only in normal rats but also in GK diabetic rats.

The 3,4-diphenyl furan derivatives were prepared as illustrated in Scheme 1. The acylhydrazide **10**[17] was condensed with various carboxylic acids to afford compounds **6a**,**c**–**e**,**g**, and **8**. Similarly, compound **10** was condensed with various benzoic acids followed by deprotection of the methyl group by BBr_3, when necessary, to afford compounds **6b**,**f**,**h**–**n**.

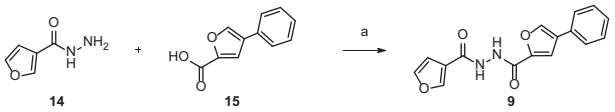
Compounds **7a**–**l** were synthesized as shown in Scheme 2. Suzuki–Miyaura reaction of the ethyl 3-bromofuran-2-carboxylate **11**[18] with various phenylboronic acids gave compound **12** in high yields. The ester group of compound **12** was converted to a hydrazide by hydrazine monohydrate under reflux conditions. Condensation of **13** with 3-nitro-4-acetoxybenzoic acid[19] followed by deprotection of the acetyl group afforded the desired compounds **7a**–**l**.

Preparation of compound **9** is shown in Scheme 3. Two commercially available fragments **14** and **15** were condensed by BOP reagent to give compound **9**.

In summary, we have found furan-2-carbohydrazides as novel scaffold of GCGR antagonists by determining SARs of a series of derivatives obtained by modifying the acidity of the phenol moiety. We have also found that the *ortho*-nitrophenol is a good scaffold for GCGR antagonistic activity. The most promising compound **7l** in our series demonstrated comparatively long-term suppression of glucose level in vivo. Further investigations are currently ongoing.



Scheme 2. Reagents and conditions: (a) phenylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, THF/H_2O, reflux, 84–99%; (b) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 47–99%; (c) (i) 3-nitro-4-acetoxybenzoic acid, WSC·HCl, DMF, rt; (ii) 10% NaOH aq, MeOH, rt, 51–77% (2 steps).



Scheme 3. Reagents and conditions: (a) BOP, Et_3N, DMF, rt, 32%.

Acknowledgments

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11. The *protocol for GCGR binding affinity test is as follows:* A liver membrane specimen was prepared from rat liver according to the method described in *Bioorg. Med. Chem. Lett.* **1992**, *12*, 915, and compounds binding affinity was measured using [^{125}I] glucagon. The liver was extirpated and added to the 20-fold volume of 50 mM Tris–HCl buffer (pH 7.2) relative to liver wet weight. The mixture was then homogenized using a glass-Tefron homogenizer. The homogenate was centrifuged at 30,000g for 15 min, and 20-fold volume of 50 mM Tris–HCl buffer (pH 7.2) relative to liver wet weight was added to the pellet. The mixture was further centrifuged at 30,000g for 15 min and the obtained pellet was used as liver membrane specimen. The specimen was suspended in the 100-fold volume of 1 mg/mL BSA relative to liver wet weight and 50 mM Tris–HCl buffer (pH7.2) containing 0.1 mg/mL bacitracin. [^{125}I] glucagon (final concd: 50 pM) was added to the specimen mixture. The mixture was then incubated at 25 °C for 30 min. [^{125}I] glucagon bound to the specimen was recovered by suction filtration using a GF/C filter pretreated

with 0.1% polyethylenimine, and the residues were washed with 50 mM Tris–HCl buffer (pH 7.4) for three times. Radioactivity was measured using an ARC-360 γ-counter. Specific binding volume was calculated by subtracting the non-specific binding volume in the presence of 1 mM glucagon from the total binding volume. Inhibition rates were calculated at each concentration of test compounds with binding volume in the absence of test compound considered as 100%. IC_{50} values were calculated using the pseudo-Hill plot.

12. *The protocol for inhibitory activity of cAMP accumulation is as follows:* Thawed hepatocytes were added to HCM™ Bulletkit™ (Takara Bio Inc.) and the mixture was centrifuged at 700 rpm, 4 °C for 3 min. After the supernatant was removed, the hepatocytes were suspended in HCM™ Bulletkit™ and the mixture was centrifuged at 700 rpm, 4 °C for 3 min. After the supernatant was removed, the hepatocytes were suspended in HCM™ Bulletkit™ and cell number was counted. The suspension was diluted to an appropriate living cell concentration, and the cells were seeded on a 24 well collagen-coat plate and incubated for 5 h. The test compound in cAMP assay buffer (Dulbecco's Modified Eagle Medium with 20 mM HEPES, 0.1% BSA and 0.5 mM IBMX) was added to the hepatocytes and the mixture was incubated at room temperature for 15 min. Glucagon or cAMP assay buffer was added, and the mixture was incubated at room temperature for 5 min. After the solvent was removed, 0.5 M-HCl was added, and the mixture was kept at room temperature for 10 min. 1 M Tris–HCl (pH 8.0) was then added for neutralization, and the solution was collected. The amount of cAMP in the supernatant was measured by a cAMP dynamic2 kit (Cisbio Bioassays).
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14. *Compound* ***7l***: Mp 159–160 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.75 (1H, br s), 10.51 (1H, s), 10.35 (1H, s), 8.47 (1H, d, J = 2.4 Hz), 8.06 (1H, dd, J = 8.7, 2.4 Hz), 7.94 (1H, d, J = 1.8 Hz), 7.66 (2H, d, J = 8.7 Hz), 7.23 (1H, d, J = 8.7 Hz), 7.19 (2H, d, J = 8.7 Hz), 6.94 (1H, d, J = 1.8 Hz), 2.57 (2H, t, J = 7.8 Hz), 1.60–1.53 (2H, m), 1.34–1.22 (4H, m), 0.85 (3H, t, J = 7.3 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 163.9, 158.3, 155.0, 144.6, 142.5, 140.2, 136.7, 134.1, 131.3, 129.2, 128.8, 128.1, 125.1, 123.3, 119.4, 114.1, 35.0, 31.1, 30.8, 22.1, 14.1; HRMS (ESI) calcd for $C_{23}H_{24}N_3O_6$ [M+H] 438.1660, found 438.1665.
15. The assay was performed by Eurofin Panlabs.
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