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Discovery of 5-Hydroxyalkyl-4-phenylpyridines as a New Class of Glucagon Receptor Antagonists

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Abstract—5-Hydroxyalkyl-4-phenylpyridines have been identified as a novel class of glucagon antagonists with potential utility for the treatment of diabetes. A lead structure with moderate activity was discovered through a high throughput screening assay. Structure–activity relationships led to the discovery of a potent antagonist, $IC_{50} = 0.11$ μM, more than 60-fold improvement over the lead structure.

Glucagon is a 29 amino acid peptidic hormone secreted by α-cells in pancreatic islets. The glucagon receptor is a member of the G-protein coupled receptor family which signals through the adenylate cyclase pathway by increasing cAMP levels in hepatocytes.[1,2] Elevated intracellular cAMP levels stimulate liver glycogenolysis and gluconeogenesis, resulting in an increase of hepatic glucose production.[3] A sustained high concentration of glucagon in the plasma is believed to be an important cause of excessive hepatic glucose production in diabetic subjects.[4,5] This alteration contributes to the elevation in blood glucose, the underlying cause of morbidity and mortality in diabetes.

Numerous modifications in the amino acid sequence of glucagon led to the identification of several potent peptide antagonists and is the subject of recent reviews.[6,7] Due to the poor oral bioavailability associated with peptides, their potential medical use has been limited. However, it was shown that some of the most potent peptide antagonists significantly decrease blood glucose levels in diabetic animal models when administered intravenously.[8,9] Thus, this implied that a selective glucagon receptor antagonist could be useful for the treatment of diabetics, and consequently, prompted a strong interest from several research groups.[10–19] Reviews covering the discovery of non-peptidic small molecule antagonists have been published.[20,21]

Our research efforts were thereby aimed at the identification of a lead structure capable of competitively altering glucagon binding to the human glucagon receptor (hGR). A high throughput screening assay resulted in the discovery of compound **1** (Fig. 1), which exhibited modest activities (binding affinity, $IC_{50} = 7$ μM and inhibition of cAMP production, $IC_{50} = 2$ μM). In this article, we describe the synthesis and the SAR of this new class of glucagon receptor antagonists. Compounds were tested in a binding assay using the human glucagon receptor[22] and selected compounds were tested in a functional assay.[23]

Most analogues were synthesized by following the general procedure described in Scheme 1, exemplified by the synthesis of compound **6**.[11,24] Several other analogues were subsequently made using compounds similar to **6** as a precursor. The synthesis of **6** started with the Hantzsch pyridine synthesis.[25] Condensation of enamine **3**, obtained from ethyl isobutyrylacetate **2**, with 4-fluorobenzaldehyde afforded pyridine **4** after oxidative treatment with DDQ (14%, three steps).

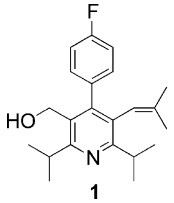

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Figure 1. Lead structure found in a high throughput screening assay.

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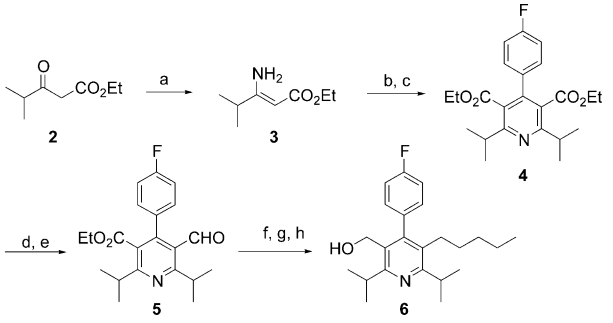
Selective reduction of one of the ester groups of pyridine **4** with Red-Al, followed by treatment with PCC, afforded aldehyde **5** (66%, two steps). A Wittig reaction of aldehyde **5** with the ylide of propyltriphenylphosphonium bromide provided the corresponding alkene ester, which was then successively reduced with LAH and catalytic hydrogenation, to afford alcohol **6**. Various ylides in the Wittig reaction could be used, leading to wide variation of alkyl groups at position 5 of the pyridine moiety (Tables 1 and 2).

A series of analogues with variation at position 5 of the pyridine moiety is listed in Table 1. We discovered that straight alkyl chains provided potent inhibitors with IC_{50} values in the sub-micromolar range. The optimum potency was obtained with alkyl chains from 3 to 7 carbon atoms in length. Addition of steric bulk by branching the alkyl group resulted in similar or decreased activity (e.g., **1** and **14**). Likewise, less lipophilic groups attached to an alkyl chain, such as hydroxy (**16**) or ether (**17**), gave either no improvement or substantially decreased the activity.

We then investigated the SAR at positions 2 and 6 of the pyridine moiety, as seen in Table 2. To avoid the formation of regioisomers and to keep the synthesis relatively simple, we limited our study to symmetrical analogues. Smaller substituents were not favorable as compounds **19** to **22**, where R^1 is methyl or ethyl, exhibited only weak activity. The diisopropyl-pyridines **23** and **24** were significantly more active with IC_{50} values just above 1 μM.

Steric bulk at this region of the molecule clearly increased the activity. It may also be important for the two isopropyl groups to shield the nitrogen atom of the pyridine moiety from a lipophilic area of the receptor. We were also interested in evaluating if the fluoro substituent found in the original lead structure (**1**) and our initial SAR (**7** to **13**) had any effect on binding affinity. This was addressed by comparison of **8** and **6**, which are the corresponding *para*-fluorophenyl analogues of compounds **23** and **24**. The compounds, **8** and **6**, exhibited approximately a two-fold increase in activity over their des-fluoro counterparts.

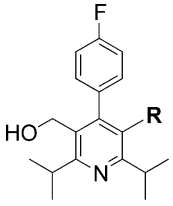


Scheme 1. Synthesis of phenylpyridine **6**. Reagents and conditions: (a) AcO–NH_4^+, cyclohexane, $-H_2O$, 92%; (b) ethyl isobutyrylacetate, 4-fluorobenzaldehyde, 20%; (c) DDQ, CH_2Cl_2, 78%; (d) Red-Al, THF, 67%; (e) PCC, CH_2Cl_2, 99%; (f) *n*BuPPh_3Br, KO*t*Bu, THF, 90%; (g) $LiAlH_4$, THF, 80%; (h) H_2, Pd/C, EtOH, 98%.

SAR at position 3 of the pyridine moiety was then explored using compound **6** as an intermediate to generate new analogues (Scheme 2). The lack of significant activity found with the methyl ether **28**, the acetate **27**, the aldehyde **25,** and the fluoromethyl derivative **30** suggested that a hydrogen donor group is required at that position. Attempts to replace the hydroxy group by other hydrogen donors also failed. Indeed, the thiol **29** and the carboxylic acid **27** exhibited no significant activities up to a concentration of 20 μM.

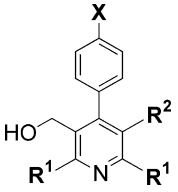
The hydroxymethyl group at position 3 of the pyridine moiety was therefore the key pharmacophoric group for activity and became the focus of further investigation (Scheme 3). A breakthrough result was obtained with the discovery of compound **31**, which exhibited an IC_{50} of 0.3 μM, or a 2-fold increase of activity over its cor-

Table 1. SAR for position 5 of the pyridine moiety

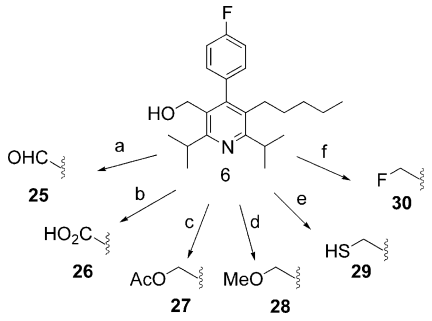


Compd	R	hGR IC_{50} (μM)
1	$CH{=}C(CH_3)_2$	7.0
7	Me	7.5
8	Et	1.0
9	Pr	0.4
10	Bu	0.75
6	Pent	0.7
11	Hex	0.35
12	Hept	0.7
13	Oct	1.8
14	$CH_2CH_2CH(CH_3)_2$	1.0
15	$CH{=}CH_2$	6.0
16	CH_2OH	>20
17	CH_2OCH_3	9.0
18	CO_2CH_3	9.5

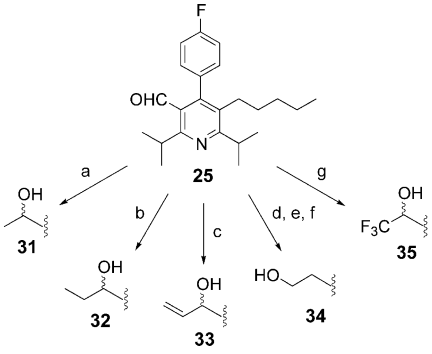
Table 2. SAR for positions 2,6 of the pyridine moiety



Compd	R^1	R^2	X	hGR IC_{50} (μM)
19	Me	Et	H	>20
20	Me	Pent	H	>20
21	Et	Et	H	>20
22	Et	Pent	H	20
23	iPr	Et	H	1.8
24	iPr	Pent	H	1.4
8	iPr	Et	F	1.0
6	iPr	Pent	F	0.7



Scheme 2. Synthesis of phenylpyridine analogues. Reagents and conditions: (a) PCC, CH_2Cl_2, 74%; (b) Jones reagent, acetone, 58%; (c) AcCl, Et_3N, CH_2Cl_2, 41%; (d) NaH, MeI, THF, 75%; (e) Lawesson's reagent, toluene, 26%; (f) DAST, CH_2Cl_2, 76%.



Scheme 3. Synthesis of hydroxyalkylpyridine analogues. Reagents and conditions: (a) MeLi, THF, 70%; (b) EtMgBr, THF, 70%; (c) CH_2=CHMgBr, THF, 70%; (d) $CH_3OCH_2PPh_3Br$, KO*t*Bu, THF, 60%; (e) HCl, THF, 50%; (f) $NaBH_4$, EtOH, 42%; (g) $TMSCF_3$, TBAF, THF, 70%.

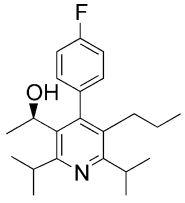
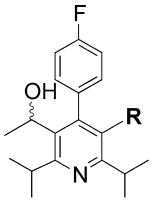


Figure 2. Absolute configuration of **36a**.

responding primary alcohol **6**. This analogue was synthesized by direct alkylation of aldehyde **25** with methyllithium to produce a racemic mixture of secondary alcohols. Larger secondary alcohols were thereafter explored (e.g., **32** and **33**), but resulted in a loss of activity, suggesting that the corresponding binding site of the receptor is relatively compact in that area. A transposition of the hydroxy group to the adjacent carbon atom (**34**) also resulted in a loss of activity. In an attempt to modify the acidity of the hydroxy group, the trifluoro isostere **35** was prepared.[26] It, however, exhibited a much weaker activity (IC_{50} = 13.0 μM) than its methyl isostere.

Since the racemic secondary alcohol **31** was the most active analogue at that point, it was resolved by chiral chromatography[27] to evaluate the activity of each enantiomer. We discovered that the faster eluting enantiomer was significantly more active (**31a**, IC_{50} = 0.15 μM) than the slower enantiomer (**31b**, IC_{50} = 3.0 μM).

Table 3. Racemates versus most active enantiomers of secondary alcohols



Compd *(racemate)*	hGR IC_{50} (μM)	R	Compd *(More potent enantiomer)*	hGR IC_{50} (μM)
36	0.22	Pr	**36a**	0.11
37	0.25	Bu	–	–
31	0.30	Pent	**31a**	0.15
38	0.24	Hex	**38a**	0.12

This interesting result prompted us to evaluate a series of hydroxyethyl analogues bearing different alkyl chains at position 5 of the pyridine moiety (Table 3). In the listed examples, a similar enantiomeric preference was observed in which compound **36a** exhibited the best activity (IC_{50} = 0.11 μM).

Finally, the absolute configuration of the secondary alcohol group was assigned by X-ray analysis of the Mosher ester derivative.[28] Thus, the secondary alcohol of compound **36a** was assigned the *R* configuration (Fig. 2) and by analogy, **31a** and **38a** were assumed to possess the same configuration. Compound **36a** was also evaluated for functional activity. In that assay, **36a** exhibited approximately the same activity (IC_{50} = 0.065 μM) as measured in the receptor binding assay.

In conclusion, SAR optimization of a lead structure found in a high throughput screening led to the discovery of new small molecule glucagon receptor antagonists with an approximately 70-fold increase of potency. Further optimization of this novel series will be reported in due course.

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23. hGR-CHO cells were grown to confluency in 24-well plates. The cells were washed twice with PBS and preincubated for 10 min at 37 °C in assay buffer as described (Feth, F.; Rascher, W.; Michel, M. C. *Arch. Pharmacol.* **1991**, *344*, 1). The cells were washed once and incubated for an additional 10 min in 250 L of assay buffer supplemented with 100 M isobutylmethylxanthine. Control DMSO (0.1%) or test compound (10 nM to 10 μM final concentration) was added to the wells followed by 50 L of glucagon to give a 10 pM final concentration. The incubation was continued for 15 min, then stopped by removal of the assay buffer and the immediate addition of 250 L ethanol (65%, v/v). After 5 min the supernatant was transferred to a microfuge tube and the ethanol was evaporated by Speed Vac. cAMP content was measured using the Amersham SPA cAMP assay kit (RPA 556) as described by the manufacturer. Results were reported as pmol per assay.
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