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Review Article

Small-molecule glucagon receptor antagonists

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CONTENTS

Abstract

The development of small-molecule glucagon antagonists reported in the scientific literature is summarized. Peptidic and nonpeptidic glucagon antagonists have been shown to attenuate glucagon-induced glucose production in animal models. To date, various classes of compounds having affinity for the glucagon receptor have been identified. Recently, a competitive human small-molecule glucagon antagonist was reported to block glucagon-stimulated glucose production in humans. Thus, small molecules that antagonize the glucagon receptor are potential therapeutic agents for the control of diabetes

Introduction

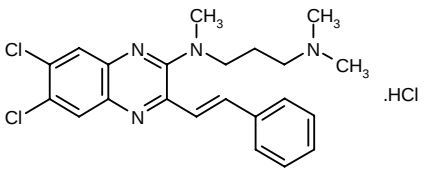
Glucagon, a 29-amino acid hormone, is secreted by the α-cells in pancreatic islets in response to falling glucose levels. The hormone binds to a specific receptor in the liver where it has numerous actions such as glycogenolysis, resulting in an increase in glucose levels, which oppose the effect of insulin. This sequence of events, mediated through cAMP, results in the generation of hepatic glucose, which helps to maintain euglycemia by preventing blood glucose levels from falling to hypoglycemic levels.

In diabetes, the bihormonal hypothesis implicates not only the lack of insulin effect but also a paradoxically elevated relative level of circulating glucagons (1). Studies using potent peptide glucagon antagonists, administered i.v., demonstrated significant decreases in blood glucose levels in diabetic animal models (2-6). Furthermore, it has been demonstrated that immunoneutralization of both exogenously and endogenously administered glucagon in animal species effectively diminished glucagon-stimulated hyperglycemia (7-9). Therefore, the continued search for selective glucagon antagonists for the treatment of type 2 diabetes is warranted.

This paper will focus primarily on small-molecule, nonpeptidic, glucagon antagonists which have been described in the literature and/or in presentations from scientific meetings. Small-molecule glucagon antagonists have also been claimed in the patent literature, such as glycoside-based compounds by Phytotech (10), Skyrin Zymogenetics (11-12), pyrimidinones by Amgen (13) and urea-based molecules by Novo Nordisk and Agouron (14-15).

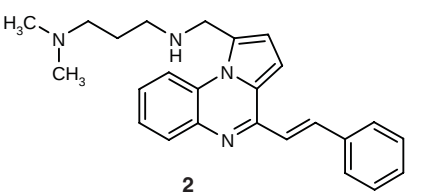
Styrylquinoxalines

The first glucagon receptor antagonist, CP-99711 (Fig. 1), was described by Pfizer in 1992 as part of a screening program (16). The styrylquinoxaline **1** displaced radiolabeled glucagon from its rat liver receptor with an IC_{50} value of 4 ± 1 μM, and it inhibited glucagon-stimulated formation of cAMP with an IC_{50} of 7 ± 1 μM. Compound **1** did not lead to an increase in cAMP in the absence of glucose. Unfortunately, further examination of other styrylquinoxalines showed that they inhibited other G-protein-coupled receptors. It was speculated that this series might interact with a common motif in G-protein-linked receptors which is most likely distinct from the glucagon-binding domain. It was postulated that the structural assembly of this compound mimics the amino terminal region of the glucagon peptide.

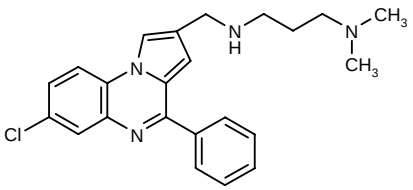


1
IC_{50} = 4 + 1 μM

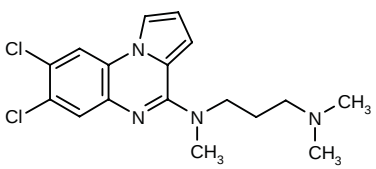
Fig. 1. The styrylquinoxaline CP-99711 as a rat glucagon receptor antagonist (16).



2
IC_{50} = 5 μM



3
IC_{50} = 10 μM



4
IC_{50} = 10 μM

Fig. 2. Examples of pyrrolo[1,2-*a*]quinoxalines having binding affinity for the rat glucagon receptor (17).

Pyrrolo[1,2-*a*]quinoxalines

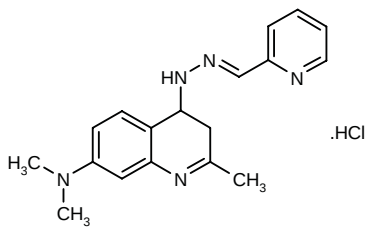
Based on the pharmacophoric requirement of CP-99711, Guillon *et al.* designed a series of pyrrolo[1,2-*a*]quinoxalines (compounds **2-4**) bearing aminoalkyl chains and aromatic substituents (17) (Fig. 2). With the exception of **2**, none of the other synthesized compounds showed any significant affinity for the glucagon receptor.

Quinoline hydrazones

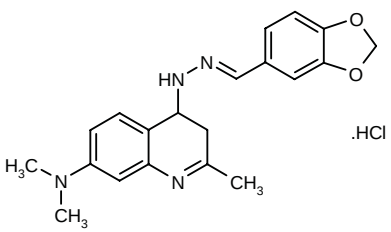
A series of quinoline hydrazones (**5-7**) having weak affinity for the human glucagon receptor was reported by Cook *et al.* (18) (Fig. 3). Systematic replacement of the hydrazine group with various isosteres in these antagonists failed to produce more active analogues. Derivatization of the distal aryl group to the piperanyl **6** led to some improvement in affinity (IC_{50} = 0.7 μM) compared to the initial lead **5**. It was found that the attachment of an annelated-cyclopentyl group in the quinoline (compound **7**) moiety was optimal for activity.

Mercapto benzimidazoles

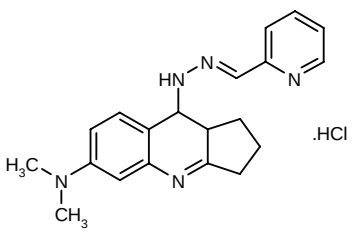
Madsen *et al.* of Novo Nordisk (19) disclosed the first nonpeptide competitive human glucagon receptor antagonist, NNC 92-1687 (Fig. 4). This antagonist **8** had an IC_{50} value of 20 μM and a functional K_i value of 9.1 μM at the human glucagon receptor. Structure-activity relationship work revealed that modification to the catechol group or to the keto linker was not well tolerated by the receptor. For example, replacement of the carbonyl oxygen for a hydroxy group led to substantial loss of activity. The presence of the hydroxy functionality in the phenyl ring



5
IC_{50} = 3 μM

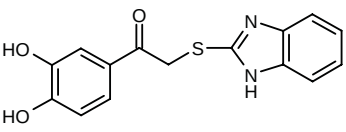


6
IC_{50} = 0.7 μM



7
IC_{50} = 0.4 μM

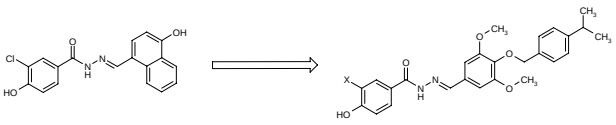
Fig. 3. Examples of quinoline hydrazones having binding affinity for the human glucagon receptor (18).



8
IC_{50} = 20 μM
K_i = 9.1 μM

Fig. 4. The mercapto benzimidazole competitive human glucagon receptor antagonist NNC 92-1687 (19).

Table I: Binding affinities of selected alkylidene hydrazides for the human glucagon receptor (21).



9
IC_{50} = 2,900 nM

No.	X	IC_{50} (nM)	Main metabolic pathway
10	Cl	23	Phase II
11	CN	20	Phase I

para to the carbonyl proved to be essential for activity. Replacements of the benzimidazole heterocycle with benzothiazole, quinoline or diphenylsubstituted imidazole or phenylsubstitued imidazole were tolerated by the receptor.

Alkylidene hydrazides

We reported (20, 21) the discovery of alkylidene hydrazide **9** (IC_{50} = 2900 nM) from the screening of our combinatorial library (Table I). Further optimization led to potent compounds, exemplified by **10** and **11**, which were competitive antagonists at the human glucagon receptor.

It was found that the presence of the benzoylhydrazide and the hydroxy group at the *para* position of the benzoyl ring is required for activity. The placement of an electron withdrawing substituent *ortho* to the hydroxy group lowered the pK_a of the hydroxy functionality, which enhanced its ability to hydrogen bond with the receptor. Substituents such as chloro and cyano substituents were optimal for activity. Compound **10** and **11** displayed mean IC_{50} values of 20 nM and 23 nM, respectively, in a competition-binding assay using [^{127}I]-radiolabeled glucagon and membranes from cells transfected with the human glucagon receptor.

In vitro metabolism studies indicated that the phenol moiety in **10** was rapidly glucuronidated (169 pmol/min/mg protein) compared to **11** (27 pmol/min/mg protein). Correspondingly, *in vivo* pharmacokinetic experiments indicated that **10** had a significantly shorter half-life than **11** ($t_{1/2}$ = 18 min *vs.* 60 min).

Compound **11** was found to be metabolized in both human and rat liver microsomes via both phase I and phase II pathways. The major metabolic pathway in rat liver microsomes was identified to be hydroxylation of the isopropyl group, followed by either further oxidation to a carbonyl or elimination of the alcohol to an alkene. Glucuronidation of the phenol moiety was found to be minor. Replacement of the chlorine atom by a cyano group therefore resulted in a metabolic swap from phase II to phase I metabolism within this series of compounds.

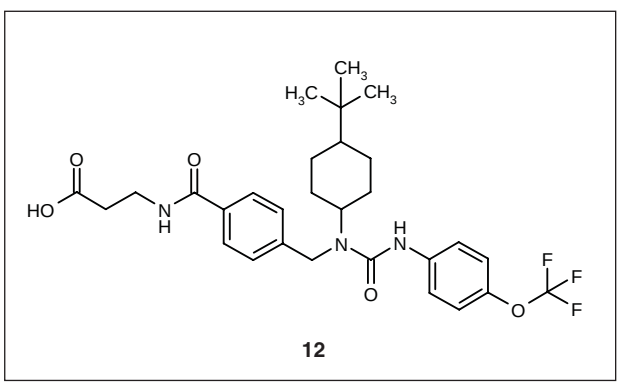
Intravenous administration of the nonpeptide glucagon antagonist **11** (3.0 mg/kg) significantly lowered blood glucose in fasted rats. Compound **11** had a mean IC_{50} value of 1.0 nM for the rat receptor. Compound **11** did not affect resting glucose levels in fed rats. However, although this series of compounds are good inhibitors of the human glucagon receptor, they are overall hydrophobic and have low oral bioavailability.

β-Alanine ureas

A series of urea-containing glucagon antagonists, exemplified by **12** (Fig. 5), was presented at the August 2002 Gordon conference by Lau *et al.* of Novo Nordisk (22).

Triaryl imidazoles

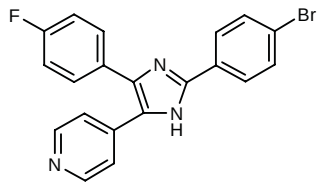
Screening of the Merck (23) sample collection of compounds identified the nonselective triarylimidazole **13** which had an IC_{50} of 270 nM in the human glucagon receptor and an IC_{50} value of 160 nM in the p38 mitogen-activated protein (MAP) kinase assay (Table II). This lead was eventually optimized for binding affinity for the glucagon receptor with selectivity over p38 MAP activity. Optimization of the lead was achieved by examining the basic imidazole hydrogen and by systematically exploring all of the 5 positions of the central imidazole core. It



12

Fig. 5. A newly disclosed urea containing human glucagon receptor antagonist (22).

Table II: Examples of triaryl imidazoles having affinity for the human glucagon receptor (23).



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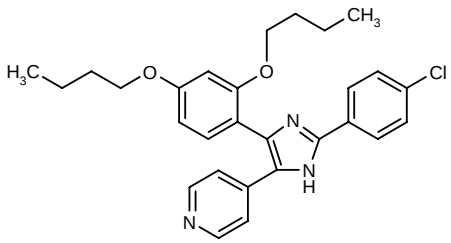


14

No.	hGluR-Mg^{2+} IC_{50} (nM)	hGluR +Mg^{2+} IC_{50} (nM)	h-p38 IC_{50} (nM)
13	270		160
14	6.5	53	20% @ 40,000

was reported that the hydrogen of the imidazole ring played a crucial role for reasonable glucagon binding since methylation of either nitrogen atom led to a decrease in activity. Likewise, the replacement of the imidazole with an oxazole gave compounds with compromised activity. Derivatization of the phenyl and pyridine ring at the C2 and C5 positions of the imidazole core indicated that halogens at the *para* position of the phenyl and the 4-pyridinyl isomer were preferred for activity at the glucagon receptor. Placement of substituents in the phenyl ring at the C4 position of the imidazole ring led to the dibutyloxy **14**, which has more than 1000-fold selectivity for the human glucagon receptor (IC_{50} = 6.5 nM) over p38 MAP activity. The activity of this class of compounds at the glucagon receptor was susceptible to the presence of Mg^{2+}, the activity of the ligands being 5- to 10-fold less active in the presence of a physiological concentration of Mg^{2+}.

2-Pyridyl-3,5-diaryl pyrroles

De Laszlo *et al.* of Merck (24) reported that the corresponding pyrrole compounds also had selectivity for the glucagon receptor over p38 MAP. The SAR requirements and biological behavior of this series, such as the cationic divalent effect, paralleled those of the imidazoles. The pyrroles appear to bind with greater affinity to the human glucagon receptor than to the mouse receptor. An example of a compound in this series is **15** (compound L-168049), which was extensively characterized and has an IC_{50} value of 170 nM at a physiological Mg^{2+} concentration. Relevant data for L-168049 are shown in Table III. L-168049 has no affinity for the GLP-1 receptor or for the rat, guinea pig or rabbit glucagon receptors. A subsequent paper (25) reported an IC_{50} value of 3.7 nM ± 3.4 nM (n=7) for L-168049 in the human glucagon receptor. This compound was demonstrated to be orally bioavailable in rats and in mice. However, no effects on blood glucose levels were observed after oral administration (50 mg/kg) in normal mice challenged with exogenously administered glucagon.

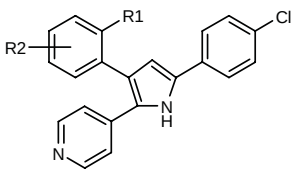
Further elaboration of the pyrrole series by appending an alkylphenyl or heteroaryl group to the C3 or C4 position of the phenyl ring on the pyrrole C3 position resulted in slightly increased affinity (26). Five of the most potent representative compounds (**16-20**) and their binding affinities for the human and mouse glucagon receptor are shown in Table III. Based on the observation that introduction of a polar group such as nitro **16** to the distal aryl group did not reduce potency, it was reported that perhaps water-solubilizing groups could be introduced in this region of the molecule. However, the paper did not contain examples of other polar/water-solubilizing substituents.

4-Arylpyridines and biphenyls

A series of 4-phenylpyridines (27) and biphenyls (28) was optimized at Bayer starting from lead **21** (IC_{50} = 7 μM), which was identified through high-throughput screening. Table IV illustrates selected structures and their binding affinities for various glucagon receptors. Most of the compounds reported have the same substituents on C2 and C6 of the pyridine ring as a means of avoiding regioisomers, the hydrophobic isopropyl group being the best substituent at these two sites. Likewise, substituents at C5 of the pyridine ring also had to be alkyl chains from 3 to 7 carbon atoms in length. SAR at C3 of the pyridine ring indicated that a hydrogen donor group was essential for activity, the 1-hydroxyethyl moiety being optimal. Resolution of the racemic compounds revealed that the (*R*)-isomer was approximately 2 times more active compared to the corresponding (*S*)-isomer. Compound **23** was highlighted in a recent report (27) as having an IC_{50} value of 110 nM.

Compound **24**, also known as Bay-27-9955, in this series was chosen for clinical development. Characterization (29) of Bay-27-9955 showed that it inhibited glucagon from the human glucagon receptor with an IC_{50} value of 110 nM. Functionally, it inhibited glucagon-stimulated cAMP generation with an IC_{50} value of 46 nM. Schild plots indicated that the inhibition was competitive with glucagon, being characterized by a pA_2 value of 7.8. This compound was reported to have little effect on the GLP-1 receptor and to be a species-selective antagonist of the glucagon receptor. Pharmacokinetic data (30) showed Bay-27-9955 to be readily absorbed after oral administration, with a bioavailability of 40%, and

Table III: The effects of Mg^{2+} on binding affinity of selected 2-pyridyl-3,5-diaryl pyrroles for various glucagon receptors (24, 26).



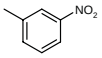
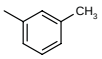
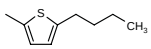
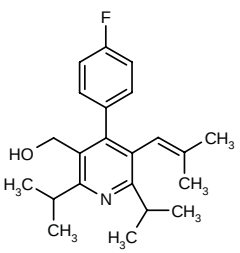
No.	R1	R_2	R_3	hGluR -Mg^{2+} IC_{50} (nM)	hGluR +Mg^{2+} IC_{50} (nM)	mGluR -Mg^{2+} IC_{50} (nM)	mGluR +Mg^{2+} IC_{50} (nM)	h-p38 IC_{50} (nM)
15 (L-168049)	OPr	H	Br	7	170	37	250	1,400
16	H		H	49	220	220	43% @ 1,000	5,600
17	H		H	9	64	100	>3,000	55% @ 5,000
18	H		H	6	34	150	2% @ 3,000	>1,000
19	F		H	2	51	120	32% @ 1,000	98% @ 40,000
20	H		H	25	810	560	>1,000	94% @ 40,000

Table IV: Binding affinities of selected 4-arylpyridines and biphenyls for various glucagon receptors (27, 28, 31, 32).



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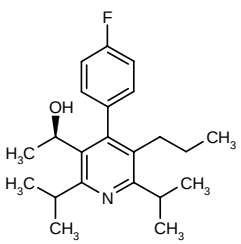

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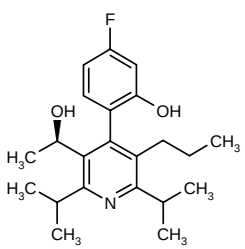
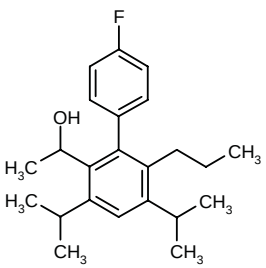


23



24

No.	Human GluR IC_{50} (nM)	Dog GluR IC_{50} (nM)	Rabbit GluR IC_{50} (nM)	Rat GluR IC_{50} (nM)	Mouse GluR IC_{50} (nM)
21	7,000				
22	110				
23	110				
24 (Bay-27-9955)	110	140	700	8,000	400

a half-life of 11-17 h in male rats. In efficacy studies, an oral dose of 200 mg of **23** almost completely blocked glucagon-induced increase in hepatic glucose output and reduced blood glucose elevation in normal males (31).

In a double-blind, placebo-controlled, crossover study subjects were administered single oral doses of Bay-27-9955 (70 mg or 200 mg) or placebo after overnight fasting and prior to infusion of somatostatin, insulin and glucagon (32). Under conditions of hyperglucagonemia, glucose production was doubled and plasma glucose concentrations significantly increased. The effects of glucagon were markedly blunted in the high-dose group

and proportionally decreased in the low-dose group. Although Bay-27-9955 was reported to be well tolerated, further development of the compound was discontinued.

Conclusions

The first small molecule having weak affinity for the rat glucagon receptor antagonist was disclosed in 1992. Since then, several classes of compounds have been described having nanomolar affinity and selectivity for the human glucagon receptor. Compounds have also been optimized in terms of oral bioavailability, solubility and other physical properties. Of most interest was Bay-27-9955, which was extensively characterized in animal models and studied in human patients. Results from the Bay-27-9955 clinical studies will help elucidate the role of glucagon in glucose homeostasis and in the design of future glucagon antagonists.

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