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Design, synthesis, structure–activity relationships, and docking studies of pyrazole-containing derivatives as a novel series of potent glucagon receptor antagonists

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ABSTRACT

Glucagon receptor antagonists possess a great potential for treatment of type 2 diabetes mellitus. A series of pyrazole-containing derivatives were designed, synthesized and evaluated by biological assays as glucagon receptor antagonists. Most of the compounds exhibited good in vitro efficacy. Two of them, compounds **17f** and **17k**, displayed relatively potent antagonist effects on glucagon receptors with IC_{50} values of 3.9 and 3.6 μM, respectively. The possible binding modes of **17f** and **17k** with the cognate receptor were explored by molecular docking simulation.

1. Introduction

Type 2 diabetes mellitus (T2DM), a chronic metabolic disorder characterized by fasting hyperglycemia consequential to dysfunction of pancreatic β-cells coupled with elevated insulin resistance and reduced insulin secretion, is affecting approximately 415 million people in 2015.[1] According to International Diabetes Federation (IDF), the estimated number of people suffering from diabetes will rise to 642 million in less than 25 years.[2] Despite many potential drug targets are continuously pursued and multiple therapeutic strategies exist for the treatment of T2DM, there is still a significant need for additional anti-diabetic agents to improve safety and efficacy.[3–6] In recent years, activation of glucagon-like peptide-1 receptor (GLP-1R) and inhibition of dipeptidyl peptidase-4 (DPP-4) to stimulate insulin secretion[7] and inhibition of sodium-glucose cotransporter-2 (SGLT-2) to regulate glucose reabsorption[8] have gained prominence in the management of T2DM, while glucagon receptor as drug target appeared to be less attractive.

Glucagon is a polypeptide hormone consisting of 29 amino acids that counteracts the action of insulin[9–13] and binds to glucagon receptor (GCGR) to trigger two transduction cascade signals resulting in stimulation of hepatic glucose production.[14–18] In patients with T2DM, inappropriately elevated glucagon levels lead to excessive hepatic glucose output, which is the main contributing factor to hyperglycemia. Therefore, blockage of glucagon-induced hyperglycemia by means of GCGR antagonism is theoretically prudent to treat T2DM.[19–25] Previous studies have validated this approach using glucagon-specific antibodies as well as peptidic and non-peptidic GCGR antagonists in animal models of diabetes.[22,25] Recently, a number of publications have claimed several chemical scaffolds as GCGR antagonists.[26–32] The biaryl containing compound **BAY-27-9955**[21] (Fig. 1) was the first active agent shown to decrease glucose output upon the administration of exogenous glucagon. Compounds **MK-3577**[33] and **MK-0893**[34,35] (Fig. 1) with β-alanine acid motif were discontinued in phase II clinical trials for treating T2DM.

In this paper, we adopted bioisosteric and conformational restraint strategies to synthesize a series of novel pyrazole-containing derivatives based on the structure of **MK-0893**. These compounds exhibited sound GCGR binding affinities and cAMP responses. Follow-up design, synthesis, structure–activity relationship (SAR) and docking studies report our efforts toward a novel series of GCGR antagonists.

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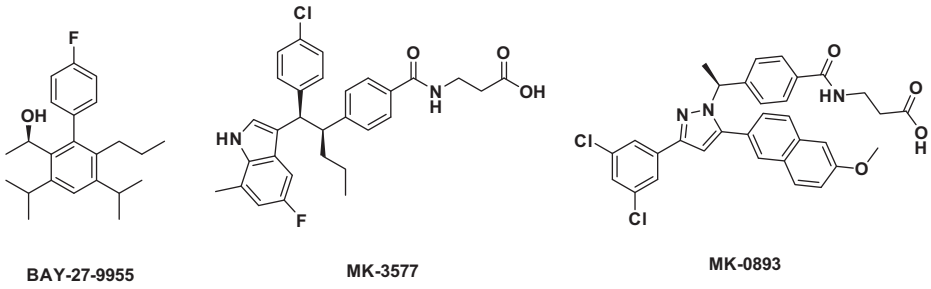

Figure 1. Structures of **BAY-27-9955**, **MK-3577** and **MK-0893**.

2. Materials and methods

2.1. Molecular design

In 2013, Fai *et al.* reported the crystal structure of the seven-transmembrane helical domain of human GCGR.[36] Comparison of the ligand-binding pocket of GCGR with that of class A GPCRs shows that GCGR has a larger binding cavity, and glucagon is capable of inducing this domain to the active state.[37] The crystal structure of GCGR (PDB ID: 4l6r) and Glide program were used for docking to study the interactions between GCGR and **MK-0893**.[38] The modeling results indicated that **MK-0893** occupied the S1 and S2 pockets and formed two hydrogen bonds in hydrogen binding districts (HB) with GCGR (Fig. 2A). In the well-defined hydrophobic S1 pocket, the 6-methoxy-2-naphthalenyl ring of **MK-0893** formed extensive hydrophobic interactions with residues Tyr149, Asp195, Ile194, Met231 and Gln232. In the S2 pocket, the 3,4-dichloro-phenyl ring formed hydrophobic interactions with residues Leu307, Val311, Tyr239, Ile235, Glu362 and Gln392. The 6-methoxy-2-naphthalenyl ring and two phenyl rings in the **MK-0893** are stacked against the side chain of Trp295 and Phe365. Moreover, 6-methoxyl and β-alanine carboxylic acid of **MK-0893** appear to form hydrogen bonds with Tyr149 and Lys381, respectively, which may account for the excellent antagonistic activity. However, the cavity formed by residues Gln232, Leu307 and Arg308 is a large pocket suggesting that introduction of steric hindrance on **MK-0893** may gain better antagonistic activity (Fig. 2B). To verify this hypothesis, we replaced β-alanine acid as substituted and cyclic β-alanine acid to obtain compound **9** (Fig. 3). Since conformational restraint is an established strategy to improve binding potency to GCGR,[39] we introduced an indane motif to obtain compound **17** (Fig. 3). A series of pyrazole-containing derivatives were subsequently synthesized and evaluated.

2.2. Chemical synthesis

The synthetic route of compound **9** was shown in Scheme 1. Briefly, esterification of commercially available compound **1** easily afforded **2**, which was subsequently transformed to compound **3** by reaction with *tert*-butyl carbazate and then reduced by sodium cyanoborohydride. Deprotection of compound **3** provided hydrazine **4**, which was smoothly reacted with ethyl 3-(3,5-dichlorophenyl)-3-oxopropanoate **5** to afford compound **6**. Triflate **7** was generated using trifluoromethanesulfonic anhydride (Tf_2O) and experienced a classical Suzuki coupling reaction with different boric acid (Z-$B(OH)_2$) to provide compound **8**. Compound **9** was then generated by hydrolysis of compound **8**, condensation with substituted 3-aminopropanoate and hydrolysis of ester.

The synthesis of compound **17** was shown in Scheme 2. Esterification of commercially available compound **10** easily afforded compound **11**, which was subsequently transformed to compound **12** by reaction with *tert*-butyl carbazate and then reduced by sodium cyanoborohydride. Deprotection of compound **12** provided hydrazine **13**, which was smoothly reacted with ethyl 3-(3,5-dichlorophenyl)-3-oxopropanoate **5** to afford compound **14**. Triflate **15** was generated using trifluoromethanesulfonic anhydride (Tf_2O) and experienced a classical Suzuki coupling reaction with boric acid (R_2-$B(OH)_2$) to provide pyrazoles **16**. Compound **17** was then generated by hydrolysis of **16**, condensation with β-alanine *tert*-butyl ester and removing of *tert*-butyl ester.

3. Results and discussion

All the synthesized compounds were evaluated for antagonism of human GCGR and the cAMP response in vitro. Antagonistic potency was measured by a competitive binding assay with *rac*-**MK-0893** as the positive control, and the results are reported as

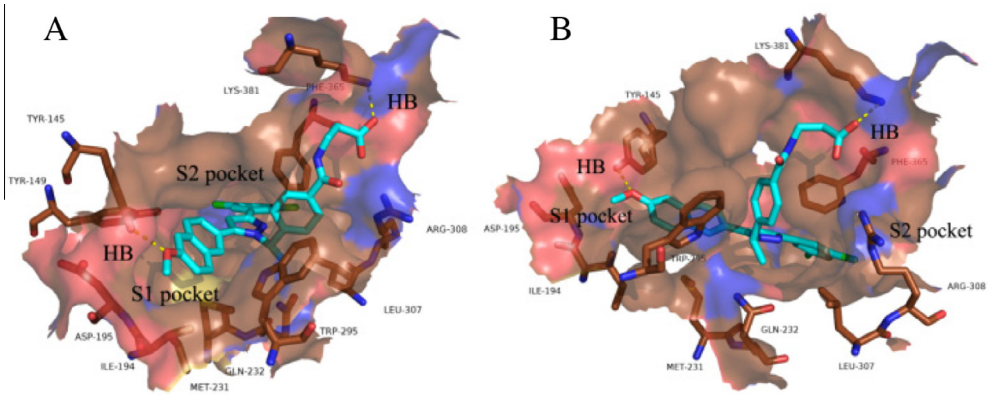

Figure 2. Docking study of **MK-0893**. All figures were prepared using PyMol (http://www.pymol.org).