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A novel series of 4-methyl substituted pyrazole derivatives as potent glucagon receptor antagonists: Design, synthesis and evaluation of biological activities

Shuangjie Shu [a,b,c,f], Antao Dai [d,f], Jiang Wang [a,b,c,f], Bin Wang [a,b,c], Yang Feng [d], Jia Li [a,b,c], Xiaoqing Cai [d], Dehua Yang [b,d], Dakota Ma [d], Ming-Wei Wang [b,d,e,*], Hong Liu [a,b,c,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China
[b] The CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (CAS), 555 Zu Chong Zhi Road, Shanghai 201203, China
[c] University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China
[d] The National Center for Drug Screening, 189 Guo Shou Jing Road, Shanghai 201203, China
[e] School of Pharmacy, Fudan University, 826 Zhangheng Road, Shanghai 201203, China

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ABSTRACT

A novel series of 4-methyl substituted pyrazole derivatives were designed, synthesized and biologically evaluated as potent glucagon receptor (GCGR) antagonists. In this study, compounds **9q**, **9r**, **19d** and **19e** showed high GCGR binding (IC_{50} = 0.09 μM, 0.06 μM, 0.07 μM and 0.08 μM, respectively) and cyclic-adenosine monophosphate (cAMP) activities (IC_{50} = 0.22 μM, 0.26 μM, 0.44 μM and 0.46 μM, respectively) in cell-based assays. Most importantly, the docking experiment demonstrated that compound **9r** formed extensive hydrophobic interactions with the receptor binding pocket, making it justifiable to further investigate the potential of becoming a GCGR antagonist.

1. Introduction

Glucagon, a 29-amino acid peptide hormone, secreted by α-cells of the pancreatic islets, was discovered by Kimball and Murlin in 1923.[1–3] As a main hormone to elevate the hepatic glucose production (HGP) by stimulating gluconeogenesis[4] and glycogenolysis,[5] glucagon, along with insulin, maintains blood glucose homeostasis.[6–8] It was reported that exogenous insulin may suppress the secretion of glucagon and immunoneutralization of insulin increased glucagon release.[9–12] Thus, in type 1 and type 2 diabetics, elevated levels of glucagon, insufficient glucagon suppression and decreased insulin secretion all contribute to hyperglycemia.[13,14]

Type 2 diabetes mellitus (T2DM) is a chronic disorder accompanied by polydipsia, polyphagia, polyuria and characterized by hyperglycemia. To reduce the risk of late-stage complications and drug resistance, new therapies for diabetes are still in need. Recently, several sodium-glucose cotransporter-2 (SGLT-2) inhibitors such as canagliflozin, dapagliflozin and embagliflozin have been approved, but they may lead to ketoacidosis.[15] Given the importance of suppressing glucagon action in treating T2DM, antagonism of glucagon receptor (GCGR) appears to be a promising approach to glycemic control.[16,17] GCGR is one of the 15 members of class B family of G-protein coupled receptors (GPCRs). In 2013, the high resolution crystal structure of seven-transmembrane (7-TM) helical domain of human GCGR was determined by research groups led by Wang and Stevens, which is conducive to understanding the molecular binding mode of the receptor.[18] 7-TM domain is the main part of GPCRs that share similar signal transduction mechanisms.[19] Compared with class A family of GPCRs, GCGR has a large ligand binding pocket. The conformational states of the full-length GCGR were revealed suggesting that glucagon binds to GCGR by a conformational selection mechanism.[20] A

Abbreviations: GCGR, glucagon receptor; cAMP, cyclic-adenosine monophosphate; T2DM, type 2 diabetes mellitus; SGLT-2, sodium-glucose cotransporter-2; HGP, hepatic glucose production; GPCRs, G-protein coupled receptors; SAR, structure-activity relationship; Ser, Serine; Arg, Arginine; Asn, Asparagine; Lys, Lysine; Leu, Leucine; Thr, Threonine; Phe, Phenylalanine; Ala, Alanine.

* Corresponding authors at: The CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (CAS), 555 Zu Chong Zhi Road, Shanghai 201203, China.

E-mail addresses: mwwang@simm.ac.cn (M.-W. Wang), hliu@simm.ac.cn (H. Liu).

[f] These authors contributed equally to this work.

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full-length GCGR structure was revealed recently demonstrating how the extracellular domain interacts with the stalk to form a compact β-sheet structure.[21]

To date, several GCGR antagonists with different chemical scaffolds were reported to display efficacies in various bioassays.[22–30] The biaryl containing compound **BAY 27-9955** (Fig. 1) was the first GCGR antagonist to blunt elevation of HGP and plasma glucose induced by exogenous glucagon.[31] Clinical trial data of compounds **MK-3577**[32] and **MK-0893**[33,34] (Fig. 1) exhibits that they are potent GCGR antagonists capable of modulating glucose homeostasis.

In this paper, a novel series of 4-methyl substituted pyrazole-containing derivatives as GCGR antagonists were designed and synthesized. Most of compounds exhibited good GCGR binding affinities and cyclic-adenosine monophosphate (cAMP) responses. Our structural optimization was dedicated to improve both GCGR binding and cAMP activities aiming at discovery of potent compounds for further development.

2. Results and discussion

2.1. Design of the target compounds

Although the structure-activity relationship (SAR) of **MK-0893** was explicit, it is very limited to understand the binding mode of this compound with GCGR. Therefore, structural modification of **MK-0893** and docking studies were carried out to make the binding information clearer. In 2016, the extra-helical binding site of **MK-0893** for GCGR was identified by a crystal complex structure.[35] Unlike glucagon binding in the 7-TM, **MK-0893** interacted with residues outside the 7-TM domain and formed interactions in two binding pockets (Fig. 2A) at allosteric sites. The β-alanine moiety formed polar interactions with residues Lys349, Ser350, Arg346, Asn404 and Lys405 including a water-mediated hydrogen bond with residues Ser350 and Leu399 between transmembrane 6 and transmembrane 7 (Fig. 2B). The hydrophobic moiety formed hydrophobic interactions with Leu329, Phe345, Leu352, Thr353 and Lys349 between transmembrane 5 and transmembrane 6. As an important strategy of lead compound optimization, introducing methyl group can modulate the physicochemical, pharmacodynamic and pharmacokinetic properties. We hypothesized that introducing methyl group on the pyrazole core may form potential hydrophobic interactions with the binding pockets (Fig. 3). Thus, a series of 4-methyl substituted pyrazole derivatives (**9** and **19**) were designed to evaluate the influence of R_1, R_2 and R_3 substituents on antagonistic activities.

2.2. Synthetic procedure of the target compounds

The desired compound **9** was synthesized according to the synthetic route shown in Scheme 1. Esterification of compound **1** produced compound **2**, which reacted with *tert*-butyl carbazate and then reduced by sodium cyanoborohydride to give compound **3**. Deprotection of compound **3** in the presence of trifluoroacetic acid afforded hydrazine **4**, which reacted with ethyl 3-(3,5-dichlorophenyl)-2-methyl-3-oxopropanoate **5** to provide compound **6**. Triflation of compound **6** with triflic anhydride generated compound **7**, which was subjected to a classic Suzuki coupling reaction to yield compound **8**. Hydrolysis of compound **8**, condensation with β-alanine *tert*-butyl ester hydrochloride and deprotection of *tert*-butyl ester obtained desired compound **9**.

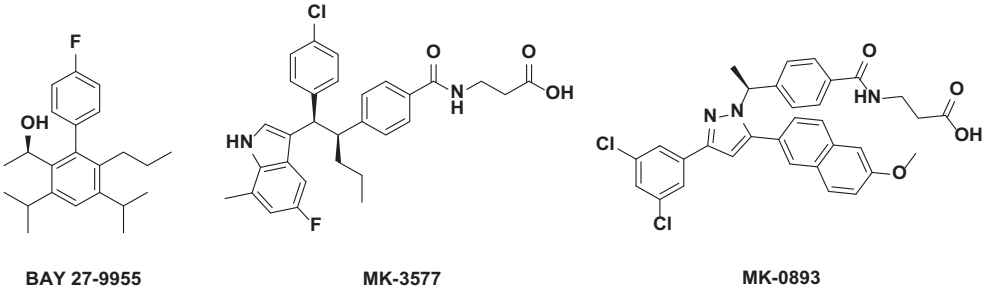

Fig. 1. Structures of **BAY 27-9955**, **MK-3577** and **MK-0893**.

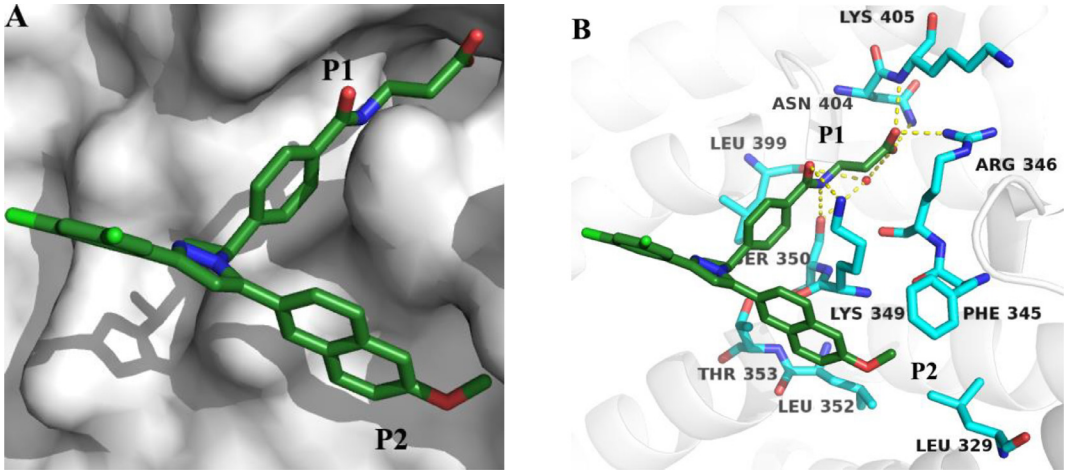

Fig. 2. **MK-0893** allosteric binding sites of GCGR. (A) surface of interactions. (B) interactions of **MK-0893** with GCGR. All figures were prepared using PyMol (http://www.pymol.org).