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Research paper

Design, synthesis, and biological evaluation of 2-(phenoxyaryl)-3-urea derivatives as novel $P2Y_1$ receptor antagonists

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ABSTRACT

A novel series of 2-(phenoxyaryl)-3-urea derivatives were designed, synthesized, and biologically evaluated for their anti-thrombotic activity. Most of compounds exhibited good inhibition against $P2Y_1$ receptor. Among them, three compounds **11**, **12**, and **13** demonstrated good $P2Y_1$ receptor antagonistic potency *in vitro* ($IC_{50} = 0.62\ \mu M$, $0.82\ \mu M$, and $0.21\ \mu M$, respectively). In antiplatelet aggregation study, four compounds **2**, **3**, **9**, and **13** showed good antiplatelet activity. The possible binding modes of compounds with $P2Y_1$ receptor were also explored by molecular docking simulation. The docking studies demonstrated that compound **13** interacted well with Phe119 through hydrophobic interaction and modestly improved the $P2Y_1$ receptor antagonistic activity, making it justifiable for further investigation.

1. Introduction

Adenosine-5′-diphosphate (ADP) is a key activator of platelets and plays a vital role in platelet activation and thrombus formation [1]. ADP activates platelets by simultaneously stimulating two G-protein coupled receptors (GPCR) $P2Y_1$ receptor and $P2Y_{12}$ receptor. $P2Y_1$ and $P2Y_{12}$ receptors are important in the process of hemostasis and thrombosis regulation [2]. $P2Y_{12}$ receptor inhibited adenylyl cyclase through Gi-dependent pathway, while $P2Y_1$ receptor facilitated platelet shape change and reversible aggregation through Gq-dependent pathway [3,4]. $P2Y_{12}$ receptor antagonists are clinically well-validated drugs for antithrombotic therapy such as clopidogrel [5–10], prasugrel [11], cangrelor [12], and ticagrelor [13]. Although there are many launched antithrombotic drugs targeting $P2Y_{12}$ receptor, these $P2Y_{12}$ receptor antagonists feature more or less bleeding liability [14]. In recent years, $P2Y_1$ receptor has emerged as an attractive target for anti-thrombotic therapies [15–19]. Léon et al. reported platelets from $P2Y_1$ receptor deficient mice were resistant to ADP-induced shape change and aggregation [20,21]. It suggests that $P2Y_1$ receptor antagonists could be a potential treatment for a variety of thrombotic diseases and may improve safety margins relative to $P2Y_{12}$ receptor antagonists.

(1′*R*,2′*S*,4′*S*,5′*S*)-4-(2-iodo-6-methylaminopurin-9-yl)-1-[(phosphato)methyl]-2(phosphato)bicycle[3.1.0]-hexane (**MRS2500**) and 1-(2-(2-*tert*-butylphenoxy)pyridin-3-yl)-3–4-(trifluoromethoxy) phenylurea (**BPTU)** are representative $P2Y_1$ receptor antagonists (Fig. 1). **MRS2500** is a potent adenine nucleotide-based $P2Y_1$ receptor antagonist ($K_i = 0.78$ nM), which inhibited ADP activated platelet aggregation *in vitro* and *in vivo*. Besides, **MRS2500** could also reduce arterial thrombosis with a moderate prolongation of the bleeding time, with no spontaneous bleeding liability [22]. However, the chemical and enzymatic stabilities of **MRS2500** and other adenine nucleotide-based $P2Y_1$ receptor antagonists are less than desirable (mainly because of their poor pharmacokinetic profiles and limited oral bioavailability), which would be expected to limit their usefulness as oral drug candidate. Thus, the discovery of novel $P2Y_1$ receptor antagonists with improved pharmaceutical characteristics could have significant utility in the treatment of a variety of thromboembolic disorders [23]. Chao et al. reported a

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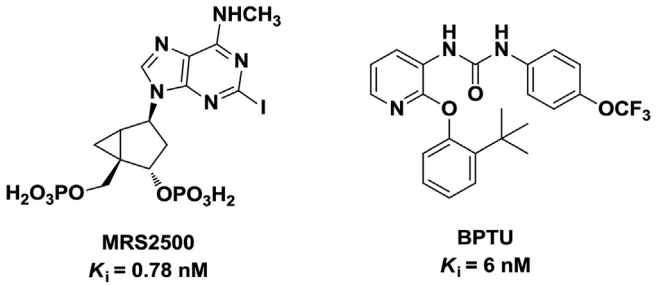

Fig. 1. Representative $P2Y_1$ receptor antagonists.

series of non-nucleotide small molecule 2-(phenoxypyridine)-3-urea analogs as $P2Y_1$ receptor antagonists [24–26]. Among them, **BPTU** showed good binding affinity with $P2Y_1$ receptor ($K_i = 6$ nM) and moderate antiplatelet activity in the ADP-induced platelet aggregation assay *in vitro*. The pharmacokinetic profile of **BPTU** revealed that this compound had moderate half-life ($t_{1/2} = 1.43$ h) and bioavailability (F = 18%) when orally dosed at 30 mg/kg in rats [24]. Other optimization and modification was reported subsequently to improve $P2Y_1$ receptor binding affinity and physicochemical property [27–32].

Zhao et al. reported the crystal structures of two different ligands (**MRS2500** and **BPTU**) binding with $P2Y_1$ receptor [33]. Nucleotide ligand **MRS2500** occupied a pocket in extracellular part of this receptor, which is orthosteric site of $P2Y_1$ receptor, while **BPTU** bound to $P2Y_1$ receptor on the lipid interface of the transmembrane domain, instead of interacting within the seven-transmembrane helical bundle (Fig. 2A and B). The location of this ligand-binding site indicates that **BPTU** acts as an allosteric modulator of $P2Y_1$ receptor. In the absence of the endogenous ligand, allosteric modulators do not exert any effect, thus preserving the physiological effects of $P2Y_1$ receptor [34]. Besides, allosteric ligands can act in a lower dosage exerting fewer side effects while orthosteric antagonists usually act in a higher dosage because their competition with endogenous ligands. Further, as allosteric ligand binding sites are located in non-conserved regions of $P2Y_1$ receptor, as opposed to the highly conserved orthosteric ligand binding sites, it is possible to develop highly-selective ligands [35]. Therefore, **BPTU** may represent a promising candidate for the development of new antiplatelet therapies. The relatively shallow ligand-binding pocket which was formed by aromatic and hydrophobic residues accommodated **BPTU** predominantly through hydrophobic interactions. The only polar interactions are represented by two hydrogen bonds between the nitrogen atoms of **BPTU**'s urea group and the mainchain carbonyl of Leu102 [10]. In addition, the pyridyl group forms hydrophobic interactions with Ala106 and Phe119. The ureido phenyl ring forms two aromatic edge-to-face interactions with Phe62 and Phe66 (Fig. 2C and D).

In this paper, a novel series of 2-(phenoxyaryl)-3-urea derivatives as $P2Y_1$ receptor antagonists were designed and synthesized. Most of compounds exhibited good $P2Y_1$ receptor binding affinities and good antiplatelet activity. Our structural optimization was dedicated to improve both $P2Y_1$ receptor binding and antiplatelet activities aiming at discovery of potent compounds for further development.

2. Chemistry

2.1. Design of 2-(phenoxyaryl)-3-urea derivatives

Although a number of **BPTU** analogs have been reported in the past few years, most of the structure optimization focuses on optimization of the phenoxy ring [36–38], however, there are few reports about the optimization of ureido phenyl ring, which is very important to form two aromatic edge-to-face interactions with Phe62 and Phe66. According to the binding mode of **BPTU** with $P2Y_1$ receptor, ureido is important as a pharmacophore. As a result, our work mainly focuses on the hydrophobic groups. Bioisosterism strategy was applied to replace trifluoromethoxyphenyl with thiophen ring. Using this strategy, compounds **1**–**7** were obtained. In addition, there is no report about alkyl substitution in ureido. It is assumed that alkyl group may enhance the hydrophobic interaction with Leu102 and well occupy the binding site, which would improve $P2Y_1$ receptor binding affinity. As a result, different alkyl groups were introduced to the ureido, and compounds **8**–**12** were synthesized. Notably, there is a cavum in the binding pocket of **BPTU** with $P2Y_1$ receptor near Phe119. It was hypothesized that introduction of a small group at the pyridine ring may accommodate well in the cavum, which therefore increases the hydrophobic interaction with Phe119 (Fig. 2C). Based on this assumption, we synthesized compounds **13**–**15**. All 2-(phenoxyaryl)-3-urea derivatives were evaluated for their inhibitory activity against $P2Y_1$ receptor (Fig. 3).

2.2. Synthesis of 2-(phenoxyaryl)-3-urea derivatives

The general synthetic route to 2-(phenoxyaryl)-3-urea derivatives is outlined in Scheme 1. First, 5-nitrothiophene-2-carboxylic acid (**16**), as the starting material, condensation with different alcohols and amines in different conditions afforded corresponding esters and amides (**17a-f**). Subsequent reduction of the nitro group with Zn/ammonium formate gave corresponding amines **18a-f**. Reacting 2-(*tert*-butyl)phenol (**19**) with 2-chloro-3-nitropyridine in DMF at 80 °C afforded the 2-(2-(*tert*-butyl)phenoxy)-3-nitropyridine (**20**). Subsequent reduction of the nitro group with Zn/ammonium formate gave the 2-(2-(*tert*-butyl)phenoxy)pyridin-3-amine **21**. Amine **21** was then reacted with diphosgene in the presence of 1,8-bis(dimethylamino)-napthalene to provide the key intermediate isocyanate **22**. Isocyanate **22** reacted with different amines (**18a-f**) at 80 °C under microwave irradiation, affording compounds **1**–**6**. Treatment of different commercially available amine with key intermediate **22** gave the corresponding target compounds **7**–**12**.

The synthesis of target compounds **13**–**15** is shown in Scheme 2. Briefly, condensation of commercially available 2-(*tert*-butyl) phenol **19** with substituted 2-chloro-3-nitro compounds **23a-c** easily afforded compounds **24a-c**, which was subsequently transformed to amines **25a-c** using Zn/ammonium formate. A nucleophilic substitution reaction of 1-isocyanato-4-(trifluoromethoxy) benzene **26** with amines **25a-c** provided compounds **13**–**15**.

3. Results and discussion

All the synthesized compounds were evaluated for inhibition rate and IC_{50} test against $P2Y_1$ receptor *in vitro* and anti-platelet activity in rats. Antagonistic potency was measured by a competitive binding assay with **BPTU** as the positive control, and the results are reported as inhibition rate at 50 μM and concentration for 50% inhibition (IC_{50}). SAR for these compounds is summarized in Tables 1 and 2.

We first focused on optimization of the ureido phenyl ring by replacing the phenyl moiety with substituted thiophen rings and alkyl groups. Initially, our SAR started from 5-substituted thiophen derivatives. The effects of substitution on the thiophen ring were explored. Compounds **1** and **2** were obtained by introduction of a small ester groups at the 5-position of the thiophen ring. These compounds displayed good inhibition activities against $P2Y_1$