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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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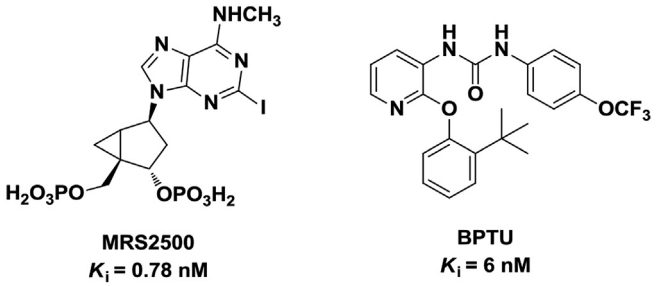


Fig. 1. Representative $P2Y_1$ receptor antagonists.

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 $P2Y_1$

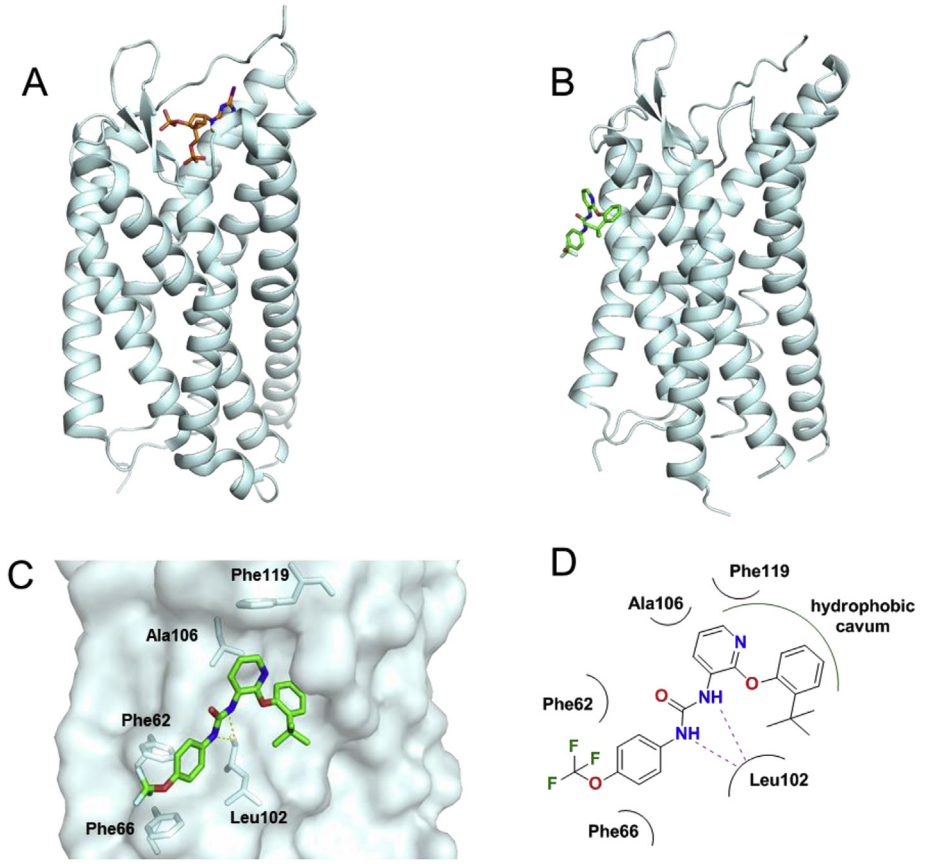


Fig. 2. (A)The binding site of **MRS2500** with $P2Y_1$ receptor (PDB ID: 4XNW); (B)The binding site of **BPTU** with $P2Y_1$ receptor (PDB ID: 4XNV); (C)The binding mode of **BPTU** with $P2Y_1$ receptor; (D) 2D interaction diagram between BPTU and $P2Y_1$ receptor (hydrogen bond was presented by purple). The interaction was drawn using Chemdraw 12.0 Software.

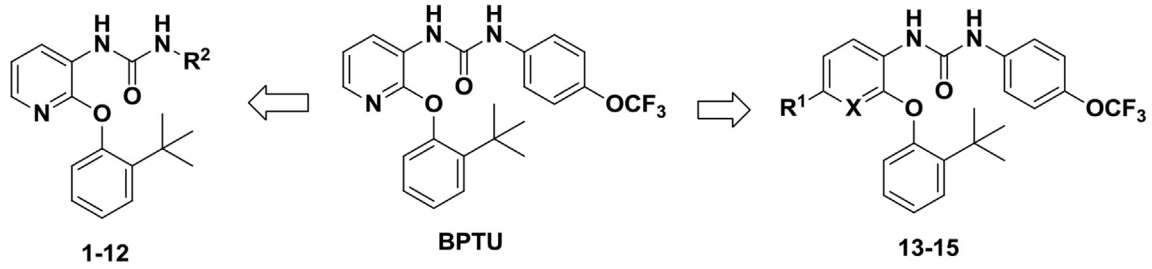
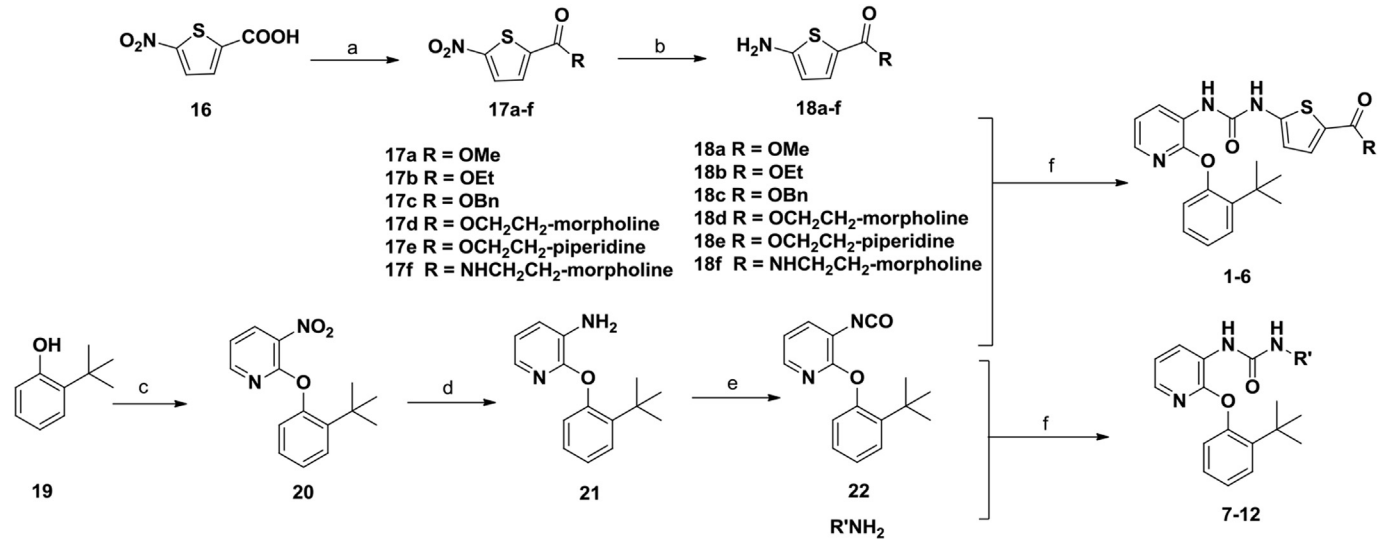


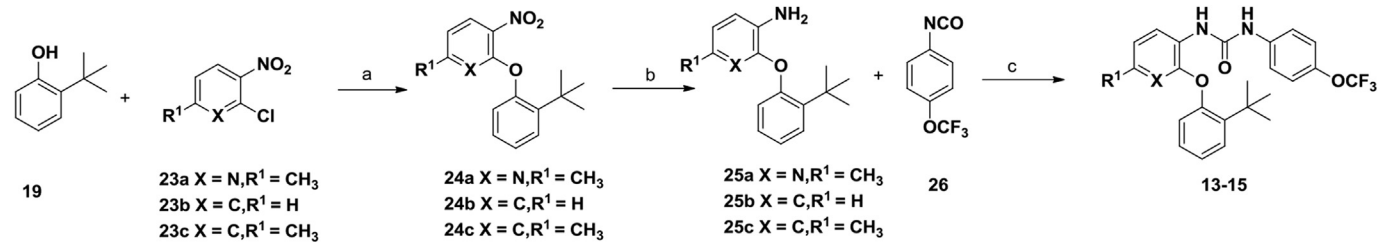
Fig. 3. Design of 2-(phenoxyaryl)-3-urea derivatives.

receptor, with 91.41% and 91.78% inhibition rate at 50 μM. Introduction of bigger ester groups gave compounds **3**–**5**. All these three compounds exhibited mild inhibition activities against $P2Y_1$ receptor, with 53.07%, 52.84%, 27.97% inhibition rate at 50 μM, respectively. While introduction of amide group and cyano group afforded compound **6** and **7**, which displayed no inhibition activities toward $P2Y_1$ receptor at the concentration of 50 μM. The effects of alkyl substitution were also explored. As shown in Table 1, most of the alkyl derivatives exhibited good *in vitro* potency against $P2Y_1$ receptor. Additionally, all of these antagonists occupied a narrow potency range, indicating tolerability for a wide variety of alkyl substituents. Basd on the crystal structure of **BPTU**, introducing small group at the *ortho*-position of the pyridine ring may increase the hydrophobic interaction with Phe119. Compound **13** exhibited good potency against $P2Y_1$ receptor, with 90.06% inhibition rate at 50 μM. While replacement the pyriding ring to the phenyl ring, compound **14** also displayed high potency (inhibition rate = 91.03%). However, introducing methyl group on the phenyl ring, the potency decreased (inhibition rate = 30.47%).

Furthermore, compounds with good inhibition rate at 50 μM were selected to test IC_{50}. The ester thiophen derivatives demonstrated the benzyl substitution was better than methyl and ethyl substitution (IC_{50} = 12.92 μM, 12.52 μM, 7.19 μM, respectively). All alkyl groups were tolerated for $P2Y_1$ receptor inhibition. For example, compounds **8** (*n*-butyl) and **9** (*trans*-4-methyl-cyclohexan) exhibited mild inhibitory activities against $P2Y_1$ receptor with IC_{50} values of 9.98 μM and 9.05 μM, respectively. Compound containing a cyclopentyl substituent (**10**) displayed good $P2Y_1$ receptor potency (IC_{50} = 2.41 μM). Introducing steric hindrance at the urea group (**11** and **12**) also provided potent $P2Y_1$ receptor inhibitory activity with IC_{50} values of 0.62 μM and 0.82 μM, respectively. Introduction of a small group like methyl group at the *ortho*-



Scheme 1. Reagents and conditions: (a) (i) for **17a** and **17b** thionyl chloride, reflux, MeOH; (ii) for **17c**, **17d**, and **17e** thionyl chloride, TEA, CH_2Cl_2, 80 °C; (iii) for **17f** HATU, DIEA, CH_2Cl_2; (b) ammonium formate, Zn, EtOH/EtOAc (4:1); (c) 2-chloro-3-nitropyridine, Cs_2CO_3, DMF, 80 °C; (d) ammonium formate, Zn, EtOH/EtOAc (4:1); (e) diphosgene, 1,8-bis(dimethylamino)-napthalene, CH_2Cl_2, 0 °C; (f) toluene (dry), 80 °C.



Scheme 2. Reagents and conditions: (a) Cs_2CO_3, DMF, 80 °C; (b) ammonium formate, Zn, EtOH/EtOAc (4:1); (c) toluene (dry), 80 °C.

position of pyridine (**13**) showed the best P2Y1 receptor potency among all the compounds ($IC_{50} = 0.21$ μM), which is the same as the positive control **BPTU**. While replacement the pyridine ring to the phenyl ring, compound **14** exhibited mild $P2Y_1$ receptor antagonistic potency ($IC_{50} = 7.53$ μM).

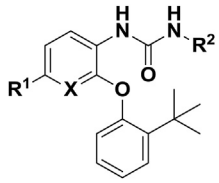
Six 2-(phenoxyaryl)-3-urea derivatives were selected to evaluate their inhibitory effect on ADP-induced platelet aggregation in rats when orally dosed of 3 mg/kg. **BPTU** was used as the positive control (Table 3). Compounds **2** and **3** showed moderate anti-platelet activity (inhibition rate = 50.60% and 69.40%). However, compounds **8** and **11** did not exhibit good anti-platelet activity, indicating that increasing lipophilicity of $P2Y_1$ receptor inhibitors reduced the *ex vivo* potency. Compound **13**, introducing the methyl group at the *ortho*-position of pyridine displayed good anti-platelet activity (inhibition rate = 68.90%), which is the same as the positive control **BPTU**.

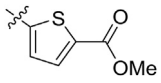
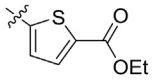
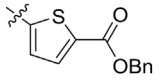
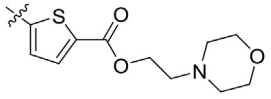
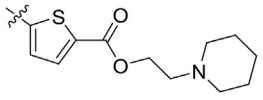
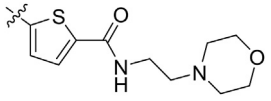
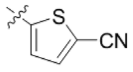
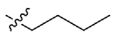
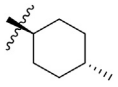
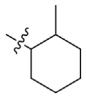
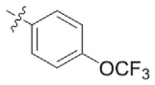
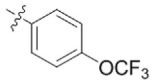
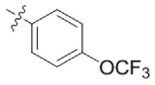
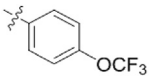
To gain insight into the binding modes and interactions of these compounds against $P2Y_1$ receptor, molecular modeling was carried out using Glide program. The crystal structure of $P2Y_1$ receptor (PDB ID: 4XNV) was used in molecular docking simulation (Fig. 4A). It was observed that compounds **11**, **12**, and **13** occupied the same binding pockets and shown similar binding modes as the positive control **BPTU**. The alkyl moiety of compounds **11** and **12** formed hydrophobic interactions with the residue Leu102 (Fig. 4B and C). Compared to compounds **11** and **12**, the 4-(trifluoromethoxy) group of compound **13** and **BPTU** (Fig. 4D) formed a more favorable π-π stacking interaction with Phe62 and Phe66. In addition, the introduction of a methyl group at the *ortho*-position of pyridine, compound **13** accommodates very well with the cavum in the binding pocket, which formed hydrophobic interaction with Phe119. Inspired by the docking results, we proposed that addition of appropriate hindrance on the pyridine moiety might modestly improve the inhibitory activity against $P2Y_1$ receptor. Molecular docking was used to investigate the binding modes of compounds **13** and **15** with ligand **BPTU**, which was illustrated in Fig. 4F. From the molecular docking result, we noted that these two compounds shared the similar binding modes with $P2Y_1$ receptor. However, there is a dihedral angle about 12–15° between the phenyl ring of compound **15** and the pyridine ring of compound **13**. As a result, the methyl group in compound **15** could not accommodate well in the cavum, compared with compound **13**, which made its inhibition rate decreased.

4. Conclusion

In conclusion, we designed, synthesized, and evaluated a series of 2-(phenoxyaryl)-3-urea derivatives as novel $P2Y_1$ receptor inhibitors. *In vitro* evaluation found that most of the compounds exhibited good *in vitro* potency against $P2Y_1$ receptor in low micromolar range. Among these, compounds **11** ($IC_{50} = 0.62$ μM), **12** ($IC_{50} = 0.82$ μM), and **13** ($IC_{50} = 0.21$ μM) showed good $P2Y_1$ receptor potency. Compounds **2**, **3**, **9,** and **13** were found to display the good anti-platelet activity in rats. Molecular docking results reveal that both hydrophobic and hydrogen bonding interactions are important for the inhibitory potency. These potent compounds represent a novel scaffold for the development of $P2Y_1$ receptor

Table 1
Cellular binding assay results for 2-(phenoxyaryl)-3-urea derivatives.



Compd.	R^1	X	R^2	Inhibition Rate[a] %
1	**H**	**N**		91.41 ± 1.71
2	**H**	**N**		91.78 ± 2.43
3	**H**	**N**		53.07 ± 8.08
4	**H**	**N**		52.84 ± 11.60
5	**H**	**N**		27.97 ± 6.93
6	**H**	**N**		3.76 ± 0.68
7	**H**	**N**		0.80 ± 9.20
8	**H**	**N**		91.09 ± 2.32
9	**H**	**N**		84.77 ± 7.74
10	**H**	**N**		95.59 ± 1.72
11	**H**	**N**		97.34 ± 0.02
12	**H**	**N**		97.31 ± 0.30
13	**CH_3**	**N**		90.06 ± 2.43
14	**H**	**C**		91.03 ± 4.52
15	**CH_3**	**C**		30.47 ± 23.62
BPTU	**H**	**N**		91.59 ± 2.63

[a] The test was carried out in 1321N1/$P2Y_1$ cell strain, **MRS2365** as $P2Y_1$ receptor agonist.

Table 2
Cellar binding assay of 2-(phenoxyaryl)-3-urea derivatives *in vitro*.

Compd.	IC_{50}[a] (μM)	Compd.	IC_{50}[a] (μM)
1	12.92 ± 0.05	**10**	2.41 ± 1.87
2	12.52 ± 0.75	**11**	0.62 ± 0.17
3	7.19 ± 1.16	**12**	0.82 ± 0.04
8	9.98 ± 0.54	**13**	0.21 ± 0.14
9	7.05 ± 1.25	**14**	7.53 ± 6.08
BPTU	0.28 ± 0.24		

[a] The test was carried out in 1321N1/$P2Y_1$ cell strain, **MRS2365** as $P2Y_1$ receptor agonist.

Table 3
Inhibitory effect of 2-(phenoxyaryl)-3-urea derivatives on ADP-induced platelet aggregation in rats at a dose of 3 mg/kg.

Compd.	Inhibition Rate[a] %	Compd.	Inhibition Rate[a] %
2	50.60%	**9**	57.60%
3	69.40%	**11**	26.67%
8	22.00%	**13**	68.90%
BPTU	67.40%		

[a] Assay details are described in Experimental Section. Aggregation data refer to *ex vivo* measurements 2 h after intragastric administration.

inhibitors. Current efforts are aimed at further modifying these compounds to achieve more potent and selective $P2Y_1$ receptor inhibitors.

5. Experimental section

5.1. General information

General procedures of the synthesis route were described as below. The reagents (chemicals) were purchased from Alfa Aesar, Sigma, Acros and Shanghai Chemical Reagent Company, and used without further purification. Analytical thin-layer chromatography (TLC) was performed on HSGF 254 (150–200 μm thickness, Yantai Huiyou Company, China). Column chromatography was performed with CombiFlash® Companion system (Teledyne Isco, Inc.). Nuclear magnetic resonance (NMR) spectra were performed on a Brucker AMX-300, AMX-400, AMX-500, and AMX-600 NMR (TMS as IS). Chemical shifts were reported in parts per million (ppm, δ) downfield from tetramethylsilane. Proton coupling patterns were described as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), triplet of doublets (td), quartet (q), multiplet (m), and broad (br). Low- and high-resolution mass spectra (MS and HRMS) were given with electrospray ionization (ESI) and electric ionization (EI) produced by Finnigan MAT-95. Melting points (mp) were measured in open capillary tubes, using an SGW X-4 melting point apparatus (−50–400 °C). All compounds were confirmed with over 90% purity which were determined by Agilent-1100 HPLC with binary pump, photodiode array detector (DAD), using Agilent Extend-C18 column (150 × 4.6 mm, 5 μm). All compounds were analyzed by, using MeOH/H_2O = 80:20 (v/v) (30 min, 1 mL/min) and calculated the peak areas at 214 nm.

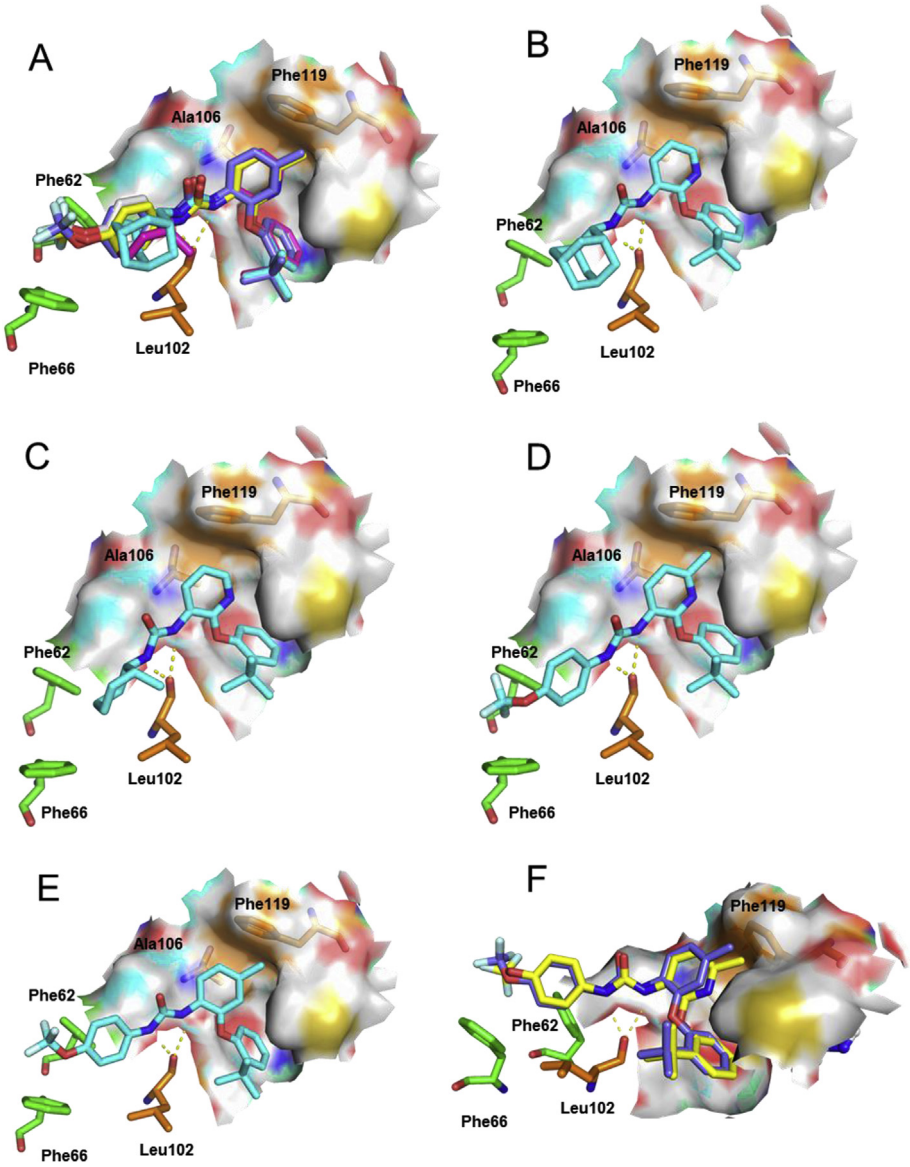


Fig. 4. (A) Superposition of compounds **11** (blue), **12** (pink), **13** (yellow), and **15** (purple) with ligand **BPTU** from 4XNV. Docking structures of compounds **11** (B), **12** (C), **13** (D) and **15** (E) in the active site of $P2Y_1$. (F) Superposition of compounds **13** (yellow) and **15** (purple). Hydrogen atoms have been omitted for clarity. All figures were prepared using PyMol (http://www.pymol.org).

5.2. General procedures

The preparation and data of key intermediate **20**–**22** have already been reported [23]. The preparation intermediate **23**–**25** are the same as the synthetic route of **20**–**22**.

5.2.1. Methyl 5-(3-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)ureido) thiophene-2-carboxylate *1*

To a solution of isocyanate **22** (100 mg, 0.81 mmol) in toluene (1 mL) in microwave tube was added methyl 5-aminothiophene-2-carboxylate (191 mg, 1.21 mmol). The resulting mixture was heated with shaking at 80 °C for 1.5 h. The solvent was removed by vacuum, extracted with ethyl acetate and water, the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by column chromatography (petroleum ether/ethyl acetate 4:1 v/v) to yield as a white solid (200 mg, 63%). m.p. 109–110 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (dd, J = 8.0, 2.0 Hz, 1H), 7.71 (dd, J = 4.8, 2.0 Hz, 1H), 7.58 (d, J = 4.0 Hz, 1H), 7.48 (dd, J = 8.0, 2.0 Hz, 1H), 7.25–7.12 (m, 2H), 7.09 (dd, J = 8.0, 4.8 Hz, 1H), 6.86 (dd, J = 8.0, 1.6 Hz, 1H), 6.53 (d, J = 4.4 Hz, 1H), 3.83 (s, 3H), 1.39 (s, 9H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 162.5, 152.9, 152.4, 151.5, 147.6, 141.1, 139.8, 132.4, 127.1, 127.0, 124.7, 124.0, 123.8, 121.0, 118.9, 110.1, 51.7, 34.3, 30.5 ppm. LC-MS: 424.0 $[M-H]^-$, 425.9 $[M+H]^+$; HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd for $C_{22}H_{22}N_3O_4S$ 424.1337; Found 424.1341. Purity: 97.9%.

Compounds **2**–**12** were prepared according to the procedures described for compound **1**.

5.2.2. Ethyl 5-(3-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)ureido) thiophene-2-carboxylate *2*

m.p. 205–206 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (dd, J = 8.0, 2.0 Hz, 1H), 7.71 (dd, J = 4.8, 2.0 Hz, 1H), 7.58 (d, J = 4.4 Hz, 1H), 7.48 (dd, J = 8.0, 2.0 Hz, 1H), 7.27–7.12 (m, 2H), 7.09 (dd, J = 8.0, 4.8 Hz, 1H), 6.86 (dd, J = 8.0, 1.6 Hz, 1H), 6.52 (d, J = 4.4 Hz, 1H), 4.29 (q, J = 7.2 Hz, 2H),1.39 (s, 9H), 1.35 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR

(125 MHz, DMSO-d_6) δ 162.1, 152.9, 152.4, 151.5, 147.4, 141.1, 139.7, 132.2, 127.1, 126.9, 124.7, 124.0, 123.8, 121.3, 118.9, 110.0, 60.2, 34.3, 30.5, 14.3 ppm. LC-MS: 438.0 $[M-H]^-$, 440.0 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{23}H_{24}N_3O_4S$ 438.1493; Found 438.1497. Purity: 97.7%.

5.2.3. Benzyl 5-(3-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)ureido) thiophene-2-carboxylate ***3***

m.p. 197–198 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.58 (dd, J = 8.0, 1.6 Hz, 1H), 7.70 (dd, J = 4.8, 1.6 Hz, 1H), 7.62 (d, J = 4.4 Hz, 1H), 7.48 (dd, J = 8.0, 2.0 Hz, 1H), 7.46–7.29 (m, 5H), 7.25–7.13 (m, 2H), 7.08 (dd, J = 8.0, 4.8 Hz, 1H), 6.86 (dd, J = 8.0, 1.6 Hz, 1H), 6.53 (d, J = 4.4 Hz, 1H), 5.29 (s, 2H), 1.38 (s, 9H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.9, 152.9, 152.4, 151.5, 147.8, 141.1, 139.8, 136.3, 132.7, 128.5, 128.0, 127.9, 127.1, 127.0, 124.7, 124.0, 123.8, 120.8, 118.9, 110.1, 65.6, 34.3, 30.5 ppm. LC-MS: 500.0 $[M-H]^-$, 501.9 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{28}H_{26}N_3O_4S$ 500.1650; Found 500.1656. Purity: 96.0%.

5.2.4. 2-Morpholinoethyl 5-(3-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)ureido)thiophene-2-carboxylate ***4***

m.p. 166–168 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.58 (dd, J = 8.0, 1.6 Hz, 1H), 7.71 (dd, J = 4.8, 1.6 Hz, 1H), 7.61 (d, J = 4.0 Hz, 1H), 7.48 (dd, J = 8.0, 1.6 Hz, 1H), 7.25–7.13 (m, 2H), 7.12–7.06 (dd, J = 8.0,4.8 Hz, 1H), 6.86 (dd, J = 8.0, 1.6 Hz, 1H), 6.53 (d, J = 4.0 Hz, 1H), 4.41 (t, J = 1.6 Hz, 2H), 3.70 (t, J = 4.8 Hz, 4H), 2.77 (t, J = 1.6 Hz, 2H), 2.60 (t, J = 4.8 Hz, 4H), 1.39 (s, 9H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.0, 152.9, 152.4, 151.5, 147.6, 141.1, 139.8, 132.4, 127.1, 127.0, 124.7, 124.0, 123.8, 121.1, 118.9, 110.1, 66.2, 61.7, 56.6, 53.4, 34.3, 30.5 ppm. LC-MS: 523.0 $[M-H]^-$, 524.9 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{27}H_{31}N_4O_5S$ 523.2021; Found 523.2030. Purity: 97.1%.

5.2.5. 2-(Piperidin-1-yl)ethyl 5-(3-(2-(2-(tert-butyl)phenoxy) pyridin-3-yl)ureido)thiophene-2-carboxylate ***5***

m.p. 180–182 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.58 (dd, J = 8.0, 1.6 Hz, 1H), 7.71 (dd, J = 4.8, 1.6 Hz, 1H), 7.64 (d, J = 4.4 Hz, 1H), 7.48 (dd, J = 8.0, 1.6 Hz, 1H), 7.24–7.14 (m, 2H), 7.09 (dd, J = 8.0, 5.2 Hz, 1H), 6.86 (dd, J = 8.0, 1.6 Hz, 1H), 6.54 (d, J = 4.0 Hz, 1H), 4.47 (t, J = 5.6 Hz, 2H), 2.98 (brs, 2H), 2.81 (brs, 4H), 1.74–1.68 (m, 4H), 1.57–1.52 (m, 2H), 1.39 (s, 9H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.8, 153.0, 152.4, 151.5, 141.1, 139.8, 127.1, 127.1, 127.0, 124.7, 124.0, 123.8, 118.9, 110.1, 59.7, 34.3, 30.5, 20.7, 14.1 ppm. LC-MS:521.0 $[M-H]^-$, 523.0 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{28}H_{33}N_4O_4S$ 521.2228; Found 521.2238. Purity: 96.6%.

5.2.6. 5-(3-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)ureido)-N-(2-morpholinoethyl)thiophene-2-carboxamide ***6***

m.p. 140–141 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (dd, J = 8.0, 1.6 Hz, 1H), 6.90 (dd, J = 4.8, 1.6 Hz, 1H), 6.68 (dd, J = 8.0, 1.6 Hz, 1H), 6.64 (d, J = 4.4 Hz, 1H), 6.44–6.34 (m, 2H), 6.28 (dd, J = 8.0, 5.2 Hz, 1H), 6.06 (dd, J = 8.0, 1.2 Hz, 1H), 5.72 (d, J = 4.0 Hz, 1H), 2.91 (t, J = 4.8 Hz, 4H), 2.69 (t, J = 6.8 Hz, 2H), 1.77 (m, 6H), 0.59 (s, 9H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 161.8, 152.8, 152.4, 151.4, 144.7, 141.1, 139.4, 128.6, 127.1, 126.6, 126.10, 124.7, 124.3, 123.8, 118.9, 109.5, 66.2, 59.7, 57.6, 53.3, 36.3, 34.3, 30.5 ppm. LC-MS: 522.0 $[M-H]^-$, 524.0 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{27}H_{32}N_5O_4S$ 522.2180; Found 522.2191. Purity: 97.7%.

5.2.7. 1-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)-3-(5-cyanothiophen-2-yl)urea ***7***

m.p. 238–240 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H), 8.90 (s, 1H), 8.48 (dd, J = 7.9, 1.7 Hz, 1H), 7.76 (dd, J = 4.8, 1.7 Hz, 1H), 7.72 (dd, J = 4.2, 2.7 Hz, 1H), 7.44 (dd, J = 7.9, 1.7 Hz, 1H), 7.21 (dtd, J = 15.1, 7.3, 1.6 Hz, 2H), 7.11 (dd, J = 7.9, 4.8 Hz, 1H), 6.91 (dd, J = 7.9, 1.4 Hz, 1H), 6.66 (d, J = 4.2 Hz, 1H), 1.32 (s, 9H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 153.1, 152.4, 151.7, 147.1, 141.1, 140.1, 137.3, 127.3, 127.1, 124.7, 123.8, 119.0, 115.7, 109.6, 96.8, 34.3, 30.5 ppm. LC-MS: 391.0 $[M-H]^-$, 392.9 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{21}H_{19}N_4O_2S$ 391.1234; Found 391.1236. Purity: 95.3%.

5.2.8. 1-Butyl-3-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)urea ***8***

m.p. 123–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (dd, J = 8.0, 1.7 Hz, 1H), 7.81 (dd, J = 4.9, 1.7 Hz, 1H), 7.50 (dd, J = 7.9, 1.8 Hz, 1H), 7.29–7.17 (m, 2H), 7.01 (dd, J = 7.8, 4.9 Hz, 1H), 6.93 (dd, J = 8.0, 1.4 Hz, 2H), 4.70 (s, 1H), 3.36–3.27 (m, 2H), 1.60–1.55 (m, 2H), 1.48–1.38 (m, 11H), 0.98 (t, J = 7.3 Hz, 3H) ppm. ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.2, 152.6, 152.2, 141.1, 137.7, 127.1, 127.0, 125.8, 125.7, 124.4, 123.8, 118.7, 34.3, 31.6, 30.5, 19.5, 13.6 ppm. LC-MS: 340.1[M-H]$^-$, 342.0[M+H]$^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{20}H_{26}N_3O_2$ 340.2031; Found 340.2036. Purity: 95.5%.

5.2.9. 1-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)-3-((1r,4r)-4-methylcyclohexyl)urea ***9***

m.p. 197–199 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (dd, J = 8.0, 1.7 Hz, 1H), 8.18 (s, 1H), 7.59 (dd, J = 4.8, 1.7 Hz, 1H), 7.42 (dd, J = 7.9, 1.7 Hz, 1H), 7.18 (dtd, J = 15.1, 7.3, 1.6 Hz, 2H), 7.04–6.92 (m, 2H), 6.84 (dd, J = 7.9, 1.4 Hz, 1H), 1.89 (d, J = 9.7 Hz, 2H), 1.67 (d, J = 11.4 Hz, 2H), 1.32 (s, 10H), 1.17 (dd, J = 9.2, 5.1 Hz, 1H), 1.14–0.94 (m, 4H), 0.87 (d, J = 6.5 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 154.5, 152.7, 152.2, 141.1, 137.6, 127.1, 125.9, 125.5, 124.4, 123.7, 118.8, 59.7, 48.3, 34.3, 33.6, 32.9, 31.4, 30.5, 22.1, 20.6, 14.1 ppm. LC-MS: 380.1[M-H]$^-$, 382.0[M+H]$^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{23}H_{30}N_3O_2$ 380.2344; Found 380.2349. $[\alpha]_D25$ = +1.33 (c = 0.1, EtOH). Purity: 95.3%.

5.2.10. 1-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)-3-cyclopentylurea ***10***

m.p. 217–219 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, J = 8.0, 1.7 Hz, 1H), 7.76 (dd, J = 4.9, 1.7 Hz, 1H), 7.45 (dd, J = 7.9, 1.7 Hz, 1H), 7.19 (dtd, J = 31.6, 7.4, 1.6 Hz, 2H), 6.97 (dd, J = 7.9, 4.9 Hz, 1H), 6.90 (s, 1H), 6.88 (dd, J = 8.0, 1.4 Hz, 1H), 4.61 (d, J = 6.8 Hz, 1H), 4.06 (m, 1H), 2.07–1.99 (m, 2H), 1.74–1.66 (m, 2H), 1.62 (m, 2H), 1.50–1.42 (m, 2H), 1.39 (s, 9H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.8, 152.7, 152.5, 141.5, 139.8, 127.7, 127.4, 127.1, 125.3, 125.1, 123.2, 119.3, 52.8, 34.9, 33.8, 30.8, 23.8 ppm. LC-MS: 352.1[M-H]$^-$, 354.0[M+H]$^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{21}H_{26}N_3O_2$ 352.2031; Found 352.2030. Purity: 97.8%.

5.2.11. 1-(Adamantan-2-yl)-3-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)urea ***11***

m.p. 228–230 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (dd, J = 8.0, 1.7 Hz, 1H), 7.74 (dd, J = 4.9, 1.7 Hz, 1H), 7.47–7.43 (m, 1H), 7.18 (dtd, J = 15.1, 7.3, 1.6 Hz, 2H), 6.95 (dd, J = 8.0, 4.9 Hz, 1H), 6.87 (dd, J = 7.9, 1.4 Hz, 1H), 6.72 (s, 1H), 4.38 (s, 1H), 2.11 (s, 3H), 2.04 (m, 6H), 1.70 (m, 6H), 1.39 (s, 9H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.8, 152.9, 152.5, 141.5, 139.5, 127.8, 127.4, 127.0, 125.6, 125.1, 123.1, 119.3, 52.0, 42.5, 36.6, 34.9, 30.9, 29.8 ppm. LC-MS: 418.1[M-H]$^-$, 420.0[M+H]$^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{26}H_{32}N_3O_2$ 418.2500; Found 418.2503. Purity: 98.6%.

5.2.12. 1-(2-(2-(tert-butyl)phenoxy)pyridin-3-yl)-3-(2-methylcyclohexyl)urea ***12***

m.p. 209–210 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 3.4 Hz, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.19 (dt, J = 33.1, 7.4 Hz, 2H), 6.97 (dd, J = 7.8, 5.0 Hz, 1H), 6.91 (t, J = 7.9 Hz, 1H), 6.83 (s, 1H), 4.38 (s, 1H), 3.29 (s, 1H), 2.11–2.01 (m, 1H), 1.76 (m, 2H), 1.67 (m, 1H), 1.46–1.31 (s, 9H), 1.29–1.06 (m, 4H), 1.03–0.91 (d, J = 6.5 Hz, 3H), 0.90–0.82 (m, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.7, 152.7, 152.5, 141.5, 139.7, 127.7, 127.4, 127.2, 125.4, 125.1,

123.2, 119.3, 39.1, 34.9, 34.6, 30.8, 25.9, 25.8, 19.4 ppm. LC-MS: 380.1 $[M-H]^-$, 382.0$[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{23}H_{30}N_3O_2$ 380.2344; Found 380.2346. Purity: 98.7%.

5.2.13. 1-(2-(2-(tert-butyl)phenoxy)-6-methylpyridin-3-yl)-3-(4-(trifluoromethoxy)phenyl)urea ***13***

To a solution of 1-isocyanato-4-(trifluoromethoxy)benzene **25** (215 mg, 1.06 mmol) in oluene (2 mL) in microwave tube was added 2-(2-(*tert*-butyl)phenoxy)pyridin-3-amine (170 mg, 0.70 mmol). The resulting mixture was heated with shaking at 80 °C for 1.5 h. The solvent was removed by vacuum, extracted with ethyl acetate and water, the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was purified by column chromatography (petroleum ether/ethyl acetate 4:1 v/v) to yield as a white solid (120 mg, 38.3%) m.p. 161–163 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (d, $J = 8.4$ Hz, 1H), 7.55–7.49 (m, 2H), 7.45 (dd, $J = 7.6$, 2.0 Hz, 1H), 7.22–7.15 (m, 3H), 7.14–7.10 (m, 1H), 6.91 (d, $J = 8.0$ Hz, 1H), 6.81 (dd, $J = 8.0$, 1.2 Hz, 1H), 2.25 (s, 3H), 1.40 (s, 9H) ppm.; ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.8, 152.6, 151.9, 148.0, 142.7, 140.9, 138.8, 127.7, 127.1, 127.0, 124.3, 123.3, 122.1, 121.8, 119.2, 117.9, 34.3, 30.5, 23.0.ppm. LC-MS: 458.1 $[M-H]^-$, 459.9 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{24}H_{23}F_3N_3O_3$ 458.1697; Found 458.1696. Purity: 96.9%.

Compounds **14**–**15** were prepared according to the procedures described for compound **13**.

5.2.14. 1-(2-(2-(tert-butyl)phenoxy)phenyl)-3-(4-(trifluoromethoxy)phenyl)urea ***14***

m.p. 143–145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (dd, $J = 8.0$, 1.6 Hz, 1H), 7.39 (dd, $J = 8.0$, 2.0 Hz, 1H), 7.31–7.25 (m, 3H), 7.15–7.04 (m, 5H), 7.01–6.96 (m, 1H), 6.76 (dd, $J = 8.0$, 1.6 Hz, 1H), 6.72–6.67 (m, 2H), 1.36 (s, 9H) ppm; ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.2, 152.4, 146.8, 140.6, 138.9, 130.6, 127.7, 127.3, 124.0, 122.9, 122.7, 121.8, 120.6, 120.0, 119.2, 117.1, 34.5, 30.3.ppm. LC-MS: 443.0 $[M-H]^-$, 444.9 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{24}H_{22}F_3N_2O_3$ 443.1588; Found 443.1588. Purity: 98.1%.

5.2.15. 1-(2-(2-(tert-butyl)phenoxy)-4-methylphenyl)-3-(4-(trifluoromethoxy)phenyl)urea ***15***

m.p. 161–162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, $J = 8.0$ Hz, 1H), 7.38 (dd, $J = 7.6$, 2.0 Hz, 1H), 7.24–7.19 (m, 2H), 7.12–7.02 (m, 5H), 6.89 (dd, $J = 8.4$,1.2 Hz, 1H), 6.84 (s, 1H), 6.67 (dd, $J = 8.0$, 1.6 Hz, 1H), 6.60–6.57 (m, 1H), 2.22 (s, 3H), 1.35 (s, 9H) ppm.; ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.4, 152.5, 146.7, 142.5, 140.4, 139.0, 132.1, 128.1, 127.7, 127.3, 123.8, 123.6, 122.3, 121.8, 121.4, 120.3, 120.3, 119.1, 117.7, 34.4, 30.3, 20.4. ppm. LC-MS: 457.0 $[M-H]^-$, 458.9 $[M+H]^+$; HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{25}H_{24}F_3N_2O_3$ 457.1745; Found 457.1750. Purity: 95.0%.

5.3. Biological experiment

5.3.1. Materials and methods

5.3.1.1. Cell culture. 1321N1 cells stably expressing $P2Y_1$ were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin G, and 100 g/mL streptomycin sulfate, 275 μg/mL G418 at 37 °C in a humidified atmosphere of 95% air and 5% CO_2.

5.3.1.2. Intracellular calcium assay. 1321N1/$P2Y_1$ cells were cultured in 96-well plates for 24 h, the cells were incubated with 2 μM Fluo-4-AM diluted in HBSS buffer at 37 °C for 50 min. After loading, cells were treated with the compounds of interest, then calcium responses were subsequently measured by **MRS2365** (selective $P2Y_1$ receptor agonist) stimulation using Flexstation 3 (Molecular Device, USA) with fluorescence excition made at 485 nm and emission at 525 nm.

5.3.2. Reagents and chemicals

Citric acid monohydrate (>99.5% purity, determined by HPLC) and Trisdium citrate dehydrate (>99% purity, determined by HPLC) were purchased from Nanjing Chemical Reagent CO., LTD.

Adenosine diphosphate (ADP) was purchased from Amresco (USA). Vicagrel was purchased from Jiangsu Vcare Pharmatech CO., LTD.

5.3.3. Animals

Experiments were performed in adult male Sprague-Dawley (SD) rats weighting 180–220 g. Rats were purchased from Qinglongshan Animmal Central (Nanjing, Jiangsu, China). The rats were housed in a room with a 12: 12 h light: dark cycle at an ambient temperature of 22–25 °C and a relative 60% humidity. Animals were allowed free access to water. Animals used in this study were maintained in accordance with the guide for care and use of Laboratory Animal published by the U.S. National institutes of Health (NIH publication NO.85-23, revised 1996) and the experiment was approved by the Animal Ethics Committee of China Pharmaceutical University (Jiangsu, China).

Blood sample was collected from rats with 3.8% sodium citrate solution (9:1, v/v). Then, the sample was centrifuged for 10 min at 1000 rpm to obtain platelet-rich plasma (PRP). The remaining material was centrifuged again for 10 min at 3000 rpm to get platelet-poor plasma (PPP).

5.3.4. Rat anti-platelet aggregation assay

The antiplatelet aggregation study was performed by measuring platelet aggregation using platelet aggregometer (LG-PABER-I). Platelet aggregation agonist ADP (100 μmol/L) was prepared with 0.9% saline solution. Forty SD rats were randomly divided into 8 group containing 5 rats in each group. The rats were treated with chemical compounds (3.0 mg/kg) and vehicle. Two hours after intragastric administration of chemical compounds and vehicle, blood samples were obtained. Then, 300 μL PPP was as blank correction while 270 μL PRP were added into a curvette and incubated at 37 °C for 60 s before the addition of 30 μL of ADP with stirring. Platelet aggregation was estimated after stirring for 240 s.

Abbreviations

ADP, adenosine-5′-diphosphate; MRS2500, (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; BPTU, 1-(2-(2-*tert*-butylphenoxy)pyridin-3-yl)-3-(4- (trifluoromethoxy)phenyl)urea; DMF, N,N-Dimethylformamide; HATU, 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; TEA, Triethylamine; DIEA, N,N-Diisopropylethylamine; SAR, structure-activity relationship.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.09.014.

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