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Benzofuran-substituted urea derivatives as novel $P2Y_1$ receptor antagonists

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ABSTRACT

Benzofuran-substituted urea analogs have been identified as novel $P2Y_1$ receptor antagonists. Structure–activity relationship studies around the urea and the benzofuran moieties resulted in compounds having improved potency. Several analogs were shown to inhibit ADP-mediated platelet activation.

Purinergic receptor antagonists have recently demonstrated therapeutic potential for the treatment of a variety of diseases, including thrombosis, diabetes, cystic fibrosis, and cancer.[1] The $P2Y_1$ and $P2Y_{12}$ receptors play key roles in platelet aggregation and thrombus formation.[2] The nucleotide adenosine diphosphate (ADP) acts as the endogenous activator for both of these receptors on the platelet surface. The binding of ADP to the $P2Y_1$ receptor results in an agonist response followed by a transitory increase in intracellular Ca^+, and finally platelet shape change and reversible aggregation. Activation of the $P2Y_{12}$ receptor reduces cyclic adenosine monophosphate (cAMP) levels causing an amplification of the platelet response and stabilization of the resulting aggregates.

Inhibition of the $P2Y_{12}$ receptor is a well-established strategy for anti-thrombotic therapy; Plavix® (clopidogrel), an irreversible $P2Y_{12}$ receptor antagonist, is the number one selling drug on the market for antiplatelet therapy.[3] However, $P2Y_1$ is a relatively new target being explored for anti-thrombotic therapies. Several studies have shown that exclusive inhibition of the $P2Y_1$ receptor can effectively prevent platelet aggregation and thrombus formation both in vitro[4] and in vivo.[5] Therefore, $P2Y_1$ receptor antagonists offer great potential as novel anti-thrombotic agents.

A number of potent nucleotide-based inhibitors of $P2Y_1$ such as **1**[6] (Fig. 1) have been reported, and a few non-nucleotide inhibitors have been disclosed.[7] In an effort to identify new non-nucleotide-based $P2Y_1$ antagonists, we conducted a high throughput screen of the GSK compound collection using a FLIPR-based cellular assay.[8] This afforded tetrahydro-quinolinamine **2**, (the lead optimization of which we have discussed in a previous publication[7a]) and pyridyl-urea analog **3** (Fig. 1) as micromolar hits. Interestingly, compound **3** is structurally related to previously reported aryl-urea antagonists of the $P2Y_1$ receptor such as **4**.[7b] Based on the promising FLIPR activity observed for **3**, we initiated lead optimization efforts to identify novel urea-based inhibitors with improved potency. Towards this end, we designed benzofuran analogs **5** (Fig. 1) in order to probe the effect of constraining the aryl-ether group of **3**. Herein, we present a structure–activity relationship (SAR) study of these novel $P2Y_1$ receptor antagonists.

The benzofuran analogs **5a–m** were prepared according to the sequence outlined in Scheme 1.[9] Selective *ortho*-iodination of 2-nitrophenol (**6**) was achieved using thallium acetate.[10] Sonagashira coupling of **7** with the appropriate alkyne[11] proceeded smoothly to form intermediate **8**. In situ cyclization afforded the desired nitro-benzofuran intermediate **9**. Reduction of the nitro-group using $SnCl_2$–H_2O, followed by addition of the appropriate isocyanate afforded benzofuran analogs **5a–m**.

All compounds were initially evaluated in the $P2Y_1$ FLIPR assay.[8] Hits were then followed up in a competitive binding assay employing radiolabeled ADP ([^{33}P]-2-SMe-ADP) to confirm $P2Y_1$ specific activity (see Tables 1 and 2).[8] SAR studies around R^1 when

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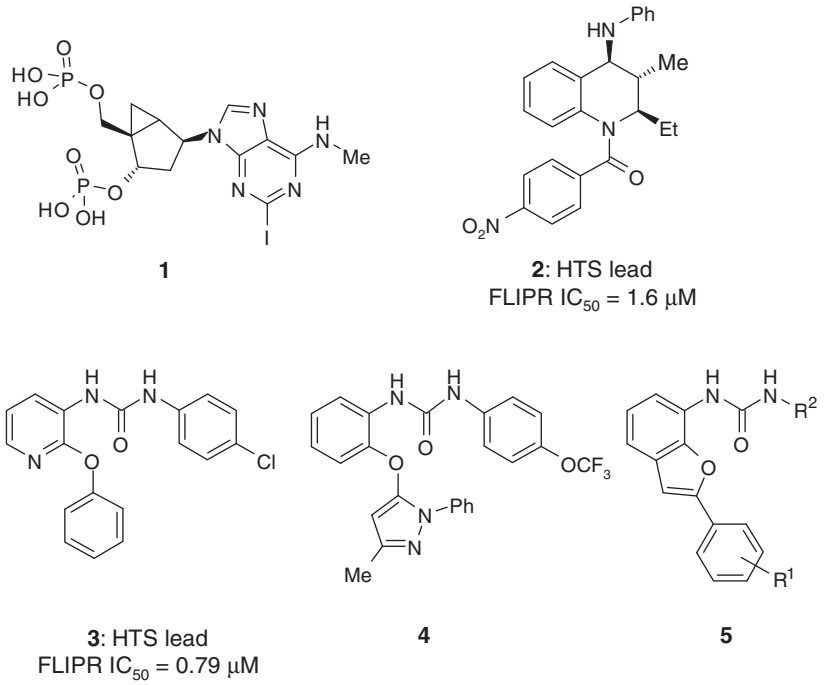
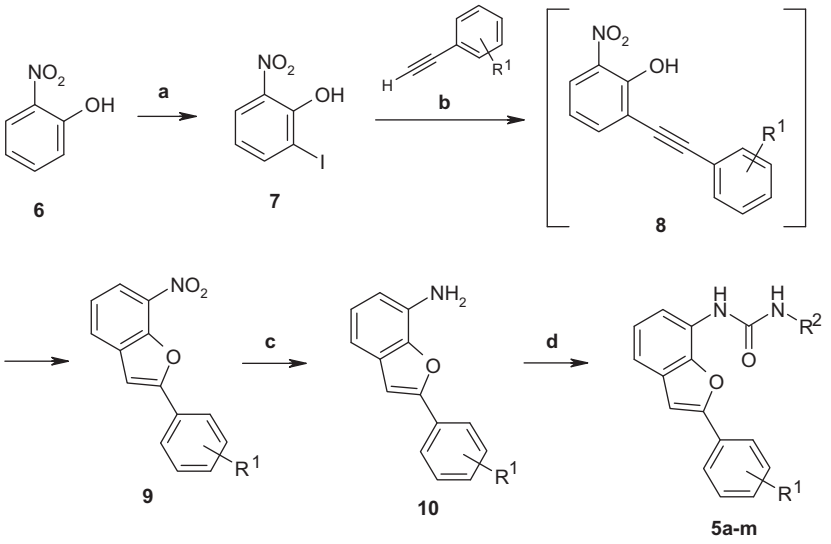


Figure 1. $P2Y_1$ receptor antagonists.



Scheme 1. Reagents and conditions: (a) $Tl(OAc)_2$, I_2, CH_2Cl_2, 25 °C; (b) $(PPh_3)_2PdCl_2$, CuI, piperidine, DMF, 60 °C; (c) $SnCl_2$–$2H_2O$, 25 °C; (d) R^2NCO, CH_2Cl_2, 25 °C.

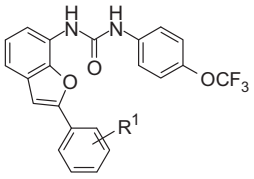
R^2 = 4-OCF_3-Ph indicate that *ortho*-substitution of the phenyl ring is essential for activity (Table 1). The 3-CF_3 analog **5a** was inactive while the 2-CF_3 analog **5b** displayed weak activity. Larger groups at the *ortho*-position seem to improve activity, and this trend is most evident in the binding assay. The 2-*i*-Pr (**5c**) and 2-*t*-Bu (**5d**) derivatives showed roughly a fivefold improvement in binding activity over the 2-CF_3 analog **5b**. The 2-Cl derivative **5e** is inactive, most likely due to the relatively small size of the chlorine atom. A similar enhancement of $P2Y_1$ activity with *ortho*-substitution was also reported for a structurally similar aryl-ether series (**4**).[7b] In the benzofuran series, electronics also seem to play an important role, as the bulky but more electron-donating 2-*i*-PrO analog **5f** is inactive.

Due to the potency and ease of synthesis of the 2-*i*-Pr analog **5c**, the 2-*i*-Pr group at the R^1 position was maintained in conducting SAR studies around R^2 (Table 2). Several new analogs (**5g–k**) were found to be more potent than **5c** in the cellular assay. Polar substituents such as NMe_2 (**5l**) result in significant reduction of activity, while non-polar groups such as *n*-pentyl (**5h**) and *t*-Bu (**5i**) improve both the cellular and binding activity relative to **5c**. The observation that polarity can disrupt activity is also seen in comparing the more polar BuO analog **5j** to the analogous *n*-pentyl derivative **5h**.

The effect of several benzofuran analogs on ADP-mediated platelet alpha-granule release was measured by detecting surface expression of P-selectin (CD62P) using flow cytometry.[8] The results are reported in Table 3. Analogs **5c**, **5g**, and **5i** all show significant inhibition of P-selectin expression demonstrating that this class of $P2Y_1$ inhibitors is functionally active.

In conclusion, we have identified benzofuran-ureas (**5**) as a novel class of $P2Y_1$ receptor antagonists. SAR studies showed that a

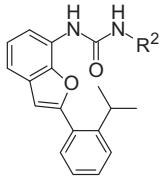
Table 1
Cellular and binding assay results for compounds **5a–f**



Compound	R^1	FLIPR IC_{50}[a] (μM)	K_i[a] (μM)
5a	3-CF_3	>25	nd
5b	2-CF_3	6.3	2.5
5c	2-*i*-Pr	4.0	0.6
5d	2-*t*-Bu	4.0	0.4
5e	2-Cl	>25	nd
5f	2-*i*-PrO	>25	nd

[a] Values are means of at least three determinations with a standard deviation ⩽ 0.3 log units (nd, not determined).

Table 2
Cellular and binding assay results for compounds **5g–m**



Compound	R^2	FLIPR IC_{50}[a] (μM)	K_i[a] (μM) or %*I* at 10 μM
5g	3-CF_3-4-Me-Ph	1.6	69%
5h	4-*n*-Pentyl-Ph	0.63	0.14
5i	4-*t*-Bu-Ph	1.3	72%
5j	4-BuO-Ph	2.0	0.64
5k	4-Cl-Ph	2.0	0.76
5l	4-Me_2N-Ph	15.8	nd
5m	3-CF_3-Ph	5.0	60%

[a] Values are means of at least three determinations with a standard deviation ⩽ 0.3 log units (nd, not determined).

Table 3
P-selectin assay results

Compound	%*I* at 10 μM
5c	49%
5g	79%
5i	100% (300 nM IC_{50}[a])

[a] Value is a mean for $n = 2$ independent donors with a standard deviation ⩽ 0.25 log units.

large, relatively non-polar *ortho*-phenyl substituent on the benzofuran ring is required for optimal activity. We also observed that alkyl-substituted aryl groups are optimal substituents on the urea. Finally, we have demonstrated that the benzofuran-substituted urea analogs are functional $P2Y_1$ inhibitors that affect ADP-mediated platelet activation.

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8. Assay protocols: *$P2Y_1$ FLIPR assay:* HEK-293 MSRII cells endogenously expressing $P2Y_1$ were maintained in DMEM/F12 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, 15 mM Hepes, and 1% penicillin–streptomycin at 37 °C in a humidified 5% CO_2 incubator. Cells were seeded at a density of 20,000 cells/well (384-well format) and cultured for 48 h prior to experiment. On the day of the experiment, growth media was removed and the cells were loaded with calcium 3 dye (Molecular Devices) in HBSS, pH 7.4, containing 2.5 mM probenecid for 1 h at 37 °C. The dye loaded cells were then incubated with compound for 10 min prior to challenge with an EC_{80} concentration of ADP (determined daily; typically 2–6 nM). Intracellular calcium fluxes were measured on a fluorescence imaging plate reader (FLIPR). Compound IC_{50} values were subsequently determined by non-linear regression analysis using activity base.
$P2Y_1$ binding assay: Membranes were prepared from BacMam-transduced U_2OS cells expressing human $P2Y_1$. [^{33}P]-2-MeS-ADP was utilized as the radioligand. Binding reactions were performed in 96-well plates in a volume of 130 μL containing 150 pM [^{33}P]-2-MeS-ADP, 0.5 μg $hP2Y_1$ expressing U_2OS cell membranes pre-bound to 0.5 mg of WGA-SPA (wheat germ agglutinin-coupled scintillation proximity assay) beads in 15 mM Hepes, 145 mM NaCl, 0.1 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl binding buffer, and various concentrations of test compound or dimethylsulfoxide vehicle control. Reactions were allowed to proceed to completion at room temperature for 1 h. Following centrifugation (2000*g*), supernatants were counted on a Perkin-Elmer Topcounter.
P-selectin assay: Platelets were obtained by standard venepuncture technique from healthy human volunteers who had not taken aspirin or non-steroidal anti-inflammatory drugs for 10 days. Blood was collected into EDTA (10 mM) anticoagulant and centrifuged at 300*g* for 4 min to obtain platelet-rich plasma (PRP). Prior to assay, PRP was diluted 1:50 in Hepes Modified Tyrodes Buffer (HMTB: 12 mM Na bicarbonate, 138 mM NaCl, 5.5 mM glucose, 2.9 mM KCL, and 10 mM HEPES, pH 7.4), in order to minimize compound binding to plasma protein binding. Platelets were incubated with compounds or dimethylsulfoxide vehicle and immediately activated with ADP (300 nM) for 5 min. Following a 15 min incubation with phycoerythrin (PE) conjugated anti-CD62P antibody, platelets were fixed for 30 min in 2% paraformaldehyde, diluted in PBS, and analyzed with a BD FACScalibur according to standard procedures.
9. *Representative experimental procedure:* A two-neck round bottom flask was charged with a solution of 2-iodo-6-nitrophenol (Ref. 10, 100 mg, 0.377 mmol) in DMF (0.4 mL). Under a stream of nitrogen, piperidine (37 μL, 0.377 mmol), $(Ph_3P)2PdCl_2$ (5.3 mg, 0.0075 mmol) and CuI (2.9 mg, 0.015 equiv) were then added. The mixture was heated to 60 °C and a solution of the 1-ethynyl-2-(1-methylethyl)benzene (Ref. 11, 65.3 mg, 0.453 mmol) in DMF (0.9 mL) was added dropwise over 1 h. The resulting solution was stirred for another 2 h, cooled to room temperature and then quenched with water. The product was extracted three times with Et_2O. The organic layers were combined and washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by reverse-phase HPLC (Sunfire, 40–100% MeCN/H_2O, 0.1% TFA, 10 min) to afford 2-[2-(1-methylethyl)phenyl]-7-nitro-1-benzofuran (**9**) as a yellow solid (70 mg, 66% yield). ^{1}H NMR ($CDCl_3$) δ 1.37 (d, J = 6.9 Hz, 6H), 3.49 (sept, J = 6.9 Hz, 1H), 6.97 (s, 1H), 7.33 (t, J = 7.3 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 7.46–7.53 (m, 2H), 7.70 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 7.9 Hz, 1H), 8.16 (d,

J = 8.1 Hz, 1H). To a solution of **9** (61 mg, 0.217 mmol, 1 equiv) in EtOAc (1.1 mL) was added $SnCl_2$–$2H_2O$ (245 mg, 1.08 mmol). The mixture was stirred overnight and then quenched with ice and 1 N NaOH (aq). The aqueous layer was extracted two times with EtOAc and the organic layers were combined, dried over Na_2SO_4, filtered and concentrated to afford 2-[2-(1-methylethyl)phenyl]-1-benzofuran-7-amine (**10**). A solution of this residue and 1-isocyanato-4-[(trifluoromethyl)oxy]benzene (36 μL, 0.239 mmol) in CH_2Cl_2 (0.43 mL) was stirred at room temperature overnight. The solvent was removed in vacuo and the residue was purified by reverse-phase HPLC (Sunfire, 40–100% MeCN/H_2O, 0.1% TFA, 10 min) to afford *N*-{2-[2-(1-methylethyl)phenyl]-1-benzofuran-7-yl}-*N*′-{4-[(trifluoromethyl)oxy]phenyl}urea (**5c**) as the TFA salt (60 mg, 61% yield over two steps). ^{1}H NMR ($CDCl_3$) δ 1.27 (d, *J* = 6.9 Hz, 6H), 3.38 (sept, *J* = 6.9 Hz, 1H), 6.85 (s, 1H), 7.19 (d, *J* = 8.8 Hz, 2H), 7.29 (m, 1H), 7.39–7.49 (m, 6H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.76 (d, *J* = 7.8 Hz, 1H).

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