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Identification of 1-{2-[4-chloro-1′-(2,2-dimethylpropyl)-7-hydroxy-1,2-dihydrospiro[indole-3,4′-piperidine]-1-yl]phenyl}-3-{5-chloro-[1,3]thiazolo[5,4-*b*]pyridin-2-yl}urea, a potent, efficacious and orally bioavailable $P2Y_1$ antagonist as an antiplatelet agent

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ABSTRACT

Spiropiperidine indoline-substituted diaryl ureas had been identified as antagonists of the $P2Y_1$ receptor. Enhancements in potency were realized through the introduction of a 7-hydroxyl substitution on the spiropiperidinylindoline chemotype. SAR studies were conducted to improve PK and potency, resulting in the identification of compound **3e**, a potent, orally bioavailable $P2Y_1$ antagonist with a suitable PK profile in preclinical species. Compound **3e** demonstrated a robust antithrombotic effect in vivo and improved bleeding risk profile compared to the $P2Y_{12}$ antagonist clopidogrel in rat efficacy/bleeding models.

Atherothrombosis is the number one cause of mortality and morbidity worldwide. Platelet activation plays a key role in the development of arterial thrombosis. The two G-protein coupled receptors $P2Y_1$ and $P2Y_{12}$ are important in regulating haemostasis and thrombosis. Synergistic activation of both $P2Y_1$ and $P2Y_{12}$ receptors is essential for a complete platelet response. Blockade of either receptor significantly decreases both in vitro and ex vivo ADP-induced platelet aggregation and reduces thrombosis formation.[1] Inhibitors of $P2Y_{12}$ have resulted in several marketed drugs such as the widely used clopidogrel, and recently approved prasugrel and ticagrelor. An attractive alternative therapeutic approach would be the discovery of antagonists of the $P2Y_1$ receptor. Preclinical models with $P2Y_1$ knock-out mice or with selective $P2Y_1$ antagonists such as the adenosine nucleotide analog MRS2500[2] and the recently disclosed diaryl ureas[3,4] showed complete blockade of ADP-induced platelet aggregation and effective reduction of arterial thrombosis with only moderate prolongation of bleeding time. With the potential of providing a reduced bleeding liability and equivalent efficacy compared to $P2Y_{12}$ antagonism, $P2Y_1$ antagonism is a promising target for antithrombotic therapy.[5]

Recently, we reported on the discovery of 1-(2-(1′-neopentylspiro[indoline-3,4′-piperidine]-1-yl)phenyl)-3-(4-(trifluoromethoxy)phenyl)urea (compound **1**)[4] as a $P2Y_1$ receptor antagonist. Compound **1** inhibited platelet aggregation (PA) in human platelet-rich plasma with an IC_{50} of 4.9 μM at 10 μM ADP, which is five-to ten-fold less potent than the desired potency range to achieve a reasonable human dose projection. Additionally, the modestly improved aqueous solubility of compound **1** (4 μg/ml, amorphous) was a desired property. Described in this article is the discovery of 7-hydroxyl substitution on the indoline that resulted in improvements in both potency and solubility. Optimization of this series resulted in the identification of compound **3e**, which showed more than 20-fold improvement in in vitro potency in FLIPR (fluorescent imaging plate reader) and PA assays and had

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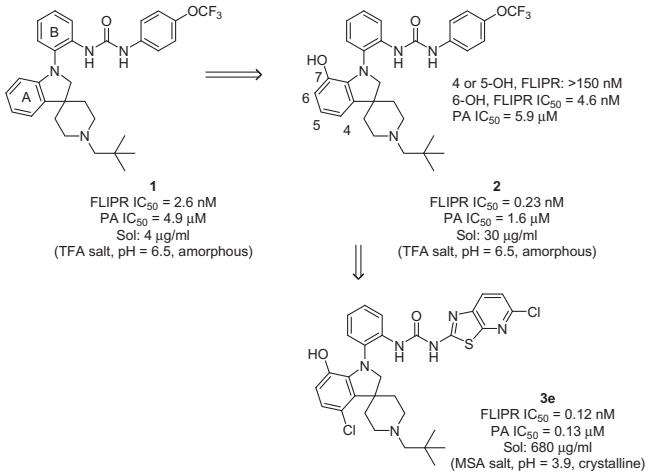
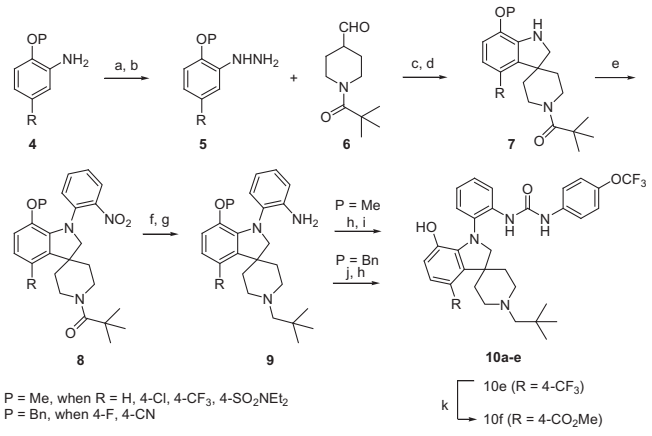


Figure 1. Identification of **3e** from compound **1**.

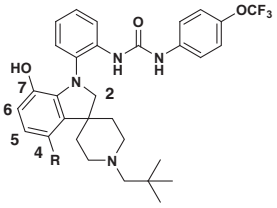
enhanced solubility compared with **1** (Fig. 1). Compound **3e** also demonstrated a robust antithrombotic effect and improved bleeding risk profile compared to $P2Y_{12}$ antagonist clopidogrel in rat efficacy/bleeding models.

Shown in Scheme 1 is a general synthesis of 7-hydroxy spiropiperidinylindoline analogs listed in Table 1. Methyl or benzyl protected anilino phenols **4** were converted to their corresponding hydrazines **5** by treatment with sodium nitrate followed by tin(II) chloride in the presence of concentrated HCl. The requisite spiropiperidinylindolines **7** were prepared via Fischer indoline synthesis by reacting hydrazines **5** with aldehyde **6** in the presence of acids, followed by reduction of the imines formed in situ with $NaBH_4$, $NaBH(OAc)_3$ or $NaCNBH_3$. N-Arylation of compound **7** with 1-bromo-2-nitrobenzene provided compound **8** which were sequentially reduced, first by Zn/NH_4Cl, then by Red-Al to anilines **9**. Treatment of **9** with 4-trifluoromethoxyphenylisocyanate afforded the diaryl ureas, which were deprotected with either BCl_3/Bu_4NI (when methyl was the protecting group) or $Pd/C/H_2$ (when benzyl was the protecting group) to provide the final compounds **10a–e**. Compound **10f** (R = CO_2Me) can be obtained by hydrolysis of compound **10e** (R = CF_3) with aqueous NaOH in methanol.[6]



Scheme 1. Synthesis of 7-OH spiroindolines. Reagents and conditions: (a) $NaNO_2$, 6 N HCl, 0 °C; (b) $SnCl_2$, concd HCl, 64–98% for 2 steps; (c) concd H_2SO_4/EtOH, or HOAc, or TFA/toluene/CH_3CN, or HCl/dioxane, rt −80 °C; (d) $NaBH_4$ or $NaBH(OAc)_3$, or $NaCNBH_3$, MeOH, −20 °C to rt, 24–88% for 2 steps; (e) *o*-Br-NO_2Ph, $Pd_2(dba)_3$, *rac*-BINAP, Cs_2CO_3, toluene, 110–120 °C, 65–96%; (f) Zn/NH_4Cl/MeOH, 82–98%; (g) Red-Al, CH_2Cl_2, 45–90%; (h) OCN-*p*-OCF_3-Ph, CH_2Cl_2, 90–98%; (i) BCl_3, tetrabutylammonium iodide, CH_2Cl_2, −50 °C to rt, 23–70%; (j) Pd/C, H_2, ammonium acetate, MeOH, 40–99%; (k) 1 N NaOH, MeOH, 70%.

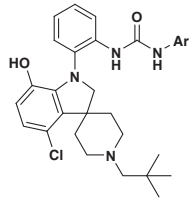
Table 1
SAR of 4-substituted 7-hydroxyl indolines

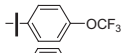
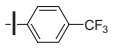
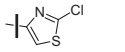
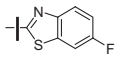
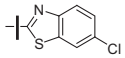
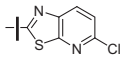


Compds	R	FLIPR[a] (nM)	PA[b] IC_{50} (μM)	LM (h, r)[c] % remaining
2	H	0.27 ± 0.11	1.6 ± 0.45	80, 87[d]
10a	F	0.37 ± 0.04	0.92 ± 0.32	78, 70[d]
10b	Cl	0.04 ± 0.02	0.45(n = 1)	79, 88
10c	CN	0.04 ± 0.00	1.1 ± 0.11	59, 51
10d	SO_2NMe_2	0.13 ± 0.01	0.42(n = 1)	21, 75
10e	CF_3	0.14 ± 0.02	0.13 ± 0.04	100, 98
10f	CO_2Me	0.15 ± 0.01	0.70 ± 0.26	48, 57

[a] FLIPR was tested with 1 μM 2-methylthio-ADP, and IC_{50} values are reported as the mean ± SD; $n \geqslant 2$.
[b] PA was tested with 10 μM of ADP, and IC_{50} values are reported as the mean ± SD; $n \geqslant 2$ unless otherwise stated. See Ref. 4 and Supporting information for detailed assay conditions.
[c] Liver microsome stability: percentage of compound remaining after incubation with 0.5 μM of human (h) or rat (r) liver microsomes.
[d] 3 μM liver microsomes were used.

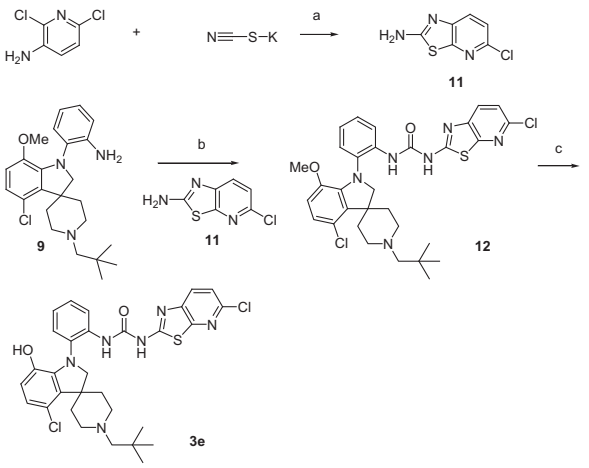
Table 2
Arylurea modifications



Compds	Ar	FLIPR (nM)	PA IC_{50} (μM)	LM (h, r) %remaining
10b		0.04 ± 0.02	0.45(n = 1)	79, 88
3a		0.08 ± 0.03	0.40(n = 1)	93, 93
3b		0.03 ± 0.01	0.17 ± 0.06	87, 66
3c		0.06 ± 0.02	0.23(n = 1)	100, 89
3d		0.17 ± 0.05	0.32 ± 0.09	96, 93
3e		0.12 ± 0.05	0.13(n = 1)	94, 93

The compounds in Table 2 with various heteroaryl ureas were prepared in a similar way as described in Scheme 2 for the synthesis of compound **3e**. 5-Chlorothiazolo[5,4-*b*]pyridin-2-amine **11** was obtained in one step by treatment of 2,6-dichloropyridin-3-amine with KSCN. The aniline **9** was first activated with *p*-nitrophenylchloroformate, and then reacted with **11** in the presence of DMAP to give **12**. Demethylation with $AlCl_3$ under microwave irradiation at 100 °C or with BCl_3–Me_2S under conventional heating at 50 °C afforded compound **3e**.

As previously reported, a nitrogen atom 'walk' around the A and B rings of compound **1** was performed in order to increase aqueous solubility, however, these aza-analogs resulted in weaker $P2Y_1$



Scheme 2. Synthesis of compound **3e**. Reagents and conditions: (a) concd HCl/EtOH, 100 °C, 24 h, 46–89%; (b) ClCOO-*p*-NO_2Ph, K_2CO_3, CH_2Cl_2, then compound **11**, DMAP, 80 °C, 5 h, 84–96%; (c) $AlCl_3$, CH_2Cl_2, microwave irradiation, 100 °C, 10 min, 63–97% or BCl_3–Me_2S, CH_2Cl_2, 50 °C to rt, 80–95%.

activity.[4] Another approach to improve solubility was to add a hydroxyl group onto compound **1**. Toward this goal, a hydroxyl group was attached to various positions of the A ring. Of the four positions surveyed on the A ring, the 4- and 5-OH analogs showed significantly decreased potency while the 6-OH substitution was about two fold weaker compared with compound **1**. The 7-OH analog (compound **2**) in contrast resulted in a 10-fold improvement in potency as measured in the FLIPR assay and was three fold more potent in the human PA inhibition assay. The FLIPR assay measured the activation of the calcium signaltransduction pathway by $P2Y_1$ in washed human platelets in a more physiologically relevant setting using the endogenously expressed receptor and a reduced amount of protein (~0.1% BSA) and was more sensitive than the binding assay (K_i). The K_i of compound **2** was determined to be 8 nM, which was similar to compound **1** (K_i = 4 nM), due to the sensitivity of the binding assay (the assay bottomed out at single digit nanomolar). Compound **2** also showed modestly enhanced aqueous solubility (30 μg/ml) compared with compound **1** (4 μg/ml) both as amorphous form.

When compound **2** was tested for in vitro bioactivation in human liver microsomes using GSH,[7] significant amounts (ca. 38%) of glutathione (GSH) addition to hydroxylindoline was observed (M+GSH).[8] The reactivity was probably due to the electron-rich nature of the hydroxyindoline ring system, which could readily undergo oxidation to form a quinone and subsequently serve as a Michael accepter for GSH. To decrease the electron density of the 7-hydroxyl indoline ring, various electron-withdrawing groups were installed at the 4-position (Table 1). Analogs containing 4-fluoro and 4-chloro substitutions (**10a** and **10b**) showed improved PA IC_{50} by approximately two and four fold respectively and maintained good metabolic stability when tested for oxidative metabolism in liver microsomes. Both compounds significantly reduced GSH adduct formation (M + GSH <1%) compared with compound **2** as measured in the assays for reactive intermediate formation.[8] Analogs with a 4-CN or 4-CO_2Me substitution (**10c** and **10f**), though still potent, had reduced liver microsomal stability. The analog with a 4-dimethylsulfonamide substitution (**10d**) showed poor liver microsomal stability and also generated 5% of the GSH adduct. The 4-CF_3 analog (**10e**) was potent and metabolically stable, but was found to be chemically unstable under basic conditions. Overall, the 4-chloro substitution demonstrated the optimal profile with the best potency, liver microsomal stability, much reduced GSH adduct formation and was chosen to serve as the spiropiperidine indoline template to study the SAR of the terminal urea substitutions.

The SAR of aryl and heteroaryl ureas were investigated and representative compounds are shown in Table 2. A variety of substituted phenyl and heteroaryl urea analogs were found to be highly potent in both the FLIPR and the PA assays. Among the ureas surveyed, the small chlorothiazole (**3b**) and 5-chloro-2-methylthiazolo[5,4-*b*]pyridine urea (**3e**) were the most potent in the PA assay. These compounds were subsequently differentiated in rat cassette PK studies with compound **3e** showing the best overall rat PK profile. Compound **3e** was further characterized in vitro and in vivo as a potential candidate for advancement.

Compound **3e** was selective against $P2Y_{12}$ and $P2Y_2$ receptors with IC_{50}s greater than 10 μM, and with over a thousand fold selectivity against the $P2X_1$ receptor in the FLIPR assay (IC_{50} = 180 nM). It generated less than 1% GSH adduct formation in the reactive intermediate assay. However, in vitro patch clamp assay indicated **3e** inhibited the cardiac hERG current by 96% at 1 μM. To follow up on this observation, an anesthetized rabbit electrophysiology (EP) study was performed to evaluate effects of **3e** on cardiac electrophysiology in vivo. At increasing IV doses of 3, 10 and 30 mg/kg which generated plasma concentrations of 24, 62, 158 μM respectively, compound **3e** had no effect on PR and QRS intervals on surface electrocardiography (ECG), but with a trend toward QTc increase at 10 and 30 mg/kg doses. However, the increase was small and signified minimal proarrhythmic risk. The lack of a significant QTc increase in vivo despite potent hERG inhibition in the patch clamp assay was probably due to the high protein binding of **3e** (rabbit: 99.3%; human: 99.3%; and rat: 99.8% bound) as hERG activity is free fraction driven.

Since the free base aqueous solubility of compound **3e** was poor (<1 μg/ml, crystalline, at pH 6.5), salt screening was performed and a mono-methanesulfonic acid (MSA) salt was identified as the preferred salt form with good aqueous solubility (680 μg/ml, crystalline, at pH 3.9). The MSA salt was used in subsequent PK studies. The PK profile of compound **3e** in different preclinical species is listed in Table 3. Compound **3e** demonstrated low clearance, good $T_{1/2}$ and moderate oral bioavailability across species and was chosen to proceed to rat efficacy and bleeding models.

Compound **3e** was evaluated in established efficacy models of $FeCl_2$-induced carotid thrombosis (AT), cuticle bleeding time (CBT) and mesenteric bleeding time (MBT) conducted in pentobarbital-anesthetized rats.[9] Vehicle (1 ml/kg of 10% *N*,*N*-dimethylacetamide/90% dextrose 5% water) or compound **3e** (0.3, 1, 3, 10 mg/kg) was administered as an oral gavage 1 h before induction of AT or BT (*n* per group indicated in figures). Blood samples were obtained at the end of the AT protocol to assess platelet aggregation responses to 10 μM ADP and obtain exposure levels (7 ± 1, 39 ± 14, 81 ± 11, 240 ± 49 nM at 0.3, 1, 3, 10 mg/kg, respectively). Compound **3e** produced a dose-dependent reduction in thrombus weight and maintenance of blood flow (Fig. 2). Dose-dependent inhibition of ex vivo platelet aggregation and prolongation of BTs were also observed (Fig. 3). The 10 mg/kg dose produced maximum efficacy with complete preservation of blood flow in all

Table 3
PK data for compound **3e**

	Rat[a]	Dog[b]	Cyno[b]
Dose (mpk) (IV/PO)	0.4/15	0.2/5	0.2/5
Clearance (ml/min/kg)	7.3	4	7.7
V_{dss} (l/kg)	3.8	2.5	3.3
$T_{1/2}$ (h)	8.4	9.4	8.2
F%	23%	27%	22%

[a] Rat IV/oral PK formulation: DMAC/cremophor/ethanol/water (1/1/1/7).
[b] Dog and cyno IV/oral PK formulation: PEG400/ethanol/water (50/5/45).

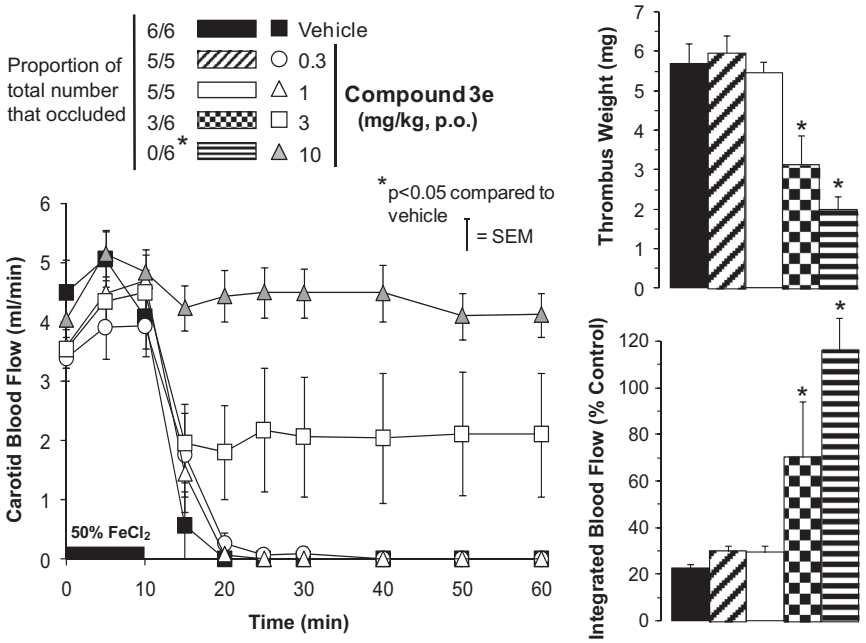


Figure 2. Effects of compound **3e** on arterial thrombosis in rats.

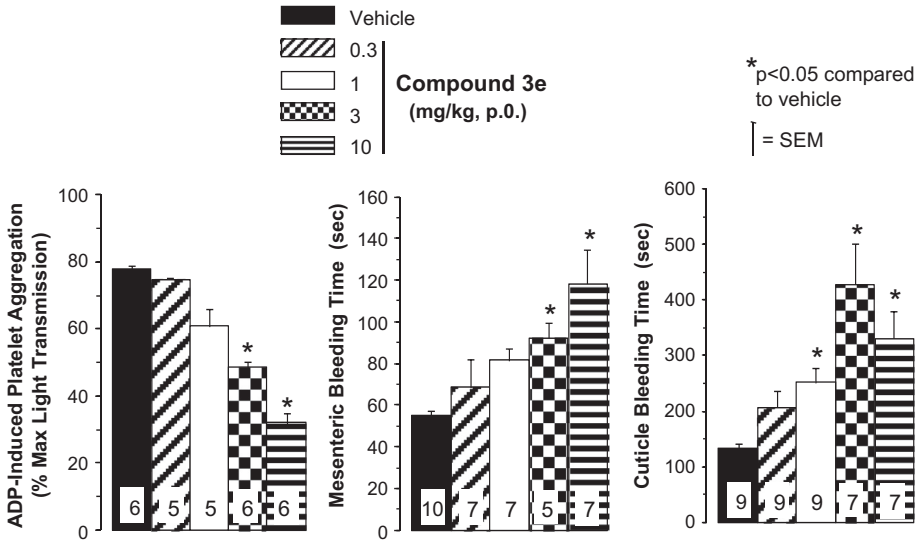


Figure 3. Effects of compound **3e** on platelet aggregation and bleeding times in rats. Number of rats per group indicated at bottom of each bar.

drug-treated rats, a 66 ± 6% reduction in thrombus weight, and a 74 ± 4% decrease in platelet aggregation. The ID_{50}s were similar for inhibition of thrombosis (7.3 mg/kg) and ex vivo platelet aggregation (6.6 mg/kg). The IC_{50} of compound **3e** for thrombus weight reduction was 0.13 μM, which compared to 0.47 μM obtained previously for oral administration of compound **1** in the same model. Relative to vehicle, compound **3e** at 10 mg/kg prolonged CBT by 2.5 ± 0.4 fold and MBT by 2.2 ± 0.3 fold. In comparison, a 10 mg/kg oral dose of clopidogrel tested in these same models reduced thrombus weight by an equivalent 67 ± 5%, but prolonged CBT by 6.2 ± 1.1 fold and MBT by 8.2 ± 0.2 fold.[9]

In conclusion, SAR studies on the spiropiperidinylindoline chemotype identified 7-hydroxyl substitution which both improved $P2Y_1$ potency and also increased solubility. Electron-withdrawing groups were introduced at the 4-position to address the issue of reactive intermediate formation. Further SAR studies of the urea group resulted in discovery of compound **3e** as a potent, orally bioavailable $P2Y_1$ antagonist with a suitable PK profile in different preclinical species for further advancement. Compound **3e** demonstrated a robust antithrombotic effect with an improved bleeding profile compared to $P2Y_{12}$ antagonist clopidogrel in rat efficacy/bleeding models.

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