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Discovery of 2-(Phenoxypyridine)-3-phenylureas as Small Molecule $P2Y_1$ Antagonists

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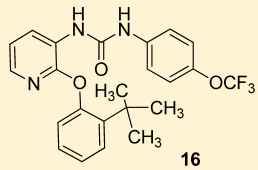
ABSTRACT: Two distinct G protein-coupled purinergic receptors, $P2Y_1$ and $P2Y_{12}$, mediate ADP-driven platelet activation. The clinical effectiveness of $P2Y_{12}$ blockade is well established. Recent preclinical data suggest that $P2Y_1$ and $P2Y_{12}$ inhibition provide equivalent antithrombotic efficacy, while targeting $P2Y_1$ has the potential for reduced bleeding liability. In this account, the discovery of a 2-(phenoxypyridine)-3-phenylurea chemotype that inhibited ADP-mediated platelet aggregation in human blood samples is described. Optimization of this series led to the identification of compound **16**, 1-(2-(2-*tert*-butylphenoxy)pyridin-3-yl)-3−4-(trifluoromethoxy)phenylurea, which demonstrated a 68 ± 7% thrombus weight reduction in an established rat arterial thrombosis model (10 mg/kg plus 10 mg/kg/h) while only prolonging cuticle and mesenteric bleeding times by 3.3- and 3.1-fold, respectively, in provoked rat bleeding time models. These results suggest that a $P2Y_1$ antagonist could potentially provide a safe and efficacious antithrombotic profile.



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INTRODUCTION

The $P2Y_1$ receptor, a G-protein-coupled receptor, expressed in the heart, skeletal, and various smooth muscles as well as the prostate, brain, and circulating blood cells,[1] is a member of the P2Y family of receptors. The P2Y receptor family is generally considered to consist of eight members, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$, encoded by distinct genes, which can be subdivided into two groups based on their coupling to specific G-proteins.[2] Several studies have suggested that modulators of specific members of the P2Y family could have therapeutic potential for the treatment of a variety of disorders,[3] such as diabetes, cancer, and ischemia-reperfusion injury.[4] Among the P2Y family, $P2Y_1$ and $P2Y_{12}$ are of particular interest because they play a major physiological role in ADP-mediated platelet aggregation, an important component of thrombosis.[5]

ADP is a key activator of platelets, and platelet activation is known to play a pivotal role in thrombus formation. ADP activates platelets by simultaneously stimulating $P2Y_1$ and $P2Y_{12}$ to produce two separate intracellular signals, which synergize to produce complete platelet activation. The antithrombotic effect of antagonizing the $P2Y_{12}$ receptor alone is very well validated clinically with the thienopyridines[6] and other drugs[7] (Figure 1), but the same level of proof of principle in humans for the $P2Y_1$ receptor has yet to be demonstrated. In 2001, Léon[8] first reported that inhibition of $P2Y_1$ activity alone could lead to an antithrombotic effect in vivo in a model of thromboplastin-induced thromboembolism using both a $P2Y_1$ knockout mouse and the $P2Y_1$ antagonist MRS2179.[9] Subsequently, Jacobson and others described a series of adenine nucleotide-based $P2Y_1$ receptor antagonists that inhibit ADP-induced platelet aggregation.[10] In general, those adenine nucleotide analogues exhibited good in vitro potency. However, their chemical and enzymatic stabilities are less than desirable, which would be expected to limit their usefulness as oral drug candidates. Thus, the discovery of novel $P2Y_1$ antagonists with improved pharmaceutical characteristics could have significant utility in the treatment of a variety of thromboembolic disorders.[11]

RESULTS AND DISCUSSION

Given the promising evidence that $P2Y_1$ receptor antagonists may represent a novel antithrombotic strategy that might complement current therapy, a discovery effort to identify small molecule $P2Y_1$ antagonists was carried out. A high throughput screening effort using the full BMS screening deck (>1 million compounds) against the human $P2Y_1$ receptor employing a binding assay with [β-^{33}P]-2-methylthioadenosine diphosphate as the ligand revealed the diaryl urea **1** (Figure 2) with good affinity and binding selectivity toward the $P2Y_1$ receptor versus $P2Y_{12}$ ($P2Y_1$ K_i = 75 nM, $P2Y_{12}$ K_i > 70 μM).[12]

The favorable profile of this compound was validated by resynthesis, and it was selected as the starting point for further structural optimization. The modular nature of this compound made it particularly well suited for utilization of array synthesis to efficiently explore the SAR of the chemotype. Optimization efforts began with exploration of the phenyl ring of the urea moiety. Scheme 1 represents the general synthetic route to the targeted compounds. First, reacting 3-CF_3PhOH **2** with 2-

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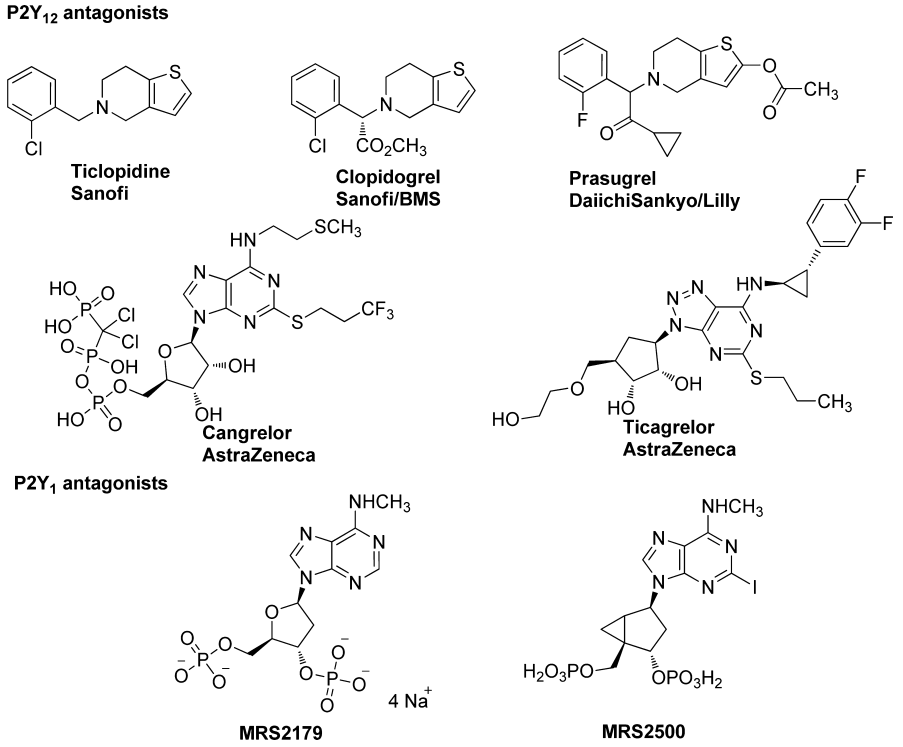
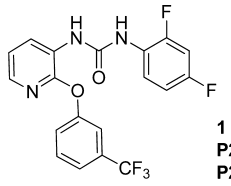


Figure 1. Known $P2Y_{12}$ and $P2Y_1$ antagonists.



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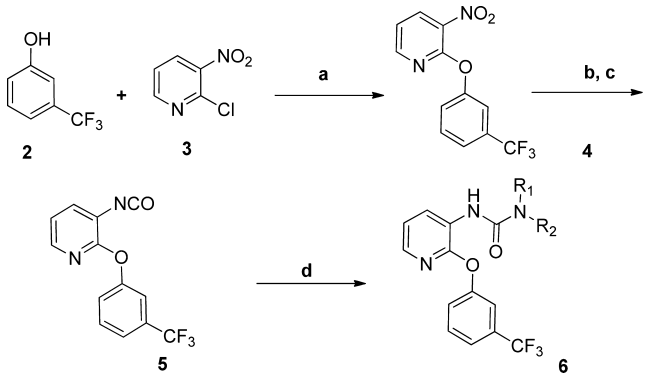
$P2Y_1$ Ki = 75 nM
$P2Y_{12}$ Ki > 70 μM

Figure 2. $P2Y_1$ lead compound.

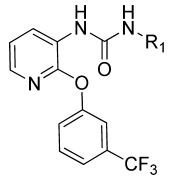
Scheme 1[a]



[a]Reagents and conditions: (a) Cs_2CO_3, DMF, 80 °C, 14 h, 60%; (b) Zn, NH_4Cl, MeOH, 92%; (c) diphosgene, N^1,N^1,N^8,N^8-tetramethylnaphthalene-1,8-diamine, CH_2Cl_2, 100%; (d) 96 R_1R_2NH, THF, 60 °C, yields in range of 12−75%.

chloro-3-nitropyridine **3** in DMF at 80 °C afforded the 3-nitro-2-phenoxypyridine **4**. Subsequent reduction of the nitro group with Zn/NH_4Cl gave the corresponing 3-aminopyridine, which was then reacted with diphosgene in the presence of N^1,N^1,N^8,N^8-tetramethylnaphthalene-1,8-diamine to provide the key intermediate isocyanate **5**.[13] In general, the isocyanate derivatives were purified by silica gel chromatography and were stable up to a year when stored at 0 °C. For subsequent array synthesis, the isocyanate was distributed into 96 vessels and reacted with 96 diverse amines in THF at 60 °C in a parallel format to afford urea analogues **6**.

All analogues of type **6** were initially screened in the human $P2Y_1$ receptor binding assay. This exploration of the distal urea



substitution demonstrated that monosubstitution was required for activity. Additionally, aliphatic substituents were not tolerated. Shown in Table 1 are selected examples from the evaluation of the urea phenyl ring. The SAR indicated that substitution of the phenyl ring and the positions of the substitution were critical for $P2Y_1$ activity. The unsubstituted analogue **6a** showed a 3-fold drop in activity compared to the lead **1**. Replacement of the *o*-fluorine (**6b**) with a larger group such as chlorine was not tolerated (**6c**). Substitution at the meta position was acceptable but, in general, provided less active analogues (**6d** and **6e**). Substitution at the para position with flourine or chlorine showed comparable activity (**6f** and **6g**), but methyl and methoxy, as well as larger lipophilic groups such as *t*-Bu, OCF_3, or Ph, provided a modest improvement in activity (**6h**−**l**). Various other functional groups such as dimethylamine, nitrile, and methyl ester were acceptable at this position but resulted in analogues with weaker binding (**6m**−**o**). Bis-substitution was also explored with analogues showing no improvement in activity (**6p** and **6q**). Insertion of one or two methylene groups between the phenyl ring and distal nitrogen atom of the urea resulted in a dramatic loss of activity (**6r** and **6s**).

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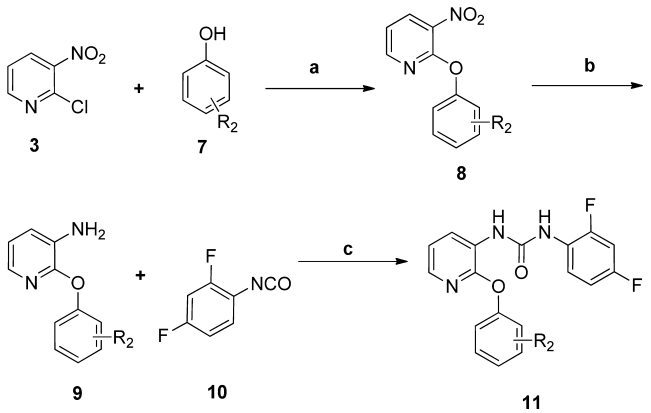
Table 1. Inhibition Data for Compounds 1 and 6a−s

compd	R_1	hP2Y$_1$ K_i^a (nM)
1	2, 4-di-F-Ph	75 ± 5
6a	Ph	171 ± 105
6b	2-F-Ph	47 ± 27
6c	2-Cl-Ph	>5000
6d	3-F-Ph	607 ± 105
6e	3-Cl-Ph	128 ± 45
6f	4-F-Ph	145 ± 28
6g	4-Cl-Ph	69 ± 23
6h	4-MePh	48 ± 28
6i	4-*t*-Bu-Ph	31 ± 4
6j	4-OCF_3-Ph	313 ± 198
6k	4-Ph-Ph	28 ± 4
6l	4-OMe-Ph	21 ± 11
6m	4-NMe_2-Ph	506 ± 85
6n	4-CN-Ph	571 ± 150
6o	4-CO_2Me-Ph	644 ± 296
6p	3,4-di-Cl-Ph	84 ± 16
6q	3,5-di-Cl-Ph	169 ± 38
6r	CH_2Ph	>5000
6s	CH_2CH_2Ph	>5000

[a]Data represent the mean ± SEM, n = 2.

Optimization efforts on the phenoxy ring and the linker were carried out, utilizing the 2,4-difluorophenyl urea present in the HTS hit **1**. The same general synthetic scheme was utilized in an array format (Scheme 2), and several analogues were thus

Scheme 2[a]



[a]Reagents and conditions: (a) Cs_2CO_3, DMF, 80 °C; (b) Zn, NH_4Cl, EtOH/EtOAc (4:1); (c) toluene, 80 °C, 1.5 h, yields in range of 22−44%.

prepared. Introduction of the variable group in the first step necessitated that all the subsequent steps be carried out in parallel for all vials. Instead of generating an isocyanate of type **5** for each core, 2,4-difluorophenyl isocyanate was condensed with each of 3-aminopyridines.

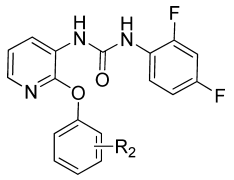
Shown in Table 2 are selected representatives from the exploration of the phenyl ether. As seen with the phenylurea, substituents and their position were pivotal to P2Y$_1$ activity. The unsubstituted phenyl analogue **11a** displayed more than a 30-fold drop in activity compared to compound **1**. On the contrary, substitution with a *tert*-butyl group at the ortho position (**11b**) improved potency by 4-fold, and at the meta position (**11c**) by 2.5-fold, respectively. Additionally, an

Table 2. Inhibition Data for Compounds 1 and 11a−h

compd	R_2	hP2Y$_1$ K_i^a (nM)
1	3-CF_3	75 ± 5
11a	H	2000 ± 1158
11b	2-*t*-Bu	18 ± 4
11c	3-*t*-Bu	33 ± 7
11d	2-*i*-Pr	28 ± 2
11e	2-Et	77 ± 6
11f	2-CF_3	172 ± 71
11g	4-CF_3	>5000
11h	4-Me	>5000

[a]Data represent the mean ± SEM, n = 2.

isopropyl group (**11d**) at the ortho position provided a modest improvement in activity, whereas the ethyl group (**11e**) displayed the same level of potency as compound **1**. Combined, the data indicated that lipophilicity at the ortho position was important for activity. In the case of the CF_3 substituent, while substituting at the ortho position maintained activity (**11f**) compared to compound **1**, moving the CF_3 substituent to the para position (**11g**) resulted in a substantial loss of activity. This was further confirmed using a methyl group as the para substituent (**11h**).



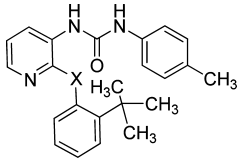
The linker between the phenyl ring and pyridyl ring was explored by replacing the oxygen atom with sulfur or nitrogen. Synthesis of these analogues utilized the same chemistry sequence except that the initial starting phenol was replaced by the correspondingly substituted thiophenol or aniline. When the oxygen atom in compound **12** was replaced by sulfur (**13**), a modest drop in activity was observed (Table 3). However,

Table 3. Inhibition Data for Compounds 12−15

compd	X	hP2Y$_1$ K_i^a (nM)
12	O	7 ± 0.1
13	S	20 ± 8
14	NH	175 ± 40
15	NMe	>5000

[a]Data represent the mean ± SEM, n = 2.

replacing with nitrogen (**14**) resulted in a 20-fold drop in activity. Furthermore, methylation of the linking nitrogen of **14** provided a more significant loss in activity (**15**).



Following the exploration of the two terminal phenyl rings, a focused library was executed based on the discovered SAR, in which preferred substituents at each position were combined. This so-called "best−best" array included eight preferred substituents each from the phenoxy moiety and the phenyl

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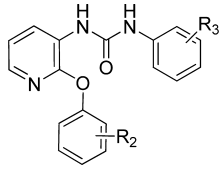
Table 4. Inhibition, Platelet Aggregation, and Selectivity Data for Compounds 1, 12, and 16−19

compd	R_2	R_3	hP2Y$_1$ K_i[a] (nM)	hPRP[b] IC_{50} (μM)	hP2Y$_{12}$ K_i[a] (nM)	hP2Y$_2$ K_i[c] (nM)	hP2Y$_6$ K_i[c] (nM)	hP2Y$_{11}$ K_i[c] (nM)	hP2Y$_{14}$ K_i[a] (nM)	metab stab., % remaining (h/r)[d]
1	3-CF_3	2,4-DiF	75 ± 5	53 ± 28 (n = 5)	>70000	–	–	–	–	75/50
12	2-*t*-Bu	4-Me	7 ± 0.1	7.2 ± 2.1 (n = 2)	>70000	2700	>15000	>15000	2350 ± 950	33/38
16	2-*t*-Bu	4-OCF_3	6 ± 0.6	2.1 ± 0.28 (n = 7)	>70000	>15000	>15000	>15000	3500 ± 1000	80/86
17	2-*t*-Bu	4-*t*-Bu	8 ± 0.4	1.2 ± 0.2 (n = 2)	>70000	>15000	>15000	>15000	5950 ± 950	58/33
18	2-*i*-Pr	4-*t*-Bu	7 ± 0.3	4.4 ± 3.8 (n = 4)	>70000	>15000	>15000	>15000	>15000	73/35
19	2-*i*-Pr	4-OCF_3	16 ± 2	9.4 ± 4 (n = 2)	>70000	6400	>15000	>15000	3300 ± 600	97/80

[a]Data represent the mean ± SEM, n = 2. [b]PRP run in human platelet-rich plasma using 2.5 μM ADP. [c]n = 1. [d]h: human; r: rat. Compounds (3 μM) were incubated with human or rat liver microsomes (1.0 mg/mL) for 10 min.

ring of the urea moiety. This array was prepared following the same chemistry outlined in Scheme 1. This "best−best" array produced several potent antagonists with a clear trend that preferred the combination of substitution of the phenoxy ring with a lipophilic group, such as *tert*-butyl or isopropyl at the ortho or meta position and substitution of the urea phenyl ring at the para position. Listed in Table 4 are a few selected examples from the array demonstrating that several compounds had single digit nanomolar binding affinity. These compounds were also evaluated in a platelet aggregation assay to assess their ability to block ADP-induced platelet aggregation in human platelet rich plasma[14] with several of these showing single digit micromolar potency. To ensure that this antiplatelet activity was due exclusively to P2Y$_1$ antagonism, all compounds were tested for their P2Y$_{12}$ activity. As shown, all compounds had excellent selectivity over P2Y$_{12}$. Additionally, these compounds also showed good selectivity over other P2Y receptors such as P2Y$_2$, P2Y$_6$, P2Y$_{11}$, and P2Y$_{14}$ (Table 4).



Compounds with good in vitro P2Y$_1$ binding affinity and functional activity were evaluated to determine their metabolic stability. Because the in vivo studies were carried out in rats, the metabolic stability in rat became an important factor for compound selection. Among those compounds tested, only compounds **16** and **19** showed reasonable metabolic stability in rats, 86% and 80% remaining after incubation with rat liver microsomes for 10 min, respectively. Although compound **19** was reasonably stable in rats, its functional activity was not quite potent enough for further consideration. The Caco-2 permeability for compound **16** was found to be low (21 nm s^{-1}) as was the aqueous solubility of the hydrochloride salt (<1 μg/mL at pH 6.5). Solubilizing vehicles that were compatible with the in vivo models were used for solution dosing for pharmacokinetic evaluation. The pharmacokinetic profile of compound **16** (Table 5) revealed this compound had reasonable plasma exposure, clearance, and half-life with moderate oral bioavailability (F = 18%) when dosed at 30 mg/kg using 10%EtOH/10% cremophor/80% H_2O as vehicle.

The antithrombotic efficacy of compound **16** was assessed in established arterial thrombosis and bleeding models in male anesthetized rats.[12] As shown in Figure 3, compound **16** clearly improved blood flow and reduced thrombus weight in a dose-dependent manner. A maximum of 68 ± 7% thrombus weight reduction compared to vehicle was observed using a 10 mg/kg bolus followed by 10 mg/kg/h infusion dosing paradigm. Furthermore, vascular occlusion was prevented in all drug-treated rats at this dose. Ex vivo platelet aggregation responses to ADP were also significantly inhibited at all antithrombotic doses. Compound **16** was also tested in provoked bleeding time models. As shown in Figure 4, the highest tested dose of 10 mg/kg plus 10 mg/kg/h prolonged cuticle and mesenteric bleeding times by 3.3- and 3.1-fold over control, respectively. In previous studies using these same rat models, the P2Y$_{12}$ antagonist clopidogrel, administered as an oral pretreatment dose of 20 mg/kg, reduced arterial thrombus weight by 67 ± 5% and prolonged cuticle and mesenteric bleeding times by 4.1- and 8.2-fold over control, respectively.[12]

CONCLUSIONS

Novel P2Y$_1$ antagonists have been identified by optimization of a high throughput screening hit. These urea analogues have demonstrated excellent in vitro P2Y$_1$ antagonist activity. Compound **16** showed inhibition of platelet aggregation in human platelet-rich plasma with no activity toward the P2Y$_{12}$ receptor. Furthermore, compound **16** provided significant reduction in thrombus weight in a rat arterial thrombosis model with a limited effect on bleeding. The antithrombotic and bleeding profiles of compound **16** were favorable in comparison to the established P2Y$_{12}$ antagonist clopidogrel,[15] suggesting that the current series of P2Y$_1$ antagonists may have potential as novel antithrombotic agents.

EXPERIMENTAL SECTION

General Methods. Starting materials, reagents, and solvents were obtained from commercial sources and used as received. All reactions were carried out with continuous stirring under an atmosphere of dry nitrogen. The resonance frequency for ^1H (^{13}C) on a Jeol JNM-ECP-500 is 500 MHz (125 MHz) and Bruker Variance 400 is 400 MHz

Table 5. Pharmacokinetic Properties for Compound 16

compd	dosage, mg/kg	C_{max} (nM)	T_{max} (h)	AUC total (nM·h)	CL, mL/min/kg	V_{ss}, L/kg	T_{half} (h)	F (%)
16[a]	1	160	0.8	169	16	5.6	15.4	7
16[b]	30	5830	2.0	15900	13	0.8	1.43	18

[a]Dose: iv: 1 mg/kg (n = 3), po: 1 mg/kg (n = 2), vehicle: 10% DMAC/10% EtOH/10% cremophor/70% H_2O. [b]Dose: iv: 1 mg/kg (n = 3), po: 30 mg/kg (n = 2), vehicle: 10% EtOH/10% cremophor/80% H_2O.

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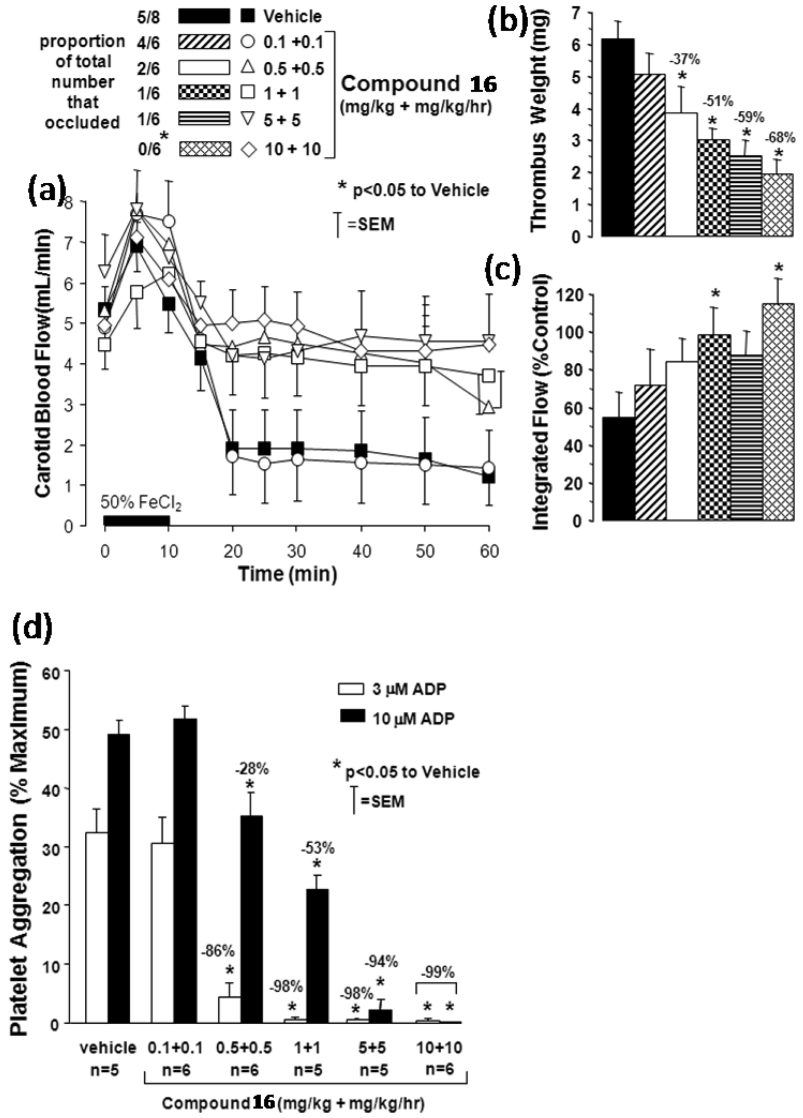

Figure 3. Effect of compound **16** on arterial thrombosis and platelet function in anesthetized rats. Vehicle or compound **16** was dosed as a bolus plus sustaining iv infusion starting 15 min prior to inducing thrombus formation by topical $FeCl_2$. (a) Carotid blood flow was measured during thrombus formation. (b) Thrombus weight, (c) integrated blood flow, and (d) ex vivo platelet aggregation responses to ADP were determined at the end of the experiment when platelet counts were sufficient for the assay (resulted in fewer replicates in some groups). Percent reductions in thrombus weight and platelet aggregation are indicated for responses that were significantly different from vehicle. The number of rats in the thrombosis model is indicated in the "proportion of total number that occluded". Compound **16** doses (mg/kg + mg/kg/h) of 0.1 + 0.1, 0.5 + 0.5, 1 + 1, 5 + 5, and 10 + 10 resulted in plasma concentrations (μM) of 0.4 ± 0.1, 1.8 ± 0.7, 4.0 ± 0.2, 16.5 ± 1.6, and 36.5 ± 2.5, respectively.

(100 MHz). Chemical shifts (δ) are reported in ppm downfield from internal tetramethylsilane (TMS), and coupling constants (J) are in hertz (Hz). Peak multiplicities are expressed as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m), broad singlet (br s). TLC was performed on EMD silica gel 60 F_{254} glass plates (2.5 × 7.5 cm). Microwave reactions were run in a Biotage initiator instrument. Products were analyzed by reverse phase analytical HPLC carried out on a Shimadzu analytical HPLC system running DiscoveryVP software using method A: Phenominex Luna C18 column (4.6 × 50 mm) eluted at 4 mL/min with a 4 min gradient from 100% A to 100% B (A: 10% methanol, 89.9% water, 0.1% TFA; B: 10% water, 89.9% methanol, 0.1% TFA, UV 220 nm), method B: Phenominex Luna C18 column (4.6 × 50 mm) eluted at 4 mL/min with a 4 min gradient from 100% A to 100% B (A: 10% acetonitrile, 89.9% water, 0.1% TFA; B: 10% water, 89.9% acetonitrile, 0.1% TFA, UV 220 nm), or method C: Zorbax SB C18 column (4.6 × 75 mm) eluted at 2.5 mL/min with methanol/water with 0.2% H_3PO_4 as a gradient of 10% to 90% methanol over 8 min followed by holding at 90% methanol for 3 min (UV 220 nm). Purification of intermediates and final products was carried out via either normal or reverse phase chromatography. Normal phase chromatography was carried out on an ISCO CombiFlash System Sq16x using prepacked SiO_2 cartridges eluted with gradients of hexanes and ethyl acetate. Reverse phase preparative HPLC was carried out using a Shimadzu preparative HPLC system running DiscoveryVP software on a Shim-PackVP-ODS column (50 × 20 mm) at 20 mL/min, 6 min gradient 100% A to 100% B with the solvent systems used for the analytical procedure. LCMS were obtained on a Shimadzu HPLC system running DiscoveryVP software, coupled with a Waters Model Platform LC mass spectrometer running MassLynx version 3.5 software using the same column and conditions as utilized for analytical procedure described above. Compounds of interest were greater than 95% pure as determined by orthogonal HPLC methods. The analysis was carried out on a Shimadzu HPLC system, equipped with orthogonal columns; Waters sun fire column (C18, 3.5 μm, 3.0 × 150 mm) and Waters Xbridge phenyl (C18, 3.5 μm, 4.6 × 150 mm) eluted at 2 mL/min with a 12 min gradient from 100% A to 100% B. For low pH, solvent A: 95% H_2O/5% CH_3CN/0.05% TFA; solvent B: 5% H_2O/95%

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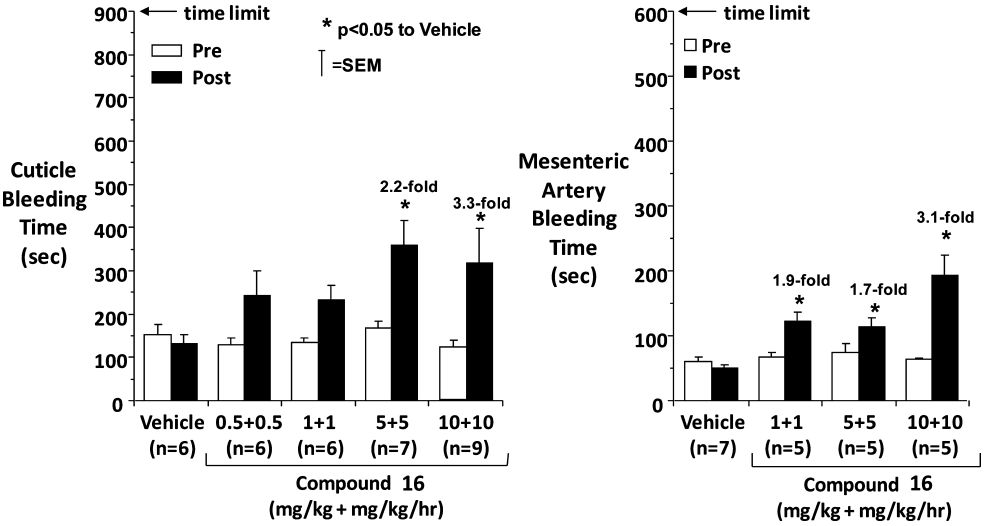

Figure 4. Effect of compound **16** on provoked bleeding times in anesthetized rats. Vehicle or compound **16** was dosed as a bolus plus sustaining iv infusion. Bleeding times were determined before and 15 min after test article administration. Fold increases over control bleeding times are indicated for responses that were significantly different from vehicle.

CH_3CN/0.05% TFA. For high pH, solventA: 95% H_2O/5% CH_3CN/0.01 M NH_4HCO_3; solvent B: 5% H_2O/95% CH_3CN/0.01M NH_4HCO_3.

1-(2,4-Difluorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (1). A solution of 2-chloro-3-nitropyridine **3** (20 g, 125 mmol) in DMF (120 mL) was treated with 3-(trifluoromethyl)-phenol **2** (21 g, 129 mmol) and cesium carbonate (50 g, 154 mmol). The mixture was heated at 70 °C for 14 h. The reaction was cooled to room temperature, and DMF was evaporated. The residue was taken up in EtOAc which was washed with 5% LiCl (3 × 100 mL) and then brine (3 × 100 mL). Drying ($MgSO_4$) and removal of solvent afforded a brown solid which was recrystallized from ethanol to afford compound **4** as an off-white solid (21 g, 60%). (M + H^+) = 285.04; ^{1}H NMR (400 MHz, CD_3OD) δ 7.2 (dd, J = 7.96, 1.39 Hz, 1H), 7.4 (m, 2H), 7.45 (m, 1H), 7.11 (m, 2H), 7.5−7.54 (td, J = 4.93, 1.64 Hz, 1H), 8.48 (dd, J = 7.83, 1.77 Hz, 1H), 8.52 (dd, J = 7.83, 1.77 Hz, 1H).

Compound **4** (21 g, 74 mmol) was dissolved in a 1:4 mixture of methanol and THF (160 mL). Palladium on activated charcoal (10%, 2.1 g, 1.93 mmol) was added, and the mixture was stirred overnight under 1 atm hydrogen pressure. The reaction mixture was filtered over Celite and concentrated to afford a white solid which was recrystallized from EtOAc to afford the 2-(3-(trifluoromethyl)phenoxy)pyridin-3-amine (17.5 g, 92%) as a white powder. (M + H^+) = 254.3. To a solution of the amine (8.2 g, 34.3 mmol) and diphosgene (5.5 g, 28 mmol) in CH_2Cl_2 (175 mL) at 0 °C was added dropwise a solution of CH_2Cl_2 (125 mL) containing N^1,N^1,N^8,N^8-tetramethylnaphthalene-1,8-diamine (14.8 g, 67 mmol). After addition was completed, the resulting mixture was stirred at 0 °C for an additional 45 min and then washed with 0.5 N HCl (3 × 150 mL), 1 N NaOH (2 × 100 mL), and brine. Drying ($MgSO_4$) and removal of solvent afforded compound **5** as a solid (9.6 g, 100%). This material was used for the subsequent reaction without further purification.

To a solution of isocyanate (**5**) (28 mg, 1.0 mmol) in toluene (1 mL) in a 2-dram vial was added 2,4-difluoroaniline (16.1 mg, 0.125 mmol). The resulting mixture was heated with shaking at 80 °C for 1.5 h. The solvent was removed by vacuum centrifugation in a speedvac, and the crude product was purified by reverse phase preparative HPLC using CH_3CN/H_2O/TFA solvent system. After removal of solvent in vacuo, the TFA salt of compound **1** was obtained as a white solid (13.0 mg, 32%). ^{1}H NMR (500 MHz, CD_3OD) δ 6.94 (t, J = 8.80 Hz, 1 H), 7.02 (ddd, J = 11.27, 8.52, 2.75 Hz, 1 H), 7.13 (dd, J = 7.70, 4.95 Hz, 1 H), 7.44 (d, J = 8.25 Hz, 1 H), 7.50 (s, 1 H), 7.53 (d, J = 7.70 Hz, 1 H), 7.62 (t, J = 7.97 Hz, 1 H), 7.73 (d, J = 4.95 Hz, 1 H), 8.09 (td, J = 9.07, 6.05 Hz, 1 H), 8.63 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 410.1 orthogonal HPLC column 1 retention time = 12.12 min, column 2 = 10.29 min.

Compounds **6a−s**, **11a−h**, **12**, and **16−19** were prepared according to the procedures described for **1**.

1-Phenyl-3-(2-(3-(trifluoromethyl)phenoxy)pyridin-3-yl)urea (6a). Yield: 23.9 mg (49%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.03 (t, J = 7.42 Hz, 1 H), 7.13 (dd, J = 7.70, 4.95 Hz, 1 H), 7.26−7.32 (m, 2 H), 7.44 (d, J = 8.25 Hz, 3 H), 7.50 (s, 1 H), 7.54 (d, J = 7.70 Hz, 1 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.72 (d, J = 3.30 Hz, 1 H), 8.61−8.66 (m, 1 H). LC-MS (ESI) m/z 374.0 (M + H^+); orthogonal HPLC column 1 retention time = 12.04 min, column 2 = 10.56 min.

1-(2-Fluorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6b). Yield: 17.2 mg (44%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.00−7.06 (m, 1 H), 7.09−7.17 (m, 3 H), 7.44 (d, J = 8.25 Hz, 1 H), 7.48−7.55 (m, 2 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.73 (d, J = 4.95 Hz, 1 H), 8.11−8.18 (m, 1 H), 8.65 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 392.0 (M + H^+); orthogonal HPLC column 1 retention time = 12.40 min, column 2 = 10.79 min.

1-(2-Chlorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6c). Yield: 19.5 mg (48%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.02−7.08 (m, 1 H), 7.14 (dd, J = 7.70, 4.95 Hz, 1 H), 7.26−7.31 (m, 1 H), 7.40 (d, J = 9.34 Hz, 1 H), 7.44 (d, J = 7.70 Hz, 1 H), 7.50 (s, 1 H), 7.54 (d, J = 8.25 Hz, 1 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.73 (d, J = 4.95 Hz, 1 H), 8.12 (d, J = 8.24 Hz, 1 H), 8.63−8.68 (m, 1 H). LC-MS (ESI) m/z 408.0 (M + H^+); orthogonal HPLC column 1 retention time = 12.81 min, column 2 = 11.05 min.

1-(3-Fluorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6d). Yield: 9.8 mg (25%). ^{1}H NMR (400 MHz, CD_3OD) δ 6.71−6.79 (m, 1 H), 7.07 (d, J = 7.70 Hz, 1 H), 7.14 (dd, J = 8.24, 4.95 Hz, 1 H), 7.23−7.31 (m, 1 H), 7.41−7.51 (m, 3 H), 7.54 (d, J = 7.70 Hz, 1 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.72 (d, J = 4.40 Hz, 1 H), 8.64 (d, J = 7.70 Hz, 1 H). LC-MS (ESI) m/z 392.0 (M + H^+); orthogonal HPLC column 1 retention time = 12.44 min, column 2 = 10.85 min.

1-(3-Chlorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6e). Yield: 15.5 mg (38%). ^{1}H NMR (400 MHz, CD_3OD) δ 6.99−7.03 (m, 1 H), 7.14 (dd, J = 8.25, 4.95 Hz, 1 H), 7.26 (d, J = 5.50 Hz, 2 H), 7.44 (d, J = 8.24 Hz, 1 H), 7.49−7.56 (m, 2 H), 7.62 (d, J = 7.70 Hz, 1 H), 7.67 (s, 1 H), 7.73 (d, J = 3.30 Hz, 1 H), 8.63 (d, J = 6.05 Hz, 1 H). LC-MS (ESI) m/z 408.0 (M + H^+); orthogonal HPLC column 1 retention time = 13.07 min, column 2 = 11.27 min.

1-(4-Fluorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6f). Yield: 14.1 mg (36%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.04 (t, J = 8.79 Hz, 2 H), 7.13 (dd, J = 7.70, 4.95 Hz, 1 H), 7.44 (dd, J = 9.07, 4.67 Hz, 3 H), 7.50 (s, 1 H), 7.54 (d, J = 7.70

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Hz, 1 H), 7.63 (t, J = 7.70 Hz, 1 H), 7.72 (d, J = 3.30 Hz, 1 H), 8.62 (d, J = 6.60 Hz, 1 H). LC-MS (ESI) m/z 392.0 (M + H^+); orthogonal HPLC column 1 retention time = 10.12 min, column 2 = 9.72 min.

1-(4-Chlorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6g). Yield: 21.6 mg (53%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.13 (dd, J = 8.25, 4.95 Hz, 1 H), 7.25−7.31 (m, 2 H), 7.40−7.48 (m, 3 H), 7.50 (s, 1 H), 7.54 (d, J = 7.70 Hz, 1 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.72 (d, J = 4.95 Hz, 1 H), 8.62 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 408.0 (M + H^+); orthogonal HPLC column 1 retention time = 12.95 min, column 2 = 11.19 min.

1-(4-Methylphenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6h). Yield: 18.06 mg (47%). ^{1}H NMR (400 MHz, CD_3OD) δ 2.29 (s, 3H), 7.08−7.15 (m, 3H), 7.29−7.34 (m, 2H), 7.40−7.46 (m, 1H), 7.48−7.56 (m, 2H), 7.61 (d, J = 7.90 Hz, 1H), 7.71 (dd, J = 4.80, 1.80 Hz, 1H), 8.61 (dd, J = 8.10, 1.80 Hz, 1H). LC-MS (ESI) m/z 388.1 (M + H^+); orthogonal HPLC column 1 retention time = 12.09 min, column 2 = 9.98 min.

1-(4-*tert*-Butylphenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6i). Yield: 15.9 mg (37%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.30 (s, 9 H), 7.13 (dd, J = 7.70, 4.95 Hz, 1 H), 7.30−7.39 (m, 4 H), 7.44 (d, J = 8.25 Hz, 1 H), 7.50 (s, 1 H), 7.54 (d, J = 7.70 Hz, 1 H), 7.62 (d, J = 8.24 Hz, 1 H), 7.71 (d, J = 3.30 Hz, 1 H), 8.62 (d, J = 6.05 Hz, 1 H). LC-MS (ESI) m/z 430.1 (M + H^+); orthogonal HPLC column 1 retention time = 13.80 min, column 2 = 11.76 min.

1-(4-(Trifluoromethoxy)phenyl)-3-(2-(3-(trifluoromethyl)-phenoxy)pyridin-3-yl)urea (6j). Yield: 18.7 mg (41%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.13 (dd, J = 7.97, 4.67 Hz, 1 H), 7.21 (d, J = 8.25 Hz, 2 H), 7.44 (d, J = 8.25 Hz, 1 H), 7.50 (s, 1 H), 7.52−7.58 (m, 3 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.71−7.75 (m, 1 H), 8.63 (d, J = 9.89 Hz, 1 H). LC-MS (ESI) m/z 458.0 (M + H); orthogonal HPLC column 1 retention time = 13.25 min, column 2 = 11.34 min.

1-(Biphenyl-4-yl)-3-(2-(3-(trifluoromethyl)phenoxy)pyridin-3-yl)urea (6k). Yield: 5.4 mg (12%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.14 (dd, J = 8.24, 4.95 Hz, 1 H), 7.29 (t, J = 7.42 Hz, 1 H), 7.38−7.48 (m, 3 H), 7.50−7.67 (m, 9 H), 7.72 (d, J = 4.95 Hz, 1 H), 8.65 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 450.0 (M + H^+); orthogonal HPLC column 1 retention time = 13.45 min, column 2 = 11.68 min.

1-(4-Methoxyphenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6l). Yield: 17.3 mg (43%). ^{1}H NMR (400 MHz, CD_3OD) δ 3.77 (s, 3 H), 6.88 (d, J = 8.79 Hz, 2 H), 7.12 (dd, J = 8.24, 4.95 Hz, 1 H), 7.33 (d, J = 9.34 Hz, 2 H), 7.43 (d, J = 7.70 Hz, 1 H), 7.49 (s, 1 H), 7.52−7.56 (m, 1 H), 7.62 (t, J = 7.97 Hz, 1 H), 7.71 (d, J = 4.95 Hz, 1 H), 8.60 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 404.1 (M + H^+); orthogonal HPLC column 1 retention time = 11.68 min, column 2 = 10.32 min

1-(4-Dimethylaminophenyl)-3-(2-(3-(trifluoromethyl)-phenoxy)pyridin-3-yl)urea (6m). Yield: 11.2 mg (27%). ^{1}H NMR (400 MHz, CD_3OD) δ 3.06 (s, 6 H), 7.13 (dd, J = 8.25, 4.95 Hz, 2 H), 7.29−7.37 (m, 1 H), 7.41−7.51 (m, 4 H), 7.52−7.57 (m, 1 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.72 (d, J = 3.30 Hz, 1 H), 8.61 (d, J = 6.05 Hz, 1 H). LC-MS (ESI) m/z 417.0 (M + H^+); orthogonal HPLC column 1 retention time = 6.98 min, column 2 = 7.65 min.

1-(4-Cyanophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6n). Yield: 14.3 mg (36%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.14 (dd, J = 7.70, 4.95 Hz, 1 H), 7.45 (d, J = 8.24 Hz, 1 H), 7.51 (s, 1 H), 7.53−7.57 (m, 1 H), 7.60−7.64 (m, 1 H), 7.66 (s, 4 H), 7.74 (d, J = 4.95 Hz, 1 H), 8.62−8.68 (m, 1 H). LC-MS (ESI) m/z 399.0 (M + H^+); orthogonal HPLC column 1 retention time = 11.82 min, column 2 = 10.41 min.

Methyl 4-(3-(2-(3-(Trifluoromethyl)phenoxy)pyridin-3-yl)-ureido)benzoate (6o). Yield: 22.8 mg (53%). ^{1}H NMR (400 MHz, CD_3OD) δ 3.87 (s, 3 H), 7.14 (dd, J = 7.70, 4.95 Hz, 1 H), 7.45 (d, J = 8.25 Hz, 1 H), 7.49−7.66 (m, 5 H), 7.74 (d, J = 4.95 Hz, 1 H), 7.96 (d, J = 8.79 Hz, 2 H), 8.65 (d, J = 6.05 Hz, 1 H). LC-MS (ESI) m/z 432.0 (M + H^+); orthogonal HPLC column 1 retention time = 12.09 min, column 2 = 10.48 min.

1-(3,4-Chlorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6p). Yield: 17.26 mg (39%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.13 (dd, J = 8.00, 5.00 Hz, 1H), 7.29 (dd, J = 8.80, 2.60 Hz, 1H), 7.38−7.46 (m, 2H), 7.48−7.56 (m, 2H), 7.62 (d, J = 8.10 Hz, 1H), 7.73 (dd, J = 5.00, 1.70 Hz, 1H), 7.83 (d, J = 2.40 Hz, 1H), 8.62 (dd, J = 8.00, 1.70 Hz, 1H). LC-MS (ESI) m/z 442.0 (M + H^+); orthogonal HPLC column 1 retention time = 14.20 min, column 2 = 11.92 min.

1-(3,5-Chlorophenyl)-3-(2-(3-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (6q). Yield: 33.1 mg (75%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.06 (s, 1 H), 7.14 (dd, J = 8.25, 4.95 Hz, 1 H), 7.44 (d, J = 8.25 Hz, 1 H), 7.47−7.52 (m, 3 H), 7.54 (d, J = 7.70 Hz, 1 H), 7.63 (t, J = 7.97 Hz, 1 H), 7.73 (d, J = 4.95 Hz, 1 H), 8.63 (d, J = 6.05 Hz, 1 H). LC-MS (ESI) m/z 442.0 (M + H^+); orthogonal HPLC column 1 retention time = 14.28 min, column 2 = 11.99 min.

1-Benzyl-3-(2-(3-(trifluoromethyl)phenoxy)pyridin-3-yl)urea (6r). Yield: 10.0 mg (26%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.41 (s, 2 H), 7.10 (dd, J = 8.24, 4.95 Hz, 1 H), 7.21−7.28 (m, 1 H), 7.32 (d, J = 4.40 Hz, 4 H), 7.39 (d, J = 8.25 Hz, 1 H), 7.45 (s, 1 H), 7.48−7.54 (m, 1 H), 7.60 (t, J = 7.97 Hz, 1 H), 7.66−7.71 (m, 1 H), 8.55 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 388.1 (M + H^+); orthogonal HPLC column 1 retention time = 11.46 min, column 2 = 10.24 min.

1-Phenethyl-3-(2-(3-(trifluoromethyl)phenoxy)pyridin-3-yl)-urea (6s). Yield: 8.1 mg (20%). ^{1}H NMR (400 MHz, CD_3OD) δ 2.83 (t, J = 7.15 Hz, 2 H), 3.46 (t, J = 7.15 Hz, 2 H), 7.09 (dd, J = 7.97, 4.67 Hz, 1 H), 7.18 (t, J = 6.87 Hz, 1 H), 7.22−7.31 (m, 4 H), 7.37 (d, J = 8.25 Hz, 1 H), 7.43 (s, 1 H), 7.51 (d, J = 7.70 Hz, 1 H), 7.60 (t, J = 7.97 Hz, 1 H), 7.67 (d, J = 4.95 Hz, 1 H), 8.52 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 402.1 (M + H^+); orthogonal HPLC column 1 retention time = 11.79 min, column 2 = 10.46 min.

3-Nitro-2-phenoxypyridines 8. Forty eight phenols **7** (1.06 mmol each in 1 mL of DMF) were dispensed into 24-well miniblocks. To these solutions was added Cs_2CO_3 (360 mg, 1.1 mmol) to each well followed by 2-chloro-3-nitropyridine (**3**) (158 mg, 1.0 mmol) in DMF (1 mL). The reactions were heated with shaking at 80 °C for 18 h. The reaction mixtures were filtered and collected into 16 × 100 mm test tubes. EtOAc (2 mL) was added to each well, shaken for 2−3 min, and then combined with the DMF filtrate. The combined solutions were dried in speedvac. The crude mixtures were dissolved in EtOAc (2.5−4 mL) and washed with H_2O (2 × 0.6 mL), saturated $NaHCO_3$ (1 × 0.5 mL), and H_2O (2 × 0.5 mL). Solvents were removed by vacuum centrifugation in a speedvac to afford crude products which were used for the subsequent reaction without further purification.

3-Amino-2-phenoxypyridines 9. The nitro compounds **8** were dissolved in a 4:1 mixed solution containing absolute EtOH (4 mL) and EtOAc (1 mL). Zn dust (1.3 g, 20 mmol) and NH_4Cl (300 mg, 5.4 mmol) were added to these solutions sequentially. The resulting mixtures were shaken at room temperature overnight. The solutions were filtered through a bed of Celite and collected into 16 × 100 mm test tubes. Solvents were removed by vacuum centrifugation in a speedvac to afford crude products which were used for the subsequent reaction without further purification.

1-(2,4-Difluorophenyl)-3-(2-phenoxypyridin-3-yl)ureas (11). To these 3-amino-2-phenoxypyridines **9** (0.085 mmol) in 2-dram vials was added 2,4-difluorophenyl isocyanate **10** (0.127 mmol) in anhydrous toluene (1 mL). The reaction mixtures were heated with shaking at 80 °C for 1.5 h. Solvents were removed by vacuum centrifugation in a speedvac, and the crude products were purified by reverse phase preparative HPLC.

1-(2,4-Difluorophenyl)-3-(2-phenoxypyridin-3-yl)urea (11a). Yield: 11.9 mg (35%). ^{1}H NMR (500 MHz, CD_3OD) δ 6.94 (t, J = 8.80 Hz, 1 H), 7.01 (ddd, J = 11.41, 8.66, 3.02 Hz, 1 H), 7.09 (dd, J = 8.25, 4.95 Hz, 1 H), 7.15 (d, J = 8.25 Hz, 2 H), 7.22 (t, J = 7.42 Hz, 1 H), 7.42 (t, J = 7.97 Hz, 2 H), 7.69 (d, J = 4.95 Hz, 1 H), 8.08 (td, J = 9.07, 6.05 Hz, 1 H), 8.61 (d, J = 7.70 Hz, 1 H). LC-MS (ESI) m/z 342.2 (M + H^+); orthogonal HPLC column 1 retention time = 11.05 min, column 2 = 9.47 min.

1-(2-(2-*tert*-Butylphenoxy)pyridin-3-yl)-3-(2,4-difluorophenyl)urea (11b). Yield: 17.5 mg (44%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.40 (s, 9 H), 6.86 (d, J = 8.25 Hz, 1 H), 6.94 (t, J = 8.80 Hz, 1 H), 7.01 (ddd, J = 11.27, 8.52, 3.30 Hz, 1 H), 7.06 (dd, J = 7.97, 4.67 Hz, 1 H), 7.13−7.19 (m, 1 H), 7.20−7.24 (m, 1 H), 7.48 (d, J = 7.70 Hz, 1 H), 7.68 (d, J = 4.95 Hz, 1 H), 8.07 (td, J = 9.07, 5.50 Hz, 1 H), 8.56 (d, J = 7.70 Hz, 1 H). LC-MS (ESI) m/z 398.2 (M +

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H^+); orthogonal HPLC column 1 retention time = 12.78 min, column 2 = 10.66 min.

1-(2-(3-*tert*-Butylphenoxy)pyridin-3-yl)-3-(2,4-difluorophenyl)urea (11c). Yield: 15.9 mg (40%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.33 (s, 9 H), 6.91−6.96 (m, 2 H), 7.01 (ddd, J = 11.27, 8.52, 3.30 Hz, 1 H), 7.08 (dd, J = 7.70, 4.95 Hz, 1 H), 7.19 (s, 1 H), 7.26−7.30 (m, 1 H), 7.34 (t, J = 7.97 Hz, 1 H), 7.69 (d, J = 3.30 Hz, 1 H), 8.08 (td, J = 9.21, 5.77 Hz, 1 H), 8.61 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 398.2 (M + H^+); orthogonal HPLC column 1 retention time = 13.03 min, column 2 = 10.92 min.

1-(2,4-Difluorophenyl)-3-(2-(2-isopropylphenoxy)pyridin-3-yl)urea (11d). Yield: 16.8 mg (44%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.20 (d, J = 6.60 Hz, 6 H), 3.12 (t, J = 7.15 Hz, 1 H), 6.94 (t, J = 8.52 Hz, 1 H), 6.99−7.07 (m, 3 H), 7.19−7.29 (m, 2 H), 7.41 (dd, J = 5.77, 3.57 Hz, 1 H), 7.62 (d, J = 4.95 Hz, 1 H), 8.09 (td, J = 9.07, 6.05 Hz, 1 H), 8.57 (d, J = 7.70 Hz, 1 H). LC-MS (ESI) m/z 384.2 (M + H^+); orthogonal HPLC column 1 retention time = 12.55 min, column 2 = 10.55 min.

1-(2,4-Difluorophenyl)-3-(2-(2-ethylphenoxy)pyridin-3-yl)-urea (11e). Yield: 15.7 mg (42%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.17 (t, J = 7.42 Hz, 3 H), 2.59 (q, J = 7.70 Hz, 2 H), 6.94 (t, J = 8.80 Hz, 1 H), 6.99−7.09 (m, 3 H), 7.16−7.28 (m, 2 H), 7.34 (d, J = 7.70 Hz, 1 H), 7.62 (d, J = 4.95 Hz, 1 H), 8.09 (td, J = 9.07, 6.05 Hz, 1 H), 8.58 (d, J = 7.70 Hz, 1 H). LC-MS (ESI) m/z 370.2 (M + H^+); orthogonal HPLC column 1 retention time = 12.10 min, column 2 = 10.22 min.

1-(2,4-Difluorophenyl)-3-(2-(2-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (11f). Yield: 13.9 mg (34%). ^{1}H NMR (400 MHz, CD_3OD) δ 6.99−6.91 (m, 1H), 6.99−6.91 (m, 1H), 7.03 (s, 1H), 7.11 (dd, J = 8.00, 4.90 Hz, 1H), 7.27 (d, J = 8.30 Hz, 1H), 7.44 (s, 1H), 7.74−7.65 (m, 2H), 7.79 (d, J = 7.80 Hz, 1H), 8.09 (d, J = 5.80 Hz, 1H), 8.61 (dd, J = 8.10, 1.80 Hz, 1H). LC-MS (ESI) m/z 410.1 (M + H^+); orthogonal HPLC column 1 retention time = 12.19 min, column 2 = 10.36 min.

1-(2,4-Difluorophenyl)-3-(2-(4-(trifluoromethyl)phenoxy)-pyridin-3-yl)urea (11g). Yield: 13.9 mg (34%). ^{1}H NMR (500 MHz, CD_3OD) δ 6.94 (t, J = 8.80 Hz, 1 H), 6.98−7.05 (m, 1 H), 7.15 (dd, J = 8.25, 4.95 Hz, 1 H), 7.34 (d, J = 8.80 Hz, 2 H), 7.71−7.77 (m, 3 H), 8.08 (td, J = 9.07, 6.05 Hz, 1 H), 8.65 (d, J = 7.70 Hz, 1 H). LC-MS (ESI) m/z 410.0 (M + H^+); orthogonal HPLC column 1 retention time = 12.46 min, column 2 = 10.81 min.

1-(2, 4-Difluorophenyl)-3-(2-(4-(methyl)phenoxy)pyridin-3-yl)urea (11h). Yield: 15.3 mg (43%). ^{1}H NMR (400 MHz, CD_3OD) δ 2.36 (s, 3 H), 6.90−6.97 (m, 1 H), 6.98−7.04 (m, 3 H), 7.06 (dd, J = 8.25, 4.95 Hz, 1 H), 7.23 (d, J = 8.25 Hz, 2 H), 7.66 (d, J = 4.95 Hz, 1 H), 8.08 (td, J = 9.21, 5.77 Hz, 1 H), 8.59 (d, J = 6.05 Hz, 1 H). LC-MS (ESI) m/z 356.2 (M + H^+); orthogonal HPLC column 1 retention time = 11.67 min, column 2 = 9.91 min.

1-(2-(2-*tert*-Butylphenoxy)pyridin-3-yl)-3-*p*-tolylurea (12). Yield: 8.6 mg (23%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.39 (s, 9 H), 2.29 (s, 3 H), 6.86 (d, J = 8.25 Hz, 1 H), 7.06 (dd, J = 8.25, 4.95 Hz, 1 H), 7.11 (d, J = 8.80 Hz, 2 H), 7.14−7.19 (m, 1 H), 7.20−7.24 (m, 1 H), 7.32 (d, J = 8.25 Hz, 2 H), 7.48 (d, J = 7.70 Hz, 1 H), 7.66 (d, J = 4.95 Hz, 1 H), 8.56 (d, J = 6.60 Hz, 1 H). LC-MS (ESI) m/z 376.2 (M + H^+); orthogonal HPLC column 1 retention time = 12.82 min, column 2 = 10.75 min.

Synthesis of 1-(2-(2-*tert*-Butylphenylthio)pyridin-3-yl)-3-*p*-tolylurea (13). To a solution of 2-chloro-3-nitropyridine **3** (158 mg, 1.0 mmol) and 2-*tert*-butylbenzenethiol (166 mg, 1.0 mmol) in DMF (3 mL) was added Cs_2CO_3 (383 mg, 1.2 mmol). The resulting mixture was stirred at 80 °C overnight. The insoluble material was removed by filtration, and the DMF solution was diluted with H_2O (5 mL) and extracted with EtOAc (2 × 6 mL). The EtOAc solution was washed with 5% aq LiCl solution (3 × 3 mL). Drying ($MgSO_4$) and removal of solvent in vacuo afforded a yellow solid which was triturated with MeOH to yield 2-((2-*tert*-butylphenyl)thio)-3-nitropyridine as a yellow solid (115 mg, 40%). HPLC purity: 100%. This material was used for the next step without further purification.

To a solution of 2-((2-*tert*-butylphenyl)thio)-3-nitropyridine (50 mg, 0.173 mmol) in 1:1 MeOH/EtOAc (3 mL) was added 10% palladium on activated charcoal (5 mg, 0.0047 mmol). The resulting mixture was stirred at 3.5 atm hydrogen pressure for 1 h. The catalyst was removed by filtration through a bed of Celite, and the solvent was removed by vacuum centrifugation in a speedvac to afford 2-((2-*tert*-butylphenyl)thio)pyridin-3-amine as a yellow solid (33 mg, 74%). (M + H^+) = 259.1.

To a solution of 2-((2-*tert*-butylphenyl)thio)pyridin-3-amine (33 mg, 0.12 mmol) in anhydrous THF (2 mL) was added *p*-tolyl isocyanate (20 μL, 0.15 mmol). The resulting mixture was stirred at 60 °C for 1.5 h. The solvent was removed by vacuum centrifugation in a speedvac, and the crude mixture was purified by reverse phase preparative HPLC. After removal of solvent in vacuo, the TFA salt of compound **13** was obtained as a yellow solid (12.5 mg, 27%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (s, 9 H), 2.28 (s, 3 H), 6.97 (d, J = 7.70 Hz, 1 H), 7.05 (d, J = 7.70 Hz, 2 H), 7.10 (t, J = 7.42 Hz, 1 H), 7.20 (d, J = 7.70 Hz, 2 H), 7.25−7.37 (m, 2 H), 7.49 (d, J = 7.70 Hz, 1 H), 8.15 (d, J = 3.85 Hz, 1 H), 8.74 (d, J = 8.25 Hz, 1 H). LC-MS (ESI) m/z 392.4 (M + H^+); orthogonal HPLC column 1 retention time =13.12 min, column 2 = 10.95 min.

1-(2-(2-*tert*-Butylphenylamino)pyridin-3-yl)-3-*p*-tolylurea (14). To a solution of 2-chloro-3-nitropyridine **3** (1.58 g, 10 mmol) and 2-*tert*-butylaniline (1.79 g, 12 mmol) in dioxane (50 mL) was added NEt_3 (1.21 g, 12 mmol). The resulting solution was reflux for 16 h, and solvent was evaporated in vacuo to afford a yellow solid which was recrystallized from MeOH to give *N*-(2-*tert*-butylphenyl)-3-nitropyridin-2-amine (1.98 g, 73%) as a yellow solid. (M + H^+) = 272.1.

The *N*-(2-(*tert*-butylphenyl)-3-nitropyridin-2-amine (272 mg, 1 mmol) was then dissolved in a 4:1 mixed solution containing absolute EtOH (4 mL) and EtOAc (1 mL). Zn dust (1.3 g, 20 mmol) and NH_4Cl (300 mg, 5.4 mmol) were added to this solution sequentially. The resulting mixture was stirred at room temperature overnight. The solution was filtered through a bed of Celite, and the solvent was evaporated in vacuo to afford the N^2-(2-*tert*-butylphenyl)pyridine-2,3-diamine (242 mg, 100%) as solid. (M + H^+) = 242.1. This material was used for the next step without further purification.

To a solution of N^2-(2-*tert*-butylphenyl)pyridine-2,3-diamine (24.2 mg, 0.1 mmol) in anhydrous THF (1 mL) in a 1-dram vial was added *p*-tolyl isocyanate (20 μL, 0.15 mmol). The resulting mixture was stirred at 60 °C for 1.5 h. The solvent was removed by vacuum centrifugation in a speedvac, and the crude mixture was purified by reverse phase preparative HPLC. After removal of solvent in vacuo, the TFA salt of compound **14** was obtained as a white solid (15.3 mg, 41%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26 (s, 9 H), 2.27 (s, 3 H), 6.70−6.76 (m, 1 H), 6.95 (d, J = 7.70 Hz, 1 H), 7.04 (d, J = 8.25 Hz, 2 H), 7.14−7.22 (m, 2 H), 7.24−7.30 (m, 1 H), 7.35 (d, J = 8.25 Hz, 2 H), 7.48 (d, J = 7.70 Hz, 1 H), 8.47 (d, J = 7.70 Hz, 1 H), 9.08 (br s, 1 H), 9.94 (d, J = 6.60 Hz, 1 H), 10.05 (br s, 1 H). LC-MS (ESI) m/z 375.2 (M + H^+); orthogonal HPLC column 1 retention time = 7.13 min, column 2 = 7.95 min.

1-(2-((2-*tert*-Butylphenyl)(methyl)amino)pyridin-3-yl)-3-*p*-tolylurea (15). To a solution of *N*-(2-*tert*-butylphenyl)-3-nitropyridin-2-amine (816 mg, 3 mmol) in anhydrous THF (6 mL) was added 60% NaH (360 mg, 9 mmol) followed by iodomethane (2.56 g, 18 mmol). After the mixture was stirred at 60 °C for 24 h, an additional 3 equiv of iodomethane (1.28 g, 9 mmo) was added. Stirring was continued at 60 °C for an additional 48 h. The reaction mixture was allowed to cool to room temperature, and solvent was evaporated to afford a crude mixture which was purified by reverse phase preparative HPLC. Removal of solvent afforded a yellow oil which was dissolved in EtOAc (20 mL) and washed with $NaHCO_3$. Drying ($MgSO_4$) and removal of solvent afforded the *N*-(2-*tert*-butylphenyl)- *N*-methyl-3-nitropyridin-2-amine (427 mg, 50%) as a light yellow oil. (M + H^+) = 286.1.

To a solution of *N*-(2-*tert*-butylphenyl)-*N*-methyl-3-nitropyridin-2-amine (427 mg, 1.5 mmol) in EtOH (10 mL) was added 10% palladium on activated charcoal (50 mg, 0.047 mmol). The resulting solution was stirred under 1 atm hydrogen pressure for 48 h. The solution was filtered through a bed of Celite, and the solvent was evaporated in vacuo to afford the N^2-(2-*tert*-butylphenyl)-N^2-

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methylpyridine-2,3-diamine (267 mg, 70%) as a dark oil. (M + H$^+$) = 256.1. This material was used for the next step without further purification.

To a solution of N^2-(2-*tert*-butylphenyl)-N^2-methylpyridine-2,3-diamine (25.5 mg, 0.1 mmol) in anhydrous THF (1 mL) in a 1-dram vial was added *p*-tolyl isocyanate (20 μL, 0.15 mmol). The resulting mixture was stirred at 60 °C for 1.5 h. The solvent was removed by vacuum centrifugation in a speedvac, and the crude mixture was purified by reverse phase preparative HPLC. After removal of solvent in vacuo TFA salt of compound **15** was obtained as a white solid (8.9 mg, 23%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.28 (s, 9 H), 2.21 (s, 3 H), 3.19 (s, 3 H), 6.80 (dd, *J* = 7.70, 4.95 Hz, 1 H), 6.96−7.07 (m, 3 H), 7.12−7.23 (m, 4 H), 7.40−7.52 (m, 2 H), 7.98 (d, *J* = 4.40 Hz, 1 H), 8.59 (br s, 1 H). LC-MS (ESI) *m*/*z* 389.3 (M + H$^+$); orthogonal HPLC column 1 retention time = 7.43 min, column 2 = 8.15 min.

1-(2-(2-*tert*-Butylphenoxy)pyridin-3-yl)-3-(4-(trifluoromethoxy)phenyl)urea (16). Yield: 15.1 mg (34%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.41 (s, 9 H), 7.05−7.12 (m, 1 H), 7.21 (d, *J* = 8.80 Hz, 2 H), 7.30−7.37 (m, 2 H), 7.43 (dd, *J* = 8.25, 5.50 Hz, 1 H), 7.54−7.66 (m, 3 H), 7.81 (d, *J* = 5.50 Hz, 1 H), 9.06 (d, *J* = 8.25 Hz, 1 H). ^{13}C NMR (125 MHz, CD_3OD), δ 29.81, 34.26, 118.68, 119.37, 119.80, 121.42, 121.90, 123.03, 124.64, 125.14, 126.96, 127.21, 127.81, 138.21, 139.10, 141.63, 144.14, 144.16, 152.82, 153.36, 153.42. LC-MS (ESI) *m*/*z* 446.1 (M + H$^+$); orthogonal HPLC column 1 retention time = 12.37 min, column 2 = 12.78 min.

1-(2-(2-*tert*-Butylphenoxy)pyridin-3-yl)-3-(4-*tert*-butylphenyl)urea (17). Yield: 19.2 mg (46%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.30 (s, 9 H), 1.40 (s, 9 H), 6.91 (d, *J* = 7.70 Hz, 1 H), 7.14 (dd, *J* = 7.97, 5.22 Hz, 1 H), 7.18−7.22 (m, 1 H), 7.25 (t, *J* = 7.70 Hz, 1 H), 7.32−7.39 (m, 4 H), 7.51 (d, *J* = 7.70 Hz, 1 H), 7.69 (d, *J* = 5.50 Hz, 1 H), 8.68 (d, *J* = 7.70 Hz, 1 H). LC-MS (ESI) *m*/*z* 418.2 (M + H$^+$); orthogonal HPLC column 1 retention time = 12.68 min, column 2 = 11.02 min.

1-(4-*tert*-Butylphenyl)-3-(2-(2-isopropylphenoxy)pyridin-3-yl)urea (18). Yield: 25.4 mg (63%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.20 (d, *J* = 6.05 Hz, 6 H), 1.31 (s, 9 H), 3.06−3.17 (m, 1 H), 6.97−7.01 (m, 1 H), 7.03 (dd, *J* = 7.70, 4.95 Hz, 1 H), 7.24 (dd, *J* = 5.50, 3.30 Hz, 2 H), 7.32−7.39 (m, 4 H), 7.40−7.44 (m, 1 H), 7.60 (d, *J* = 4.95 Hz, 1 H), 8.57 (d, *J* = 7.70 Hz, 1 H). LC-MS (ESI) *m*/*z* 404.2 (M + H$^+$); orthogonal HPLC column 1 retention time = 13.52 min, column 2 = 12.00 min.

1-(2-(2-Isopropylphenoxy)pyridin-3-yl)-3-(4-(trifluoromethoxy)phenyl)urea (19). Yield: 24.1 mg (57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25−1.16 (m, 6H), 3.07 (dt, *J* = 13.60, 6.70 Hz, 1H), 7.05 (d, *J* = 8.20 Hz, 1H), 7.15 (s, 2H), 7.36−7.22 (m, 3H), 7.42 (d, *J* = 7.70 Hz, 1H), 7.55 (d, *J* = 3.30 Hz, 2H), 7.74 (d, *J* = 4.90 Hz, 1H), 9.11 (dd, *J* = 14.80, 8.20 Hz, 1H). LC-MS (ESI) *m*/*z* 424.3 (M + H$^+$); orthogonal HPLC column 1 retention time = 12.32 min, column 2 = 12.73 min.

P2Y$_1$ Binding Assay. The P2Y$_1$ binding assay was used to identify inhibitors of [β-^{33}P]-2MeS-ADP binding to cloned human P2Y$_1$ receptors. The cDNA clone for human P2Y$_1$ was obtained from Incyte Pharmaceuticals and its sequence confirmed by established techniques (for a compendium of techniques used, see Ausubel, F. et al. *Current Protocols in Molecular Biology*; John Wiley and Sons: New York, 1995). The essential coding sequences were subcloned into pCDNA 3.1 (Invitrogen) to produce a P2Y$_1$ expression construct. This construct was then transfected into the human embryonic kidney cell line HEK-293, and stable transfectants were selected in GENETICIN (G418 sulfate; Life Technologies). Several lines were screened for binding activity, and one (HEK293 #49) was selected for further characterization. Membranes were prepared by growing HEK293 #49 in 150 mm dishes in DMEM/10% FBS in the presence of 1 mg/mL G418 until cells were 80−90% confluent. Plates were then washed with cold (4 °C) D-PBS twice and cells harvested by scraping into 10 mL of D-PBS. Cells were pelleted by centrifugation (1000*g*, 10 min, 4 °C), and the resulting pellet was resuspended in lysis buffer (10 mM Tris (7.4), 5 mM $MgCl_2$ containing Complete protease inhibitor cocktail (Roche cat. no. 1873580). The suspension was then homogenized in a Dounce homogenizer (10−15 strokes; B pestle, on ice) and the homogenate spun at 1000*g*, 4 °C, 5 min to pellet large debris. The supernatant was centrifuged at 150 000*g*, 4 °C, for 1 h and the resulting membrane pellet resuspended in 0.5−1 mL of buffer B (15 mM HEPES (7.4), 145 mM NaCl, 0.1 mM $MgCl_2$, 5 mM EDTA, 5 mM KCl), and stored at −70 °C until used. A typical procedure which was adequate for solubilizing all urea compounds consisted of adding a 10 μL aliquot of DMSO containing the test compound diluted to the final assay concentrations of 0.01 nM to 1 mM in the bottom of a 96-well WGA Flashplate (NEN) and adding 190 μL of assay buffer containing sufficient [β-^{33}P]-2MeS-ADP and membrane preparation to produce final concentrations of 0.5 nM and 0.025 μg/μL, respectively. Binding reactions were allowed to proceed to completion at room temperature for 1 h, and then the aqueous solution was aspirated. Plates were sealed, and the residual [β-^{33}P] bound to the plate was determined by scintillation counting. Dose−response curves (IC_{50}) were fit by nonlinear regression (XLFit, ID Business Solutions Ltd.) and binding constants (K_i) calculated using the Cheng−Prusoff relationship ($K_i = IC_{50}/(1 + L/K_d)$ in which a K_d for 2MeS-ADP to the P2Y$_1$ receptor was determined to be 14 nM).

P2Y$_{12}$ Binding Assay. The P2Y$_{12}$ cell binding assay is designed for testing compound selectivity. The cDNA of P2Y$_{12}$ was obtained from Incyte Pharmaceticals and its sequence confirmed by established technique. The essential coding sequences were subcloned into pCDNA 3.1 (Invitrogen) to produce a P2Y$_{12}$ expression construct. This construct was then transfected into the human embryonic kidney cell line HEK-293, and transfected cells were selected in hygromycin B (Invitrogen cat. no. 10687-010).

HEK293-P2Y$_{12}$ cells (50K/well) in a 50 μg/mL poly-D-lysine-precoated 96-well assay plate (Costar cat. no. 3917) with selective medium (0.5 mg/mL hygromycin, 10% FBS, DMEM) were plated overnight.The cell culture medium was aspirated, and the cells were washed with 100 μL of serum-free DMEM once. Then 98 μL of serum-free DMEM, 2 μL of compound solution, and 100 μL of 1 nM [β-^{33}P]-2MeS-ADP were added to each well. The assay plate was incubated at 5% CO_2, 37 °C, for 1 h. At the end of the incubation, the aqueous medium was aspirated and the cells were washed with 200 μL of DPBS twice. The assay plate was dried by inverting it on a paper towel, 100 μL of scintillation fluid (Ultima Gold, Perkin-Elmer cat. no. 603329) was added to each well, and the assay plate was shaken for 15 min. [β-^{33}P] bound to the cells was determined by scintillation counting in TopCount. Dose−response curves (IC_{50}) were fit by nonlinear regression (XLFit, ID Business Solutions Ltd.), and binding constants (K_i) were calculated using the Cheng−Prusoff relationship ($K_i = IC_{50}/(1 + L/K_d)$ in which a K_d for 2MeS-ADP to the P2Y$_{12}$ receptor was determined to be 1.4 nM.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ADP, adenosine diphosphate; AUC, area under curve; CL, clearance; iv, intravenous; HEK, human embryonic kidney; HPLC, high-pressure liquid chromatography; metab stab., metabolic stability; po, per os (by mouth); PRP, platelet-rich plasma; P2Y, G-protein-coupled purinergic receptors; SAR, structure−activity relationship; TFA, trifluoroacetic acid; V_{ss}, volume of distribution; WGA, wheat germ agglutinin

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