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2-Aminothiazole based $P2Y_1$ antagonists as novel antiplatelet agents

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ABSTRACT

ADP receptors, $P2Y_1$ and $P2Y_{12}$ have been recognized as potential targets for antithrombotic drugs. A series of $P2Y_1$ antagonists that contain 2-aminothiazoles as urea surrogates were discovered. Extensive SAR of the thiazole ring is described. The most potent compound **7j** showed good $P2Y_1$ binding (K_i = 12 nM), moderate antagonism of platelet aggregation (PA IC_{50} = 5.2 μM) and acceptable PK in rats.

Adenosine-5′-diphosphate (ADP) released from platelets, red blood cells and damaged blood vessels is a key activator of platelets and plays a crucial role in generation of arterial thrombi at the site of vascular injury.[1] Two G-protein coupled receptors, $P2Y_1$ and $P2Y_{12}$, are required for full ADP-induced platelet aggregation, but each of these receptors plays a different role in this process.[2] $P2Y_1$ triggers a rapid and transient intracellular calcium increase which causes platelet shape change and initiates the process of platelet activation.[3] $P2Y_{12}$ mediates a slower and more sustained decrease in cyclic adenosine monophosphate (cAMP), which amplifies and consolidates ADP-driven platelet activation.[4] Co-activation of both $P2Y_1$ and $P2Y_{12}$ is required for a complete platelet response, however, blockade of either receptor significantly decreases ADP-induced platelet aggregation and thrombosis formation.[5]

Given their key roles in platelet aggregation, $P2Y_1$ and $P2Y_{12}$ receptors have drawn increasing attention as potential targets of antithrombotic therapy. The $P2Y_{12}$ receptor is a well-established target of marketed antithrombotic drugs,[6] for example, clopidogrel[7], prasugrel[8] and ticagrelor.[9] Recently the $P2Y_1$ receptor has emerged as a new target for novel antithrombotic agents.[10] Mice that either lacked the $P2Y_1$ gene or had been treated with the selective $P2Y_1$ antagonist, MRS-2500,[11a] demonstrated a significant antagonism of ADP-induced platelet aggregation. MRS-2500 administered intravenously showed strong efficacy in a thrombosis model with only a moderate prolongation of bleeding time.[11b]

These studies suggest that novel $P2Y_1$ antagonists could provide an alternative or complement to current antithrombotic strategies and our efforts focused on the search for orally bioavailable $P2Y_1$ antagonists for chronic treatment.[12] This paper describes our initial effort towards the design, synthesis, and biological evaluation of a series of novel 2-aminothiazole based $P2Y_1$ antagonists.[13]

Biarylurea **1**[12d] (Fig. 1) was identified by our lead discovery group as a potent $P2Y_1$ antagonist in vitro with a K_i of 6 nM. This compound showed moderate antagonism in the platelet aggregation assay with a platelet aggregation (PA) IC_{50} of 2.1 μM using 2.5 μM ADP. In search of structurally distinct urea isosteres that could act as $P2Y_1$ antagonists, we designed aminooxazole **2**,[14] aminoimidazole **3** and aminothiazole **4**[14] (Fig. 1) as potential urea surrogates. Compared to biarylurea **1**, aminooxazole analog **2** ($P2Y_1$ K_i = 59 nM) showed a threefold decrease in binding while 2-aminoimidazole **3** ($P2Y_1$ K_i > 23 μM) did not show significant $P2Y_1$ binding affinity. Aminothiazole **4** ($P2Y_1$ K_i = 15 nM)

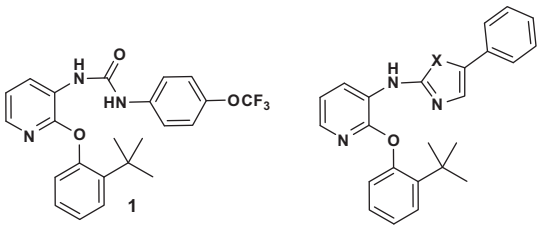

Figure 1. 2-Aminoheterocycles as $P2Y_1$ antagonists.

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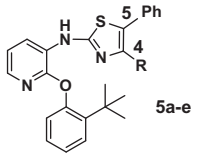
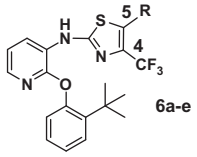
Table 1
In vitro $P2Y_1$ activity of **5a–e** versus **4**

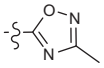


Entry	R	$P2Y_1$ K_i (nM)[a]	PA %Ctrl[b]@ 10 μM
4	H	15	87
5a	Et	12	78
5b	*t*-Bu	31	86
5c	CF_3	7	61
5d	$C(Me)_2CH_2CO_2H$	207	89
5e	$CONMe_2$	186	100

[a] $n = 2$, variation in individual values <20%.
[b] Platelet aggregation (PA) AUC% control (%Ctrl) was tested with 10 μM of drug and 2.5 μM of ADP.

Table 2
In vitro $P2Y_1$ activity of **6a–e** versus **5c**



Entry	R	$P2Y_1$ K_i (nM)[a]	PA %Ctrl[b]@ 10 μM
5c	Ph	7	61
6a	Me	11	96
6b	CO_2Et	5	50
6c	CN	21	101
6d		6	56
6e	$CONH_2$	4850	––

[a] $n = 2$, variation in individual values <20%.
[b] Platelet aggregation (PA) AUC% control (%Ctrl) was tested with 10 μM of drug and 2.5 μM of ADP.

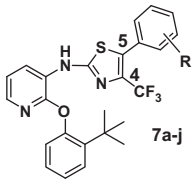
maintained similar $P2Y_1$ binding potency to that shown by **1**. However, compounds **2–4** did not show functional activity in a human platelet aggregation assay.[12d] As described herein, we selected aminothiazole **4** based on binding affinity to optimize further for increased functional assay potency.

For initial optimization of compound **4** at the C4 position nonpolar ethyl (**5a**), *t*-butyl (**5b**) and trifluoromethyl (**5c**) analogs were synthesized which maintained good $P2Y_1$ binding affinity compared to the parent compound **4** (Table 1). However, polar groups such as carboxyl (**5d**) or amide (**5e**) at this position led to significant loss of binding affinity compared to compound **4**. Compounds with $P2Y_1$ K_i values less than 300 nM were tested for platelet aggregation at 10 μM with 2.5 μM ADP. In the C4 substituted series, compound **5c** (PA %Ctrl = 61) with a CF_3 group was the most potent showing a modest level of antagonism in the platelet aggregation assay with an IC_{50} of 12 μM.

Further optimization of **5c** at the *C*5 position is shown in Table 2. A variety of groups at the *C*5 position such as methyl (**6a**), ethoxycarbonyl (**6b**), cyano (**6c**), and oxadiazole (**6d**) maintained good $P2Y_1$ binding affinity. Ester **6b** was equipotent in the platelet aggregation assay (PA %Ctrl = 50%) to compound **5c**. However, converting the ethoxycarbonyl (**6b)** to the corresponding primary amide (**6e**) resulted in significant loss of $P2Y_1$ binding potency.

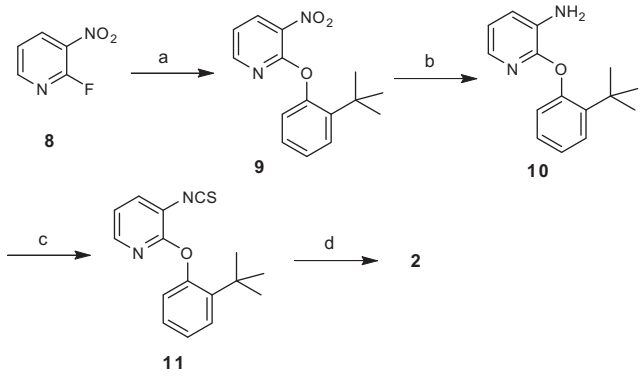
Since the phenyl substituent was well tolerated at *C*5, we sought to improve the potency further by exploring phenyl group substitutions. A number of small groups such as fluoro, methyl, methoxy, trifluoromethoxy and cyano (**7a–g**) at different positions on the phenyl ring were equipotent in $P2Y_1$ binding affinity to **5c** (Table 3). However, both hydroxyl analogs **7h** and **7i** showed significantly reduced binding affinity. Methoxy compounds **7d** and **7e** showed improved potency compared to **5c** in the platelet aggregation assay. We explored additional ether linked groups with

Table 3
In vitro $P2Y_1$ activity of **7a–j** versus **5**c

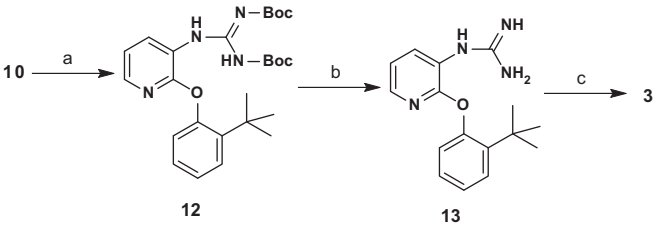


Entry	R	$P2Y_1$ K_i (nM)[a]	PA %Ctrl[b]@ 10 μM
5c	H	7	61
7a	2-Me	11	54
7b	4-Me	8	32
7c	2-F	7	41
7d	3-OMe	8	41
7e	4-OMe	8	30
7f	$4\text{-}OCF_3$	12	85
7g	4-CN	13	79
7h	2-OH	435	99
7i	3-OH	195	98
7j	$3\text{-}O(CH_2)C(Me)_2CH_2N(Me)_2$	12	17

[a] $n = 2$, variation in individual values <20%.
[b] Platelet aggregation (PA) AUC% control (%Ctrl) was tested with 10 μM of drug and 2.5 μM of ADP.



Scheme 1. Reagents and conditions: (a) 2-*t*-butylphenol, K_2CO_3, DMF, 130 °C, 6 days, 76%; (b) H_2, 10% Pd/C, MeOH/THF, 95%; (c) di(1*H*-imidazol-1-yl)methanethione, DCM, rt, 1 h, 80%; (d) 2-azido-1-phenylethanone, PPh_3-resin, dioxane, 95 °C, 4 h, 64%.



Scheme 2. Reagents and conditions: (a) *N*,*N*′-Di-Boc-*S*-methylisothiourea, $HgCl_2$, TEA, DMF, rt, 4 h, 77%; (b) TFA/DCM (1:1), rt, 2 h, 91%; (c) 2-bromo-1-phenylethanone, TEA, DMF, 80 °C, 16 h, 26%.

amine substituents to improve the physical chemical properties. Compound **7j** maintained strong binding and improved antagonism of platelet aggregation compared to compound **5c** and demonstrated an IC_{50} of 5.2 μM in the platelet aggregation assay.

Compound **7j** was the most potent compound identified in this series for platelet aggregation and was therefore advanced to a rat pharmacokinetic study. Compound **7j** demonstrated 27% oral bioavailability, had a half-life ($t_{1/2}$) of 2.1 h and a clearance of 6.7 mL/min/kg after intravenous dosing at 5 mg/kg in a solution of EtOH/cremophor/water (1:1:8). This compound also showed a high level of binding to human serum protein (99.9%).

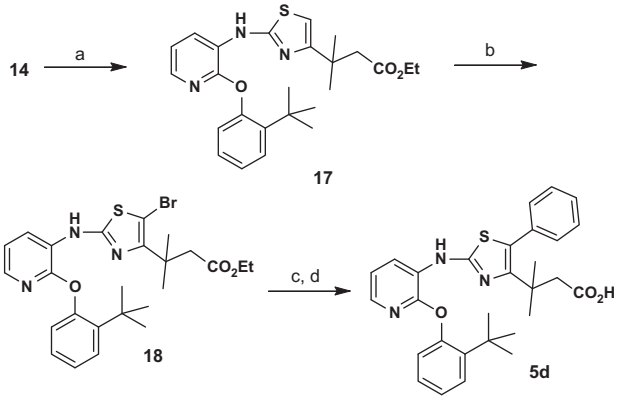
The synthesis of 2-aminooxazole **2** is shown in Scheme 1. Commercially available 2-fluoronitrobenzene **8** was treated with 2-*t*-butylphenol and potassium carbonate in DMF at 130 °C to provide **9** which was hydrogenated to give the aniline intermediate **10**. Treatment of **10** with di(1H-imidazol-1-yl)methanethione in dichloromethane at room temperature afforded the isothiocyanate (**11**) in 80% yield. Intermediate **11** was treated with 2-azido-1-phenylethanone in the presence of triphenyl phosphine resin in dioxane at 95 °C for 4 h to generate **2** in 64% yield.

Synthesis of 2-aminoimidazole **3** (Scheme 2) was accomplished from intermediate **10** by treatment with *N*,*N*′-di-Boc-*S*-methylisothiourea, mercury (II) chloride and TEA to afford **12** which was deprotected to give the guanidine intermediate **13**. Cyclization of **13** with 2-bromo-1-phenylethanone in DMF at 80 °C afforded 2-aminoimidazole **3**.

The aminothiazoles were synthesized from the amino intermediate **10**. Intermediate **10** was condensed with benzoyl isothiocyanate and subsequently hydrolyzed to the thiourea **14** (Scheme 3). Compound **14** was then condensed with appropriate α-bromoketones or α-bromoaldehydes in the presence of 2,6-lutidine at 90 °C to provide analogs **4**, **6a** and **15a**–**c**. Bromination of **15a**–**c** with NBS in acetic acid generated the corresponding bromides **16a**–**c** which were coupled with phenyl boronic acid in the presence of $Pd(PPh_3)_4$ and 2 M Na_2CO_3 at 95–100 °C to generate analogs **5a**–**c**.

The synthesis of **5d** is illustrated in Scheme 4. Cyclization of the thiourea **14** with ethyl 5-chloro-3,3-dimethyl-4-oxopentanoate in the presence of 2,6-lutidine provided ester **17** which was treated with NBS in acetic acid to give the bromide **18**. Coupling of **18** with phenyl boronic acid followed by subsequent hydrolysis provided **5d**. The amide analog **5e** was synthesized from **14** following a similar procedure to that described for **5d** using ethyl bromopyruvate.

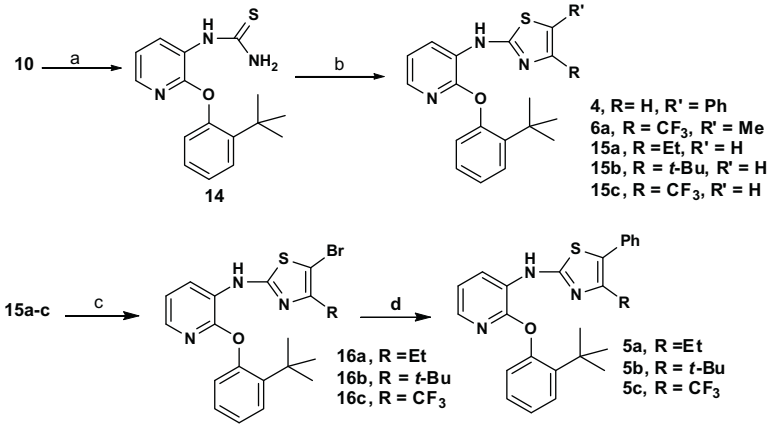
Cyclization of **14** with ethyl 2-chloro-4,4,4-trifluoro-3-oxobutanoate at 90 °C in ethanol gave compound **6b** (Scheme 5). Hydrolysis of **6b** followed by treatment with thionyl chloride and ammonium hydroxide provided primary amide **6e** which was then dehydrated in the presence of trifluoroacetic anhydride and pyridine to provide the nitrile (**6c**). Reaction of **6c** with hydroxylamine followed by cyclization in the presence of acetic acid, DIC and HOBt afforded the oxadiazole analog **6d**.



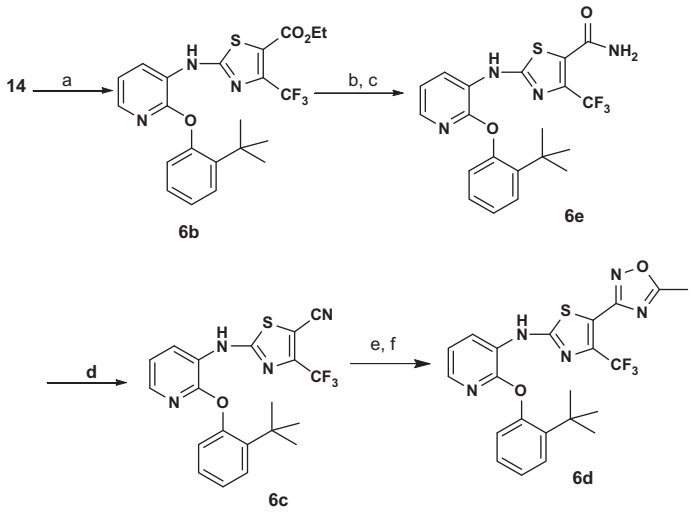
Scheme 4. Reagents and conditions: (a) ethyl 5-chloro-3,3-dimethyl-4-oxopentanoate, EtOH, 2,6-lutidine, 90 °C, 24 h, 95%; (b) NBS, HOAc, THF, rt, 83%; (c) phenyl boronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, EtOH/toluene (1:2), 80 °C, 92%; (d) 1 N NaOH, THF/MeOH (3:1), rt, 16 h, 55%.

Coupling of **16c** with the appropriately substituted phenyl boronic acids in the presence of $Pd(PPh_3)_4$ and 2 M Na_2CO_3 at 95–100 °C yielded 5-phenyl analogs **7a**–**i** (Scheme 6). Treatment of **16c** with Boc_2O and DMAP in tetrahydrofuran gave the protected aminothiazole **19**. Coupling of **19** with 3-hydroxyphenylboronic acid in the presence of $Pd(PPh_3)_4$ and 2 M Na_2CO_3 solution in 1:2 EtOH/toluene at 80 °C provided the phenol intermediate **20**. Compound **20** was treated with 3-(dimethylamino)-2,2-dimethylpropan-1-ol, PPh_3-resin and di-*tert*-butyl azodicarboxylate to yield **21** which was converted to **7j** using 20% TFA in dichloromethane.

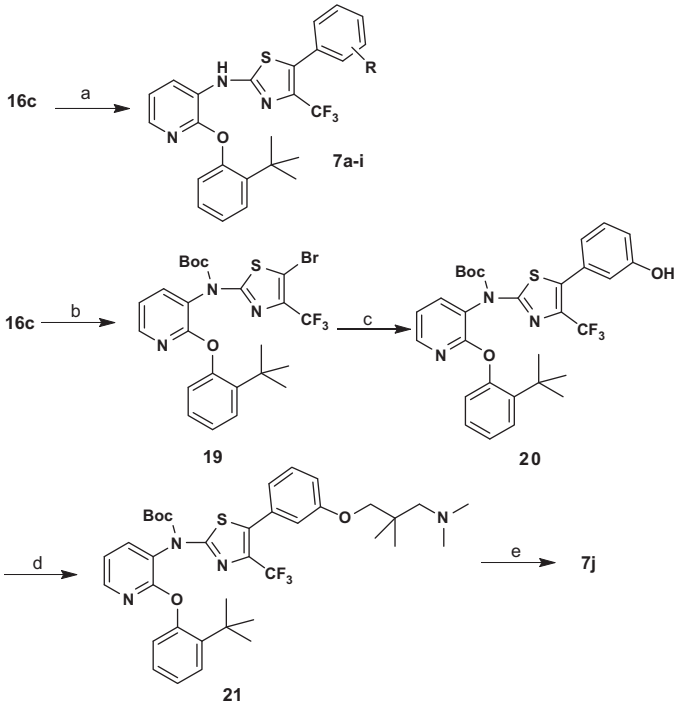
In summary, a novel series of 2-aminothiazole derivatives was discovered with excellent $P2Y_1$ binding activity. Several 4-trifluoromethyl-5-phenyl analogs demonstrated moderate antagonism of platelet aggregation of which compound **7j** was the most potent. Compound **7j** also showed acceptable oral bioavailability, clearance and half-life in rats. However, high protein binding and low solubility due to high lipophilicity may have contributed to the moderate antagonism of platelet aggregation and this compound was not advanced further. Further optimization of this series including replacement of the thiazole with different heterocyclic rings and modification of the *t*-butyl phenoxy group to improve



Scheme 3. Reagents and conditions: (a) i) Benzoyl isothiocyanate, DCM, 50 °C, 2 h, 97%; ii) 2 M LiOH, THF/MeOH (3:1), 50 °C, 2 h, 84%; (b) appropriate α-bromoketones or α-bromoaldehydes, 2,6-lutidine, EtOH, 90 °C, 48–91%; (c) NBS, HOAc/THF (1:5), rt, 45-86%; (d) phenyl boronic acid, $Pd(PPh_3)_4$, 2 M $NaCO_3$, toluene/ethanol (2:1), 95–100 °C, 24 h, 36–53%.



Scheme 5. Reagents and conditions: (a) Ethyl 2-chloro-4,4,4-trifluoro-3-oxobutanoate, EtOH, 90 °C, 4 days, 77%; (b) 1 N NaOH, MeOH/THF (3:1), rt, 91%; (c) i) $SOCl_2$, DCM, rt; ii) 28% ammonium hydroxide, THF, rt, 88%; (d) trifluoroacetic anhydride (4 equiv), pyridine (6 equiv), THF, 0 °C-rt, 1 h, 76%; (e) hydroxylamine hydrochloride, DIPEA, EtOH, 60 °C, 5 h, 87%; (f) acetic acid, DIC, HOBt, CH_3CN, 160 °C, microwave, 15 min, 37%.



Scheme 6. Reagents and conditions: (a) appropriate boronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3, toluene/ethanol (2:1), 95–100 °C, 24 h, 55–96%; (b) Boc_2O, DMAP, THF, rt, 87%; (c) 3-hydroxyphenylboronic acid, 2 M Na_2CO_3, $Pd(PPh_3)_4$, EtOH/toluene (1:2), 80 °C, 92%; (d) 3-(dimethylamino)-2,2-dimethylpropan-1-ol, PPh_3-resin, DBAD, THF, rt, 3 days, 81%; (e) 20% TFA/DCM, 1 h, 96%.

the physical properties of these molecules will be reported in due course.

References and notes

1. Murugappan, S.; Kunapuli, S. P. *Front. Biosci.* **2006**, *11*, 1977.
2. (a) Packham, M. A.; Mustard, J. F. *Semin. Thromb. Hemost.* **2005**, *31*, 129; (b) Burnstock, G.; Williams, M. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 862; (c) Gachet, C. *Ann. Med.* **2000**, *32*, 15; (d) Cattaneo, M.; Gachet, C. *Arterioscler. Thromb. Vasc. Biol.* **1999**, *19*, 2281.
3. Wilde, J. I.; Retzer, M.; Siess, W.; Watson, S. P. *Platelets* **2000**, *11*, 286.
4. Gachet, C. *Purinergic Signal.* **2012**, *8*, 609.
5. (a) Hechler, B.; Gachet, C. *Purinergic Signal.* **2011**, *7*, 293; (b) Jacobson, K. A.; Balasubramanian, R.; Deflorian, F.; Gao, Z.-G. *Purinergic Signal.* **2012**, *8*, 419; (c) Wijeyeratne, Y. D.; Heptinstall, S. *Br. J. Clin. Pharmacol.* **2011**, *72*, 647; (d) Gachet, C. *Thromb. Haemost.* **2008**, *99*, 466.
6. (a) Giorgi, M. A.; Arazi, H. C.; Gonzalez, C. D.; Di Girolamo, G. *Expert Opin. Pharmacother.* **2011**, *12*, 1285. and references cited therein; (b) Fintel, D. J. *Vasc. Health Risk Manag.* **2012**, *8*, 77. and references cited therein.
7. (a) Passacquale, G.; Ferro, A. *Br. J. Med.* **2011**, *342*, 1415; (b) Plosker, G. L.; Lyseng-Williamson, K. A. *Drugs* **2007**, *67*, 613.
8. (a) Sugidachi, A.; Yamaguchi, S.; Jakubowski, J. A.; Ohno, K.; Tomizawa, A.; Hashimoto, M.; Niitsu, Y. *J. Cardiovasc. Pharmacol.* **2011**, *58*, 329; (b) Angiolillo, D. J.; Suryadevara, S.; Capranzano, P.; Bass, T. A. *Expert Opin. Pharmacother.* **2008**, *9*, 2893; (c) Jakubowski, J. A.; Winters, K. J.; Naganuma, H.; Wallentin, L. *Cardiovasc. Drug Rev.* **2007**, *25*, 357.
9. (a) Sibbing, D.; Orban, M.; Massberg, S. *Haemostaseologie* **2013**, *33*, 12; (b) Di Minno, M. N. D.; Momi, S.; Di Minno, A.; Russolillo, A. *Curr. Drug Targets* **2013**, *14*, 3.
10. (a) Thalji, R. K.; Aiyar, N.; Davenport, E. A.; Erhardt, J. A.; Kallal, L. A.; Morrow, D. M.; Senadhi, S.; Burns-Kurtis, C. L.; Marino, J. P., Jr. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4104; (b) Pfefferkorn, J. A.; Choi, C.; Winters, T.; Kennedy, R.; Chi, L.; Perrin, L. A.; Lu, G.; Ping, Y.-W.; McClanahan, T.; Schroeder, R.; Leininger, M. T.; Geyer, A.; Schefzick, S.; Atherton, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3338; (c) Morales-Ramos, A. I.; Mecom, J. S.; Kiesow, T. J.; Graybill, T. L.; Brown, G. D.; Aiyar, N. V.; Davenport, E. A.; Kallal, L. A.; Knapp-Reed, B. A.; Li, P.; Londregan, A. T.; Morrow, D. M.; Senadhi, S.; Thalji, R. K.; Zhao, S.; Burns-Kurtis, C. L.; Marino, J.-P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6222; (d) Houston, D.; Costanzi, S.; Jacobson, K. A.; Harden, T. K. *Comb. Chem. High Throughput Screening* **2008**, *11*, 410.
11. (a) Houston, D.; Ohno, M.; Nicholas, R. A.; Jacobson, K. A.; Harden, T. K. *Br. J. Pharmacol.* **2006**, *147*, 459; (b) Hechler, B.; Nonne, C.; Roh, E. J.; Cattaneo, M.; Cazenave, J.-P.; Lanza, F.; Jacobson, K. A.; Gachet, C. *J. Pharmacol. Exp. Ther.* **2006**, *316*, 556. and references cited therein.
12. (a) Bird, J. E.; Wang, X.; Smith, P. L.; Barbera, F.; Huang, C.; Schumacher, W. A. *J. Thromb. Thrombolysis* **2012**, *34*, 199; (b) Wong, P. C.; Crain, E. J.; Jiang, X.; Bostwick, J. S.; Thibeault, C.; Ruel, R.; Rehfuss, R.; Schumacher, W. A.; Ogletree, M. L. *Circulation* **2006**, *114*. II_248, abstract 1310; (c) Schumacher, W. A.; Bostwick, J. S.; Ogletree, M. L.; Stewart, A.; Steinbacher, T. E.; Hua, J.; Price, L. A.; Wong, P. C.; Rehfuss, R. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 1; (d) Chao, H.; Turdi, H.; Herpin, T. F.; Roberge, J. Y.; Liu, Y.; Schnur, D.; Poss, M. A.; Rehfuss, R.; Hua, J.; Wu, Q.; Price, L. A.; Abell, L. M.; Schumacher, W. A.; Bostwick, J. S.; Steinbacher, T. E.; Stewart, A. B.; Ogletree, M. R.; Huang, C. S.; Chang, M.; Cacace, A. M.; Arcuri, M. J.; Celani, D.; Wexler, R. R.; Lawrence, R. M. *J. Med. Chem.* **2013**, *56*, 1704.
13. The compounds reported herein were disclosed in: Sutton, J.; Pi, Z.; Ruel, R.; L'Heureux, A.; Thibeault, C.; Lam, P.Y.S. US2006/0173002A1.
14. Ruel, R.; L'Heureux, A.; Thibeault, C.; Daris, J.-P.; Martel, A.; Price, L.; Wu, Q.; Hua. J.; Wexler, R. R.; Rehfuss, R.; Lam, P.Y.S *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3519.