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Optimization of ketone-based $P2Y_{12}$ receptor antagonists as antithrombotic agents: Pharmacodynamics and receptor kinetics considerations

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ABSTRACT

Modification of a series of $P2Y_{12}$ receptor antagonists by replacement of the ester functionality was aimed at minimizing the risk of in vivo metabolic instability and pharmacokinetic variability. The resulting ketones were then optimized for their $P2Y_{12}$ antagonistic and anticoagulation effects in combination with their physicochemical and absorption profiles. The most promising compound showed very potent antiplatelet action in vivo. However, pharmacodynamic–pharmacokinetic analysis did not reveal a significant separation between its anti-platelet and bleeding effects. The relevance of receptor binding kinetics to the in vivo profile is described.

The P2 purinergic receptors are normally classified into two families: metabotropic P2Y receptors (G-protein coupled receptors) and ionotropic P2X receptors (ligand gated ion channels).[1] Among the P2Y receptors described in humans, the $P2Y_1$ and $P2Y_{12}$ receptors are expressed in platelets and are activated by adenosine diphosphate (ADP),[2] following platelet stimulation. ADP is released from the platelet dense-granules and results in increased concentration of cytosolic calcium via activation of $P2Y_1$ and decreased concentration of cyclic adenosine monophosphate (cAMP) via activation of $P2Y_{12}$.[3] These combined actions stabilize the formed platelet aggregates, which suggests a critical role of $P2Y_{12}$ in regulating platelet function, thereby offering an opportunity for pharmacological intervention.[4,5] Indeed, common anti-platelet aggregation therapies include $P2Y_{12}$ receptor antagonists[6] such as the thienopyridine derivative clopidogrel (Fig. 1). Clopidogrel is a prodrug and exerts its pharmacological effect through an active metabolite that binds irreversibly to the $P2Y_{12}$ receptor.[7] Data suggests that reversible $P2Y_{12}$ antagonism results in a faster off-set of effect and could thus lead to a wider therapeutic window, as defined by anti-thrombotic effect vs. risk of bleeding.[8,9] Comparative clinical studies have confirmed those observations.[10]. The reversibly binding $P2Y_{12}$ receptor antagonist ticagrelor[11] (Fig. 1) has been approved as an antiplatelet agent for the treatment of patients with acute coronary syndrome (ACS).

We recently reported our discovery of novel reversible $P2Y_{12}$ receptor antagonists,[12,13] leading to the identification of **1**, an explorative clinical candidate with strong anti-thrombotic effect and substantial therapeutic window in preclinical models.[14] Given the potential limitations imposed by the ester functionality of **1** (i.e., hydrolysis by esterases to give the corresponding nicotinic acid derivative which showed limited intestinal absorption and no anti-platelet effects), we set out to identify reversibly binding $P2Y_{12}$ antagonists devoid of such liabilities. Earlier in the program

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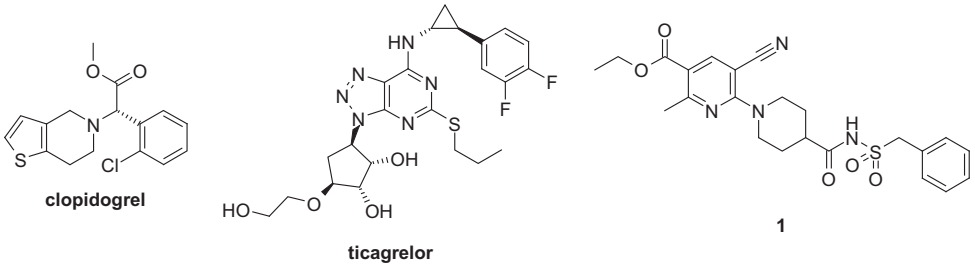
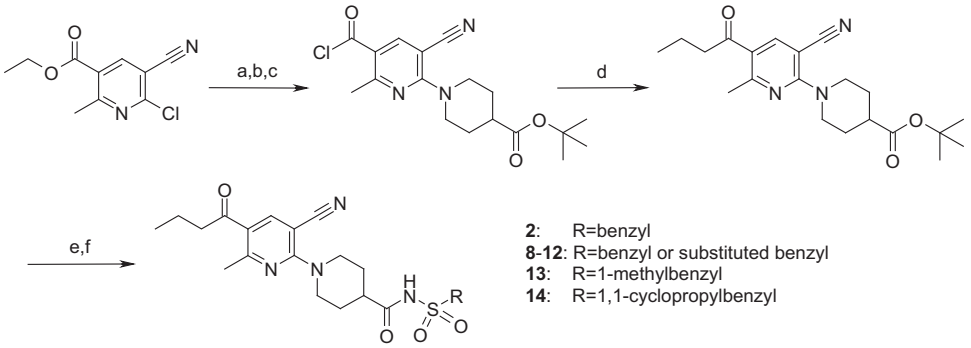


Figure 1. Selected antagonists of the P_2Y_{12} receptor.

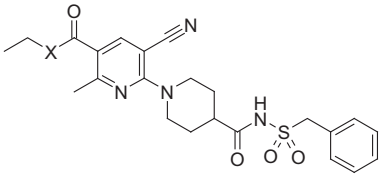
we explored the potential for ester bioisosters, but overall no tangible improvements were obtained.[12,13] Based on the available structure-activity relationships (SAR), we wanted to reevaluate ketones, being the ester bioisosters with the highest steric and electronic similarity to the parent ester compounds.

Despite still possessing the ability to accept hydrogen bonds from the $P2Y_{12}$ receptor, the propyl ketone analogue **2** (Scheme 1) showed a decreased potency compared to the corresponding ethyl ester **1**, as previously reported[13] (Table 1). Since **2** and **1** are equivalent in size (as approximated by the number of heavy atoms) and lipophilicity (based on log*D* measurements), this translated into reduced ligand efficiencies for the ketone derivative **2** (Table 1). In line with expectations, **2** displayed increased microsomal stability but the permeability was somewhat reduced, as summarized in Table 1. The overall results with **2** clearly indicated directions for optimization. Specifically, we wanted to improve functional activity and maintain adequate physicochemical properties, in order to assess whether non-ester containing $P2Y_{12}$ antagonists in the present series could show efficacy in vivo and be developed further. Compound **2** demonstrated favorable microsomal stability and solubility, but borderline permeability and suboptimal activity at the $P2Y_{12}$ receptor. These properties are normally affected by the lipophilic character of molecules. We therefore hypothesized that addition of small lipophilic substituents to structure **2** could give sufficient increases in $P2Y_{12}$ antagonism and permeability, while retaining sufficient solubility and microsomal stability to warrant in vivo testing. We thus explored chemical variations of the pyridine 2-position and the benzylic substituents, respectively,



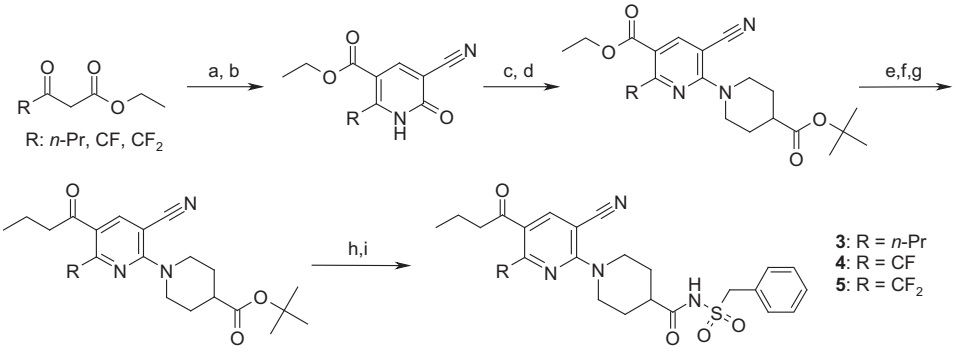
Scheme 1. Reagents and conditions: (a) *tert*-butyl piperidine-4-carboxylate, TEA, MeCN, microwave oven, single node heating, 100 °C, 5 min (77%); (b) NaOH, water, 80 °C, (98%); (c) $(COCl)_2$, cat. DMF, DCM, 0 °C to rt, 20 min (98%); (d) *n*-PrMgBr, $Fe(acac)_3$, THF, 0 °C, not isolated; (e) TFA, DCM (75%, 2 steps); (f) R-SO_2NH_2, PyBOP, DIPEA, DCM (25–48%).

Table 1
Experimental profile for the initial ethyl ester—*n*-propyl ketone matched-pair

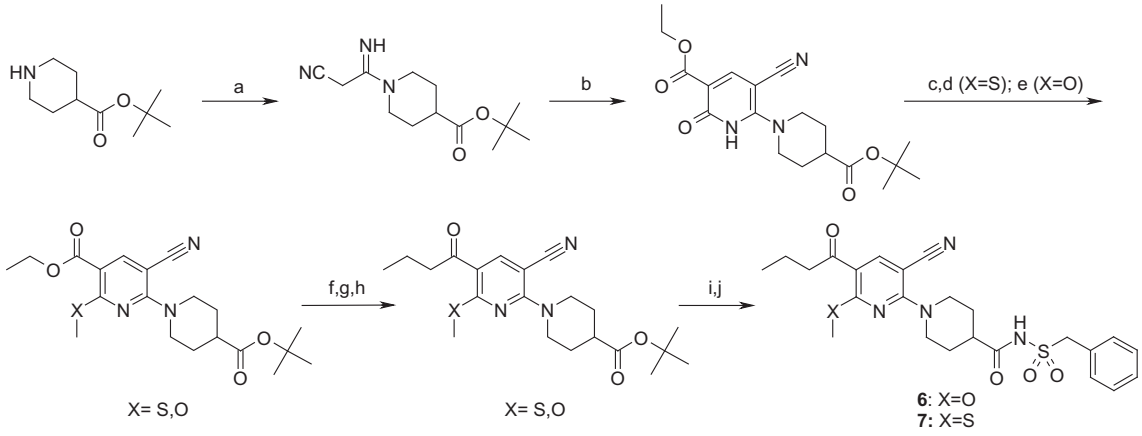


Compound	X	GTPγS IC_{50}[a] (μM)	RPC IC_{50}[a] (μM)	HLM $T_{1/2}$[b] (min)	Caco-2 P_{app} (10^{-6} cm/s)[c]	LE[d]	LLE[e]	Log *D*[f]
1	O	0.025	3.2	60	1	0.31	4.8	2.8
2	C	0.100	7	>115	0.32	0.29	4.2	2.8

[a] Results are mean of at least two experiments (Supplementary information).
[b] Intrinsic clearance of test compounds after incubation for 30 min with human liver microsomes (HLM) (1 mg/mL) at 37 °C.
[c] Permeability measured in Caco-2 cells in the apical to basolateral (A to B) direction, pH = 7.4 (Supplementary information).
[d] Ligand efficiency, calculated as −RTln(GTPγS IC_{50})/heavy atoms count.
[e] Lipophilic ligand efficiency, calculated as GTPγS pIC_{50}—log*D*.
[f] Experimental log*D* at pH = 7.4 using HPLC.[15]

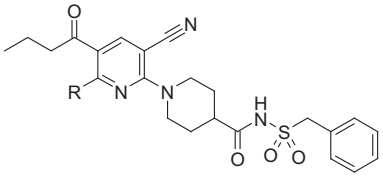


Scheme 2. Reagents and conditions: (a) 1,1-dimethoxymethyl-*N*,*N*-dimethylmethanamine, EtOH, rt, over night; (b) NaOEt, 2-cyanoacetamide/EtOH rt, over night (41–56%); (c) $(COCl)_2$, DMF, DCM, 50 °C to reflux, over night (60–90%); (d) *tert*-butyl piperidine-4-carboxylate, TEA, MeCN, microwave oven, single node heating, 100 °C, 5 min; (e) NaOH, water, 80 °C; (f) $(COCl)_2$, cat. DMF, DCM, 0 °C to rt, 20 min; (g) *n*-PrMgBr, $Fe(acac)_3$, THF, 0 °C, not isolated; (h) TFA, DCM. rt; (i) R-SO_2NH_2, PyBOP, DIPEA, DCM.



Scheme 3. Reagents and conditions: (a) ethyl 2-cyanoethanecarboximidate, DIPEA, EtOH, microwave oven, single node heating, 100 °C, 30 min, not isolated; (b) diethyl (ethoxymethylene)malonate, rt, 16 h (32%, two steps); (c) Tf_2O, TEA, DCM, 0 °C, 40 min, not isolated; (d) NaSMe THF, microwave oven, single node heating, 120 °C, 5 min, not isolated; (e) MeI, Ag_2CO_3/DMSO, microwave oven, single node heating, 100 °C, 20 min (72%); (f) NaOH (1 M), THF, EtOH, microwave oven, single node heating, 120 °C, 5 min; (g) cyanuric fluoride, pyridine, DCM, rt, 30 min, not isolated; (h) *n*-PrMgBr, Cu(I) iodide dimethylsulfide complex, 0 °C 20 min; (i) TFA, DCM, rt; (j) R-SO_2NH_2, PyBOP, DIPEA, DCM, rt, 2.5 h (60–75%).

Table 2
Experimental profile for the variation set at the pyridyl-2-position



Compound	R	GTPγS IC_{50}[a] (μM)	RPC IC_{50}[a] (μM)	Caco-2 P_{app} (10^{-6} cm/s)[b]	LE[c]	LLE[d]	Log*D*[e]
2	–Me	0.1	7	0.3	0.29	4.2	2.8
3	–*n*-Pr	0.27	>30	1.2	0.26	2.9	3.7
4	$-CH_2F$	0.093	8	<0.1	0.28	4.3	2.7
5	$-CHF_2$	0.08	11.5	<0.1	0.28	4	3.1
6	–OMe	0.076	10.3	ND[f]	0.29	4.1	3
7	–SMe	0.017	1.4	0.3	0.31	4.7	3.1

[a] Results are mean of at least two experiments (Supplementary information).
[b] Permeability was measured in human Caco-2 cells in the apical to basolateral (A to B) direction, pH = 7.4 (Supplementary information).
[c] Calculated as $-RT\ln(P2Y_{12}$ GTPγS $IC_{50})/HAC$.
[d] Calculated as $P2Y_{12}$ GTPγS $pIC_{50}-\log D$
[e] Experimental log*D* at pH = 7.4 using HPLC.[15]
[f] Not determined.

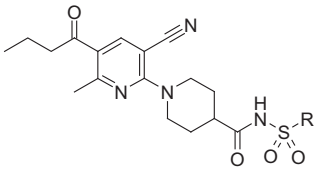
and then combined the best identified modifications in a final set for further profiling, as outlined below.

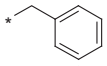
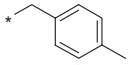
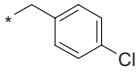
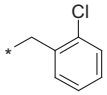
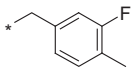
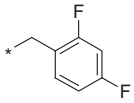
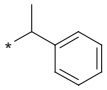
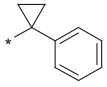
A series of analogues with variation of the pyridine 2-substituent (**3**–**7**) was thus synthesized following Schemes 2 and 3. Table 2 summarizes the results for the pyridyl-2-substituent variation set (**3**–**7**). Homologation of 2-methyl (**2**) to 2-*n*-propyl (**3**) increased the lipophilicity and, in turn, the apparent permeability, but did not result in increased potency. Fluoromethyl (**4**) and difluoromethyl (**5**) 2-substituents led to retained functional activity at the $P2Y_{12}$ receptor, but both compounds showed a further reduced permeability (P_{app} <0.1 × 10^{-6} cm/s) compared to **2**. The 2-methoxy derivative (**6**) did not afford any greater $P2Y_{12}$ antagonism than expected from its size and lipophilic contribution (cf. ligand efficiency (LE) and ligand lipophilicity efficiency (LLE)). Interestingly, compared to **2** and **6**, the methylthio substituent (**7**) resulted in a significant improvement in functional $P2Y_{12}$ antagonism (GTPγS IC_{50}: 0.017 μM), which also translated into a better platelet inactivation in plasma (RPC IC_{50}: 1.4 μM), as shown in Table 2.

Having identified the methylthio substituent as an appropriate lipophilic addition leading to potent $P2Y_{12}$ antagonism, high solubility (>100 μM) and metabolic stability (HLM $T_{1/2}$ >115 min), variation of the benzyl substituents was explored by synthesizing compounds **8**–**14** following the procedure outlined in Scheme 1. As detailed in Table 3, *p*-methyl (**8**) and *p*-chloro (**9**) analogues of **2** showed increased functional antagonism of the $P2Y_{12}$ receptor (GTPγS IC_{50}-values of 0.059 and 0.057 μM, respectively), but no improvement in platelet inactivation (RPC IC_{50}-values of 11 and 10.5 μM, respectively). Movement of the chlorine atom from the *para* (**9**) to the *ortho* (**10**) position resulted in significantly reduced activity (Table 3). Disubstitution, as exemplified by **11** and **12**, or introduction of substituents at the benzylic α-position (**13**, **14**) did not offer any advantage over the unsubstituted parent molecule (**2**). However, the cyclopropyl derivative **14**, due to its higher lipophilicity, showed the highest passive diffusion (P_{app}: 5.2 × 10^{-6} cm/s) of all compounds, with retained high aqueous solubility (>100 μM) and microsomal stability (HLM $T_{1/2}$ >115 min), and was thus considered further.

From the findings above, we combined the pyridyl-2-methylthio substituent and the 1-phenylcyclopropanesulfonamide side chain into one molecule by synthesizing compound **15** (Supplementary information) to verify any additive or synergistic SAR effects. We were pleased to see that **15** maintained similar $P2Y_{12}$ antagonism to **7** (GTPγS IC_{50}: 0.019 and 0.017 μM, respectively), but, more importantly, that it was a much more potent platelet inactivator than **1** (RPC IC_{50}: 0.64 μM; WPA IC_{50}: 0.001 μM), as summarized in Table 4. Additionally, **15** was stable in HLM ($T_{1/2}$ >115 min), soluble in water (78 μM) and reasonably permeable (P_{app}: 2 × 10^{-6} cm/s), therefore being an attractive compound to be evaluated in vivo. We were interested in benchmarking **15** against our initial clinical candidate **1** in terms of anti-platelet effect and bleeding risk before embarking on further compound optimization. Compound **15** was thus dosed to conscious dogs (N = 2) to record its pharmacodynamic (PD) profile, as summarized in Figure 2.

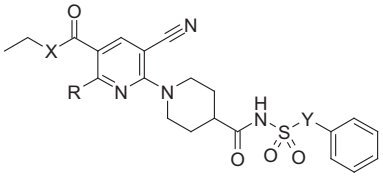
Table 3
Effects on potency, permeability, and lipophilicity by variation of the benzyl substituent



Compound	R	$P2Y_{12}$ GTPγS IC_{50}[a] (μM)	RPC IC_{50}[a] (μM)	Caco-2 P_{app} (10^{-6} cm/s)[b]	Log$D$[c]
2		0.1	7	0.3	2.8
8		0.059	11	1.4	3.4
9		0.057	10.5	0.4	3.4
10		0.27	ND	0.4	3.1
11		0.13	ND	0.7	3.4
12		0.042	11	0.3	3
(±)-**13**		0.19	28	1	3.3
14		0.16	20	5.2	3.9

[a] Results are mean of at least two experiments (Supplementary information).
[b] Permeability was measured in human Caco-2 cells in the apical to basolateral (A to B) direction, pH = 6.5 (Supplementary information).
[c] Experimental log$D$ at pH = 7.4 using HPLC.[15]

Table 4
Experimental profile comparison for the initial ester **1** and ketone derivatives **2**, **15**



Compound	R	X	Y	GTPγS IC_{50}[a] (μM)	RPC IC_{50}[a] (μM)	WPA IC_{50}[b] (μM)	HLM $T_{1/2}$[c] (min)	Caco-2 P_{app} (10^{-6} cm/s)[d]	PPB F_u[e] (%)	$T_{1/2}$[f] (h)	AUC[f] (μmol h/L)
1	Me	O	CH_2	0.025	3.2	0.009	60	1	0.35	5.1	5.9
7	MeS	C	CH_2	0.017	1.4		>115	0.3	ND[g]	ND[g]	ND[g]
15	MeS	C	cPro	0.019	0.64	0.001	>115	2	0.41	5.2	6.7

[a] Results are mean of at least two experiments (Supplementary information).
[b] Ref. 8.
[c] Intrinsic clearance of test compounds after incubation for 30 min with human liver microsomes (HLM) (1 mg/mL) at 37 °C.
[d] Permeability was measured in human Caco-2 cells in the apical to basolateral (A to B) direction, pH = 7.4 (Supplementary information).
[e] % fraction unbound in Beagle male dogs plasma measured by equilibrium dialysis (18 h at 37 °C).
[f] Pharmacokinetic parameters calculated from noncompartmental analysis concentrations in fasted Beagle male dogs (N >2; iv dose: 1 μmol/kg).
[g] Not determined.

Compound **15** displayed a strong, dose-dependent platelet inhibition action in dogs, with an estimated ID_{80} of 0.052 μmol/kg/min, and is thus a more potent anti-thrombotic agent than **1** (ID_{80}: 0.44 μmol/kg/min). However, infusion of **15** also resulted in a marked increase in bleeding time at the highest dose (1.6 μmol/kg/min, 6.7-fold increase in bleeding time), as shown in Figure 2. This finding was in stark contrast to **1**, which did not show any significant bleeding time prolongation in the tested dose range (0.02 nmol/kg/min–8.7 μmol/kg/min). The PD results in conscious dogs confirmed **15** as an effective antithrombotic agent, but also indicated the risk for a narrow therapeutic window. In order to fully characterize the risk profile of **15**, we evaluated it in a modified Folt's model of arterial thrombosis,[9,16] as summarized in Figure 3.

Disappointingly, the modified Folt's model profile of **15** confirmed the result of the PK–PD experiment in conscious dogs. Despite an evident, full anti-platelet effect (ID_{80}: 0.005 μmol/kg/min) following administration of **15** (0.001–0.15 μmol/kg/min), a sharp increase in bleeding time ($ED_{3.5\text{-fold}}$: 0.015 μmol/kg/min) was also observed. Although **1** was confirmed to be a weaker (p <0.05) inhibitor of platelet activation (ID_{80}: 0.009 μmol/kg/min), its therapeutic window was far superior to **15**, as no significant increase in bleeding time was observed. The differences in bleeding time prolongation observed for **1** and **15** in vivo could not be rationalized based on the in vitro potency and pharmacokinetic data generated, as summarized in Table 4. Here, $T_{1/2}$ and AUC values following intravenous administration to Beagle dogs (N = 2) were comparable, lending support to the hypotheses that no hysteresis

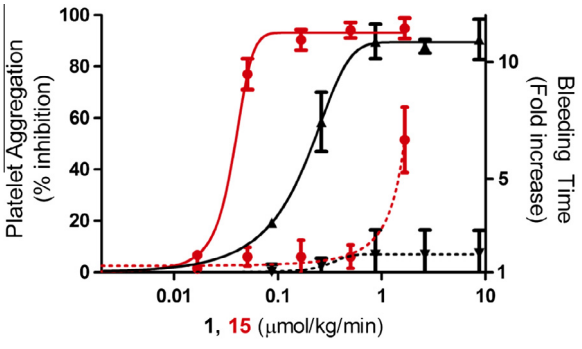


Figure 2. Platelet aggregation (% inhibition, solid lines) and bleeding time (fold increase, dashed lines) profiles for increasing doses of **1** (black) and **15** (red), measured in conscious dogs (N = 2).

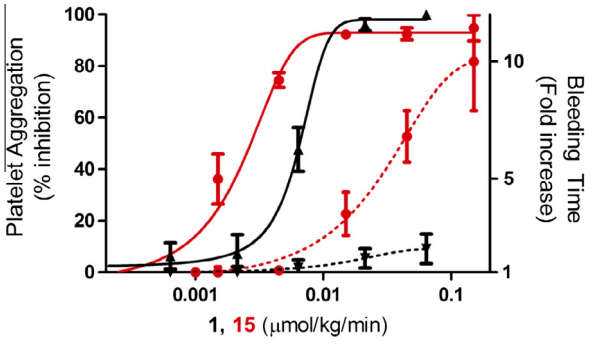


Figure 3. Platelet aggregation (% inhibition, solid lines) and bleeding time (fold increase, dashed lines) profiles for increasing doses of **1** (black) and **15** (red), measured in the Folt's dog model of arterial thrombosis (N>2).

or PK–PD disconnect confounded the results. Likewise, the main metabolites of **1** and **15**, originating from CYP450-mediated oxidation of the benzyl ring, that were characterized in vitro were devoid of $P2Y_{12}$ antagonism, thus ruling out their direct contribution to the observed in vivo PD effects. **1** and **15** did not show any significant binding to more than 100 different enzyme, receptors and ion channels at 10 μM compound concentration.[17] These included GPCRs known to be involved in platelet regulation such as the $P2Y_1$ receptor, the platelet activating factor receptor (PAFr) and thromboxane A2 receptor (TxA_2).[18] This reduced the risk of any non-$P2Y_{12}$-mediated effects. We further evaluated the binding profile of the two compounds to the $P2Y_{12}$ receptor since binding kinetics have been suggested to play a major role in differentiating PD action.[19]

Our previously described $P2Y_{12}$ radiometric binding assay[8] served to monitor the ability of test compounds to inhibit binding of a radio-ligand to the $P2Y_{12}$ receptor. The assay was run at different time points (10, 30 and 60 s) and the corresponding IC_{50} values were then compared to the IC_{50} values using a normal 60 min incubation time to reflect equilibrium conditions (Table 5). Although **15** showed a two-fold higher affinity than **1** in binding to the $P2Y_{12}$ receptor at 60 min (IC_{50}-values: 4 ± 1 and 9 ± 1 nM, respectively), it is significantly weaker than **1** at 10, 30 and 60 s, as summarized in Table 5. Additionally, both **1** and **15** displayed time-dependent binding profiles, with increasing binding affinities as function of the incubation time. Taken together, these results indicate that, under the tested in vitro conditions, **15** is a slow $P2Y_{12}$ binder and significantly slower than **1**, as illustrated in Figure 4.

Table 5
Time-dependent binding of compounds **1** and **15** (N >4) to the $P2Y_{12}$ receptor

Time	$P2Y_{12}$ IC_{50} (±SD) (nM)			
	10 s	30 s	60 s	60 min
1	44 (±5)	22 (±3)	14 (±2)	9 (±1)
15	126 (±33)	41 (±5)	23 (±3)	4 (±1)

Radiometric binding assay using the Chinese hamster ovary cell line (CHO-K1) expressing the human $P2Y_{12}$ receptor.[8]

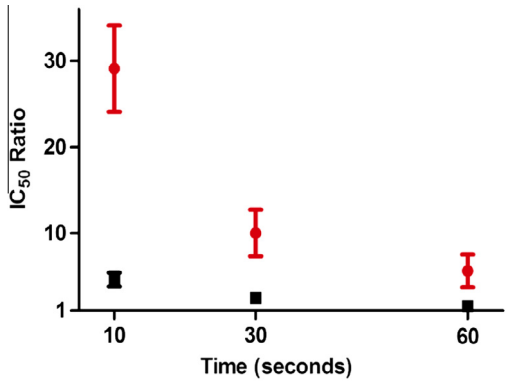


Figure 4. Ratio between $P2Y_{12}$ binding IC_{50}-values at a given incubation time (i.e., 10, 30 and 60 s) over the corresponding IC_{50}-values at 60 min (Table 5) for compounds **15** (red circles) and **1** (black squares), respectively. Standard deviations (N >4) are displayed.

The relevance of the observed kinetic profile for compound **15** to functional, in vivo $P2Y_{12}$ antagonism needs to be further investigated. However, it is tempting to speculate that in order to sufficiently separate anti-platelet effect from bleeding complications, prolonged $P2Y_{12}$ antagonism might not be desirable, as already demonstrated with examples of irreversibly binding $P2Y_{12}$ antagonists and a reversibly binding $P2Y_{12}$ antagonist with rapid receptor kinetics.[6,8,10,20]

In summary, we have developed ketone analogs of our ester-based lead series with the aim to reduce PK/PD variability in a clinical setting. Judicious addition of lipophilic substituents served to increase antagonism of the $P2Y_{12}$ receptor and anticoagulation effects, while maintaining favourable physicochemical and pharmacokinetic properties. The best compound (**15**) identified by this approach showed effective inhibition of platelet aggregation in vivo, but also a marked prolongation of bleeding time. The difference in bleeding time between compounds **15** and **1** was possibly the result of their different kinetic profiles in binding to the $P2Y_{12}$ receptor. Based on the narrow therapeutic window of **15** and the anticipated difficult balance between lipophilicity and compound developability, namely metabolic stability and oral absorption, we decided to cease any further optimisation of the series.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.001.

References and notes

1. Abbracchio, M. P.; Burnstock, G.; Boeynaems, J. M.; Barnard, E. A.; Boyer, J. L.; Kennedy, C.; Knight, G. E.; Fumagalli, M.; Gachet, C.; Jacobson, K. A.; Weisman, G. A. *Pharmacol. Rev.* **2006**, *58*, 281.
2. (a) Mahaut-Smith, M. P.; Ennion, S. J.; Rolf, M. G.; Evans, R. J. *Br. J. Pharmacol.* **2000**, *131*, 108; (b) Vial, C.; Pitt, S. J.; Roberts, J.; Rolf, M. G.; Mahaut-Smith, M. P.; Evans, R. J. *Blood* **2003**, *102*, 3646.
3. Storey, R. F. *Curr. Pharm. Des.* **2006**, *12*, 1255.
4. Gachet, C. *Ann. Rev. Pharmacol. Toxicol.* **2006**, *46*, 277.
5. Hollopeter, G.; Jantzen, H.-M.; Vincent, D.; Li, G.; England, L.; Ramakrishnan, V.; Yang, R.-B.; Nurden, P.; Nurden, A.; Julius, D.; Conley, P. B. *Nature* **2001**, *409*, 202.
6. Cattaneo, M. *Eur. Heart J. Suppl.* **2008**, *10*, 133.
7. (a) Savi, P.; Pereillo, J. M.; Uzabiaga, M. F.; Combalbert, J.; Picard, C.; Maffrand, J. P.; Pascal, M.; Herbert, J. M. *Thromb. Haemost.* **2000**, *84*, 891; (b) Sugidachi, A.; Ogawa, T.; Kurihara, A.; Hagihara, K.; Jakubowski, J. A.; Hashimoto, M.; Niitsu, Y.; Asai, F. *J. Thromb. Haemost.* **2007**, *5*, 1545; (c) Yusuf, S.; Zhao, F.; Mehta, S. R.; Chrolavicius, S.; Tognoni, G.; Fox, K. K. N. *Engl. J. Med.* **2001**, *345*, 494.
8. van Giezen, J. J. J.; Nilsson, L.; Berntsson, P.; Wissing, B. M.; Giordanetto, F.; Tomlinson, W.; Greasley, P. J. *J. Thromb. Haemost.* **2009**, *7*, 1556.
9. van Giezen, J. J. J.; Berntsson, P.; Zachrisson, H.; Björkman, J.-A. *Thromb. Res.* **2009**, *124*, 565.
10. Wallentin, L.; Becker, R. C.; Budaj, A.; Cannon, C. P.; Emanuelsson, H.; Held, C.; Horrow, J.; Husted, S.; James, S.; Katus, H.; Mahaffey, K. W.; Scirica, B. M.; Skene, A.; Steg, P. G.; Storey, R. F.; Harrington, R. A. N. *Engl. J. Med.* **2009**, *361*, 1045.
11. (a) Springthorpe, B.; Bailey, A.; Barton, P.; Birkinshaw, T. N.; Bonnert, R. V.; Brown, R. C.; Chapman, D.; Dixon, J.; Guile, S. D.; Humphries, R. G.; Hunt, S. F.; Ince, F.; Ingall, A. H.; Kirk, I. P.; Leeson, P. D.; Leff, P.; Lewis, R. J.; Martin, B. P.; McGinnity, D. F.; Mortimore, M. P.; Paine, S. W.; Pairaudeau, G.; Patel, A.; Rigby, A. J.; Riley, R. J.; Teobald, B. J.; Tomlinson, W.; Webborn, P. J. H.; Willis, P. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6013; (b) van Giezen, J. J. J.; Humphries, R. G. *Semin. Thromb. Hemost.* **2005**, *31*, 195.
12. Bach, P.; Boström, J.; Brickmann, K.; van Giezen, J. J. J.; Hovland, R.; Petersson, A. U.; Ray, A.; Zetterberg, F. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2877.
13. (a) Bach, P.; Boström, J.; Brickmann, K.; van Giezen, J. J. J.; Groneberg, R. D.; Harvey, D. M.; O'Sullivan, M.; Zetterberg, F. *Eur. J. Med. Chem.* **2013**, *65*, 360; (b) Bach, P.; Boström, J.; Brickman, K.; Burgess, L. E.; Clarke, D.; Groneberg, R. D.; Harvey, D.; Laird, E. R.; O'Sullivan, M.; Zetterberg, F. *Future Med. Chem.* **2013**, *5*, 2037.
14. Bach, P.; Antonsson, T.; Bylund, R.; Björkman, J.-A.; Österlund, K.; Giordanetto, F.; van Giezen, J. J. J.; Andersen, S. M.; Zachrisson, H.; Zetterberg, F. *J. Med. Chem.* **2013**, *56*, 7015.
15. Ungell, A.-L.; Karlsson, J. Cell Cultures in Drug Discovery: An Industrial Perspective. In *Drug Bioavailability. Estimation of Solubility, Permeability, Absorption and Bioavailability*; van de Waterbeemd, H., Lennernäs, H., Artursson, P., Eds., 1st ed.; Wiley-VCH GmbH, 2003; p 90. Chapter 5.
16. Folts, J. *Circulation* **1991**, *83*, 3.
17. Ricerca Biosciences, 7528 Auburn Road, Concord, OH 44077, USA.
18. Offermanns, S. *Circ. Res.* **2006**, *99*, 1293.
19. (a) Swinney, D. C.; Anthony, J. *Nat. Rev. Drug Disc.* **2011**, *10*, 507; (b) Copeland, R. A. *Future Med. Chem.* **2011**, *3*, 1491.
20. (a) Wallentin, L. *Eur. Heart J.* **2009**, *30*, 1964; (b) Lindholm, D.; Varenhorst, C.; Cannon, C.; Harrington, R.; Himmelmann, A.; Maya, J.; Husted, S.; Katus, H.; Steg, P.; Storey, R.; Wallentin, L.; James, S. K. *J. Am. Coll. Cardiol.* **2013**, *61*, E1.