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From ATP to AZD6140: The discovery of an orally active reversible $P2Y_{12}$ receptor antagonist for the prevention of thrombosis

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Abstract—Starting from adenosine triphosphate (ATP), the identification of a novel series of $P2Y_{12}$ receptor antagonists and exploitation of their SAR is described. Modifications of the acidic side chain and the purine core and investigation of hydrophobic substituents led to a series of neutral molecules. The leading compound, **17** (AZD6140), is currently in a large phase III clinical trial for the treatment of acute coronary syndromes and prevention of thromboembolic clinical sequelae.

Platelet aggregation, superimposed on plaque rupture, is a critical mechanism involved in transforming otherwise clinically stable atherosclerotic disease into an acute, potentially life-threatening arterial thrombotic event, such as unstable angina, myocardial infarction or thrombotic stroke. Platelets adhere to the ruptured arterial plaque and aggregate in response to a variety of local and systemic stimuli, including mediators such as adenosine diphosphate (ADP), adrenaline, thrombin and 5-hydroxytryptamine. Whatever the stimulation, the final common steps are exposure of the GPIIb/IIIa receptor complex and subsequent cross-linking of the platelets by fibrinogen.[1] The platelet aggregate is further consolidated by formation of fibrin, resulting in a firm, adherent clot.

ADP has a pivotal role in this process since the stimuli exert much of their effect by release of ADP from storage granules into the medium. The consequences of this are that the $P2Y_{12}$ receptor (formerly known as the P_{2T} receptor) on the platelet is stimulated, shape change occurs and the GPIIb/IIIa complex is exposed. Nearby platelets are also activated and the initial pro-aggregatory signal is amplified. Therefore, the ADP/$P2Y_{12}$ pathway plays a key amplifying role in the overall platelet response. Against this background, it is clear that inhibiting the effect of ADP on the $P2Y_{12}$ receptor significantly inhibits platelet aggregation and thereby prevents the formation of a firm, cross-linked thrombus.[2] The discovery and clinical use of the irreversible $P2Y_{12}$ antagonist clopidogrel[3] (Fig. 1) has confirmed the clinical impact of inhibiting this target. Although this anti-platelet agent has improved the management of acute and sub-acute coronary artery disease, several features of this agent leave room for improvement, such that more effective treatments are predicted to further improve clinical outcomes.

Using ATP, the natural antagonist ($pIC_{50} = 3.5$) of the $P2Y_{12}$ receptor, as a chemical starting point, we discovered **1** (AR-C69931MX, cangrelor, Fig. 1)[4] as a potent

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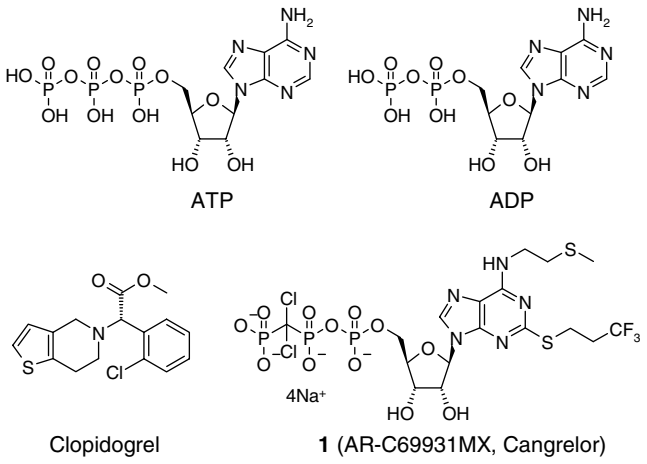


Figure 1. Structures of ATP, ADP, clopidogrel and cangrelor.

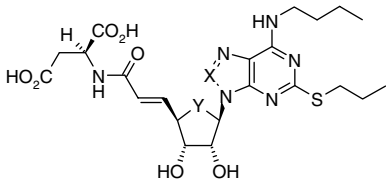
and selective $P2Y_{12}$ receptor antagonist suitable for intravenous use only. Compound **1** is currently in phase III clinical trials as an ultra-short acting, intravenously administered, antithrombotic agent.

This letter describes how the physical and chemical properties of **1** were modified in order to find potent, selective and orally active $P2Y_{12}$ antagonists.

Since ATP, the endogenous antagonist of the $P2Y_{12}$ receptor, differs from the agonist ADP in having a γ-phosphate group, we hypothesized that this terminal acidic group was essential for antagonism. Efforts were therefore directed towards finding alternative acidic groups which could mimic the polyphosphate chain and particularly the γ-phosphate unit of ATP. This initial strategy led to the discovery of an aspartic acid-derived dicarboxylate **2** (Table 1), which was ~300-fold less potent ($pIC_{50} = 7.0$) than the triphosphate **1** ($pIC_{50} = 9.4$).

Further extensive variation of the substituents on the adenosine core of **2** did not lead to improved potency and it was decided to make more fundamental changes to the core structure.

Table 1. $P2Y_{12}$ antagonist potency of aspartic acid derivatives (**2**–**4**)



Compound	X	Y	$hP2Y_{12}$ pIC_{50}[a]
2	C	O	7.0
3	N	O	9.5
4	N	CH_2	9.3

[a] ADP-induced aggregation assay using washed platelets as described in Ref. 7.

The triazolopyrimidine heterocycle has been identified as an isostere of purine and has been successfully employed in the anticancer and antiviral areas.[5] Utilising this heterocycle led to **3** in which the $P2Y_{12}$ potency had unexpectedly increased ($pIC_{50} = 9.5$). This change resulted in the identification of the first potent and selective non-phosphate $P2Y_{12}$ antagonist **3** having activity equivalent to that of triphosphate **1**.

In response to the potential instability of the glycosidic bond to enzymatic cleavage we investigated replacing the ribose sugar with a cyclopentyl unit, a structural change that has been successful in a number of areas, including the antiviral field.[6] In compound **4** we have retained the potencies of **1** and **3** but have now successfully moved away from a classic purinergic core structure.

While these changes provided improved potency over compound **2**, compounds **3** and **4** were cleared rapidly in the rat by the biliary route. The properties of these molecules are not favourable for oral absorption: doubly negatively charged, molecular weight >500, >5 H-bond donors. Variation of the substituents on the triazolopyrimidine core of structure **4** did not improve potency nor, more importantly, metabolic properties, so attention was turned once more to the acidic side chain, with the goal of reducing its complexity.

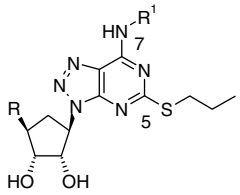
The parallel chemistry approach employed required an assay able to tolerate higher concentrations of DMSO and with higher throughput than the traditional ADP-induced aggregation in washed platelets.[7] A radiolabelled displacement binding assay was developed[8] to support this increased throughput and all subsequent data were generated using this binding assay. The switch from the functional to binding assay was validated with earlier, suitably soluble compounds, which demonstrated similar potency in both systems.

An example of the stepwise truncation from compound **4** is the monocarboxylic acid **5**, which was surprisingly potent ($pK_i = 8.3$), a result at odds with the γ-phosphate theory. Unfortunately, compound **5** was still cleared by the biliary route and had low bioavailability. However, more surprising and of crucial importance was the observation that the neutral molecules **6** and **7** (Table 2) had modest but significant $P2Y_{12}$ affinity.

Significantly, these neutral molecules were cleared in the rat solely by metabolic processes, which we felt able to understand and manipulate. While the affinity of **6** and **7** was reduced compared with compound **5**, these molecules were more amenable to rapid parallel synthesis at the 5- and 7-positions as a means of improving affinity (Schemes 1 and 2: synthesis of the intermediates according to our published procedures).[9] In all, some 6000 analogues were synthesised.

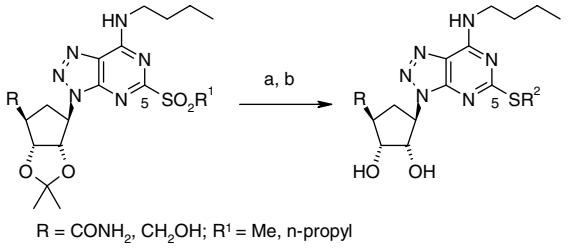
Position 5 was varied by displacement of a sulfone-leaving group by S, O and N nucleophiles. It was found that polar substituents were not well tolerated and sulfur linked groups were consistently more potent than their oxygen and nitrogen analogues. From the results it

Table 2. $P2Y_{12}$ affinity and rat pharmacokinetics of compounds with simplified C4′ side chains (**5**–**10**)

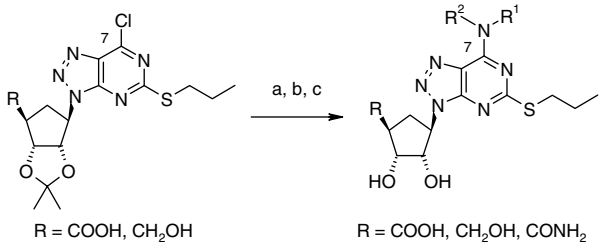


Compound	R	R^1	hP2Y$_{12}$ pK_i[a]	Rat Clp[b]	Rat V_{ss}[b]	Rat $T_{1/2}$[b]	Rat F%[c]
5	COOH	Butyl	8.3	30	0.4	0.5	<5
6	$CONH_2$	Butyl	7.7	21	1.6	1.4	<5
7	CH_2OH	Butyl	7.1	29	2.3	1.2	<5
8	COOH		9.6	16	0.5	0.5	
9	$CONH_2$		8.8	20	2.2	2.0	<5
10	CH_2OH		8.3	16	2.9	2.5	35

Units: Clp, mL/min/kg; V_{ss}, L/kg; $T_{1/2}$, h; F, bioavailability.
[a] Assay described in Ref. 8.
[b] Dosed at ~3 mg/kg (iv).
[c] Dosed at 3–10 mg/kg (po).



Scheme 1. Reagents and conditions: (a) $NaSR^2$, DMF, 20 °C, 1 h; (b) TFA–H_2O (9:1), 20 °C, 2 h.



Scheme 2. Reagents and conditions: (a) R^1R^2NH, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 20 °C, 16 h; (b) R = (COOH → $CONH_2$), oxalyl chloride, CH_2Cl_2, 20 °C, 2 h, 35% aq ammonium hydroxide, CH_2Cl_2, 20 °C, 2 h; (c) TFA–H_2O (9:1), 20 °C, 2 h.

was concluded that *S*-propyl substitution at position 5 was optimal.

Investigation of substitution at the 7-position was accomplished by displacement of chloride by amines. Using amines bearing polar substituents, or secondary amines, led to reduced affinity. In only one case was affinity significantly increased over that of the butyl substituent, but the enhancement found with racemic *trans*-2-phenylcyclopropylamine was crucial for achieving our objectives of potency and good preclinical metabolic properties.

Separation[10] of the enantiomers of *trans*-phenylcyclopropylamine showed that the 1*R*,2*S* isomer was more potent than the 1*S*,2*R* isomer.

Not only did this discovery lead to a >10-fold increase in affinity (**5** vs **8**, **6** vs **9** and **7** vs **10**), but the alcohol derivative **10** showed significant oral bioavailability in the rat (Table 2) and a sustained inhibition of ADP-induced platelet aggregation ex vivo in the dog following oral administration.

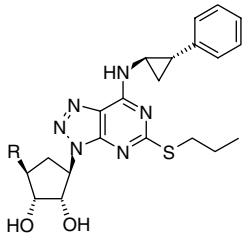
While the alcohol **10** had acceptable $P2Y_{12}$ affinity and good preclinical pharmacokinetics in both the rat and the dog, investigation of the metabolism of the compound in hepatocytes demonstrated marked species differences in biotransformation profiles.[11] Whereas oxidation was the predominant route of metabolism in rat hepatocytes, glucuronidation at the C4′ side chain hydroxyl group was the major biotransformation pathway in hepatocytes from dogs and humans.

This observation led us to set up a primary assay for glucuronidation using UDPGA supplemented rat, dog and human microsomes, in addition to the traditional NADPH supplemented microsomal assays used to measure oxidation.[11,12] These assays were applied in parallel to aid compound selection for final evaluation in human hepatocytes. Relative figures for resistance to glucuronidation and oxidation were determined by the inclusion in the assay of known standards: zileuton for the glucuronidation assay and dextromethorphan for the oxidation assay, the aim being to identify compounds with ratios of >20 × zileuton and >10 × dextromethorphan. In vitro–in vivo modelling had suggested that compounds showing such figures could be expected to have a metabolic clearance via glucuronidation and oxidation equivalent to <10% hepatic extraction (<2 mL/min/kg).

The hydroxymethyl compound **10**, although having good bioavailability, did not reach our desired criterion for resistance to glucuronidation. Further neutral alcohols were examined (Table 3) and the hydroxyethoxy **11** and hydroxyethyl **13** analogues achieved the desired metabolic stability ratios while retaining good bioavailability.

Of particular note is compound **14** (Table 3, R = H) which has a pK_i of 8.6. This is surprising since all functionality has been removed from this position and yet affinity is only 1/10 that of the triphosphate **1**. We propose that while in the triphosphate series activity is dominated by the electrostatic interactions of the acid side chain, new receptor-binding interactions have been introduced utilising the triazolopyrimidine and the lipophilic phenylcyclopropyl group. Compound **14** is, however, unstable to glucuronidation and overall the

Table 3. $P2Y_{12}$ antagonist affinity, metabolic stability and bioavailability of C4′ side chain alcohols



Compound	R	hP2Y$_{12}$ pK_i	UDPGA ratio[a]	NADPH ratio[b]	Rat F%
10	CH_2OH	8.3	5	>30	35
11	$O(CH_2)_2OH$	8.5	24	13	26
12	OH	8.7	3	Stable	50
13	$(CH_2)OH$	8.2	19	27	37
14	H	8.6	1	NT	38

Stable, turnover below limit of quantification; NT, not tested.
[a] UDPGA assay: required ratio to zileuton >20.
[b] NADPH assay: required ratio to dextromethorphan >10.

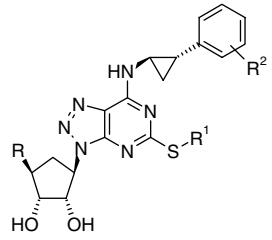
data in Table 3 show the hydroxyl side chain has an important effect on metabolic stability but little effect on affinity.

Finally, further optimisation by fine-tuning the substitution of the phenyl ring and 5-*S*-propyl substituent of **10** and **11** provided compounds **15**–**18** (Table 4) with acceptable affinity and metabolic stability suitable for further progression.

Preclinical pharmacokinetic data for 3,4-difluorophenyl compounds **16**–**18** are shown in Table 5.

Table 4. Optimising metabolic stability by substitution of the phenylcyclopropyl group

15,16: R = CH_2OH
17,18: R = $O(CH_2)_2OH$



Compound	R^1	R^2	hP2Y$_{12}$ pK_i	UDPGA ratio[a]	NADPH ratio[b]
15	$(CH_2)_2CF_3$	H	8.3	19	Stable
16	$(CH_2)_2CF_3$	3,4-DiF	8.3	Stable	>30
17	$(CH_2)_2CH_3$	3,4-DiF	8.7	Stable	24
18	$(CH_2)_2CF_3$	3,4-DiF	9.2	Stable	32

Stable, turnover below limit of quantification.
[a] UDPGA assay: required ratio to zileuton >20.
[b] NADPH assay: required ratio to dextromethorphan >10.

Table 5. Rat and dog pharmacokinetics for compounds **16**–**18**

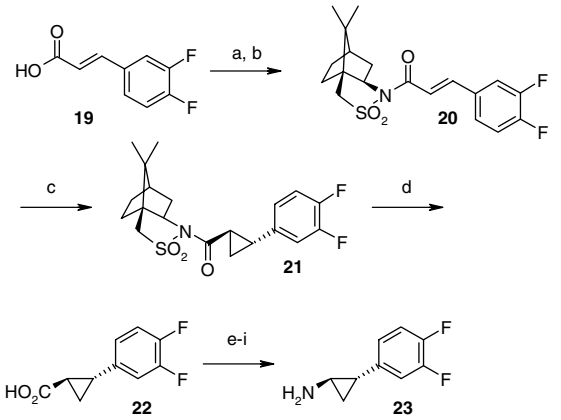
	Rat Clp[a]	Rat V_{ss}[a]	Rat $T_{1/2}$[a]	Rat F%[b]	Dog Clp[a]	Dog V_{ss}[a]	Dog $T_{1/2}$[a]	Dog F%[b]
16	11	2.7	3.0	17	16.5	5.6	4.8	59
17	21	3.8	2.6	24	9.0	3.0	2.9	72
18	12	2.1	2.5	9	NT	NT	NT	NT

Units: Clp, mL/min/kg; V_{ss}, L/kg; $T_{1/2}$, h; F, bioavailability; NT, not tested.
[a] Dosed at ~3 mg/kg (iv) in rat and 1 mg/kg (iv) in dog.
[b] Dosed at 3–10 mg/kg (po) in rat and 1 mg/kg (po) in dog.

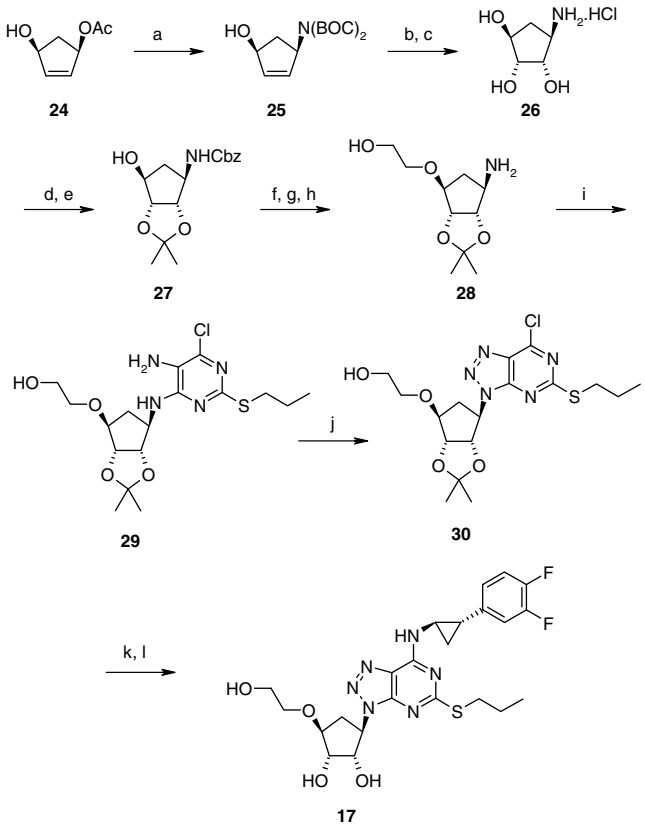
Compound **17** (AZD6140) was chosen over **16** and **18** for progression into human testing on the basis of a lower predicted dose (driven by a combination of potency and pharmacokinetic properties in pre-clinical species).

AZD6140 can be synthesised using the route outlined in Schemes 3a and 3b. Synthesis of the (1*R*,2*S*)-*trans* phenylcyclopropylamine begins with derivatisation of substituted cinnamic acid **19** with Oppolzer's sultam[13] to give **20**. Diastereoselective cyclopropanation[14] then provides, after recrystallisation, cyclopropylamide **21** in high chiral purity which is readily saponified to acid **22**. A four-step Curtius rearrangement[15] gives the cyclopropylamine **23**, which is conveniently isolated as a tartrate salt.

Synthesis of the core utilises commercially available acetate **24** which is cleanly converted to the protected amine **25** under palladium catalysis.[16] Osmium catalysed *cis* dihydroxylation[17] occurs with good diastereoselectivity to give, following amine deprotection, the aminotriol **26**. Reprotection of the amine as a benzyl carbamate and protection of the diol as an acetonide gives alcohol **27** suitable for alkylation. The hydroxyethyl side chain is next incorporated by reaction with ethyl bromoacetate followed by reduction to give key intermediate **28**. Reaction of **28** with 4,6-dichloro-2-propylthio-pyrimidine-5-amine[9] gives **29** which is converted to the triazolopyrimidine **30** under diazotization conditions. Finally,



Scheme 3a. Reagents and conditions: (a) $SOCl_2$; (b) sultam salt (80%; two steps); (c) CH_2N_2, $Pd(OAc)_2$ then recrystallise from EtOH (50%); (d) LiOH, H_2O, THF, 50 °C (99%); (e) $ClCO_2Et$, TEA, acetone, H_2O; (f) NaN_3, H_2O; (g) toluene, reflux; (h) 6 M HCl, reflux; (i) L-tartaric acid, EtOH (62%, five steps).



Scheme 3b. Reagents and conditions: (a) $(BOC)_2NNa$, $Pd(PPh_3)_4$, THF (92%); (b) OsO_4, NMO, THF, H_2O (100%); (c) HCl, MeOH, H_2O (96%); (d) dimethoxypropane, pTSA, acetone (86%); (e) CbzCl, DIPEA, MIBK (95%); (f) Bu^{t}OK, ethyl bromoacetate, THF; (g) $LiBH_4$, THF (86%; two steps); (h) H_2, Pd/C, EtOH (99%); (i) 4,6-dichloro-2-propylthio-pyrimidine-5-amine, DIPEA, DMF (75%); (j) isoamylnitrite, CH_3CN (88%); (k) **23**, DIPEA, DCM (99%); (l) TFA, H_2O (90%).

chloro displacement with amine **23** followed by deprotection gives **17**.

Phase I and phase II studies have confirmed the predicted pharmacokinetic and pharmacodynamic profile of the compound and a double blind comparison with clopidogrel, on a background of low-dose aspirin, has shown **17** to have superior anti-platelet activity as measured ex vivo by light transmission aggregometry.[18]

In summary, beginning with ATP, a poor lead for an oral programme, we have discovered oral $P2Y_{12}$ antagonists with clinical potential. Key elements in the medicinal chemical journey from ATP to **1** to **17** were:

(a) introducing affinity-enhancing 5,7-hydrophobic substituents;
(b) replacement of the labile triphosphate group;
(c) changing the core purine to a triazolopyrimidine, increasing affinity >100-fold;
(d) finding the first nonacidic reversible antagonists (e.g., **6** and **7**);
(e) introducing the *trans*-2-phenylcyclopropylamino substituent, increasing affinity >10-fold; and
(f) identifying metabolically stable neutral compounds by modifying the hydrophobic phenylcyclopropyl group and the hydroxylic side chain substituent.

In making these changes, the structure–activity relationships moved away from dependency on the acidic side chain, allowing identification of potent and orally bioavailable non-nucleotide reversible $P2Y_{12}$ antagonists. Compound **17** has now progressed to phase III clinical trials in acute coronary syndromes.

Acknowledgments

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Supplementary data

Experimental details for the General Schemes 1 and 2 and supporting analytical data (NMR, MS and elemental analyses) for compounds **2**–**18** can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.057.

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7. Aggregation of human washed platelets was assessed turbidimetrically in 96-well plates as a decrease in absorbance (650 nm). The washed platelets were incubated with the test compounds for 5 min before addition of 30 μM ADP. Antagonist potency was estimated as a % inhibition of the control ADP response to obtain a pIC_{50}. Full assay details are given in: Humphries, R. G.; Tomlinson, W.; Ingall, A. H.; Cage, P. A.; Leff, P. *Br. J. Pharmacol.* **1994**, *113*, 1057.
8. Binding data were obtained in washed platelets in 96-well plates, each well containing [^{125}I] radiolabelled $P2Y_{12}$ antagonist, test compounds and washed platelets. After 30 min incubation, the reaction was terminated by filtration, washing of the platelets and the bound radioactivity was measured and used to derive a pK_i. Full assay details

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