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Adenosine analogues as inhibitors of $P2Y_{12}$ receptor mediated platelet aggregation

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Abstract—Modified adenosine derivatives may lead to the development of $P2Y_{12}$ antagonists that are potent, selective, and bind reversibly to the receptor. Analogues of 2′,3′-*trans*-styryl acetal-*N*6-ureido-adenosine monophosphate were prepared by modification of the 5′-position. The resulting analogues were tested for $P2Y_{12}$ antagonism in a platelet aggregation assay.

Plavix® (clopidogrel bisulfate tablets), an inhibitor of ADP-induced platelet aggregation,[1] acts by irreversible inhibition of activation of the $P2Y_{12}$ receptor.[2] It has been shown clinically that the incidence of myocardial infarction and stroke are reduced by irreversible inhibition of ADP-induced platelet aggregation.[3]

However, several limitations regarding the use of irreversible $P2Y_{12}$ receptor antagonists have been identified which include (1) a low level of inhibition in approximately one third of the patient population, (2) variability of the response from patient to patient with the available dosing regimens,[4] (3) the current drugs require hepatic activation, and (4) irreversible inhibition complicates the drug's usefulness when procedures, such as coronary artery bypass graft surgery (CABG), become necessary in a patient's treatment protocol.[5] In the past decade, there have been several efforts to design and develop improved irreversible $P2Y_{12}$ receptor antagonists and to explore the usefulness of reversible intravenous and orally administered $P2Y_{12}$ receptor antagonists. Most of these efforts focus on the fact that adenosine triphosphate (ATP) acts as an antagonist of the $P2Y_{12}$ receptor. This invites the possibility of exploring ATP mimetics as a foundation for the discovery of new chemotypes that antagonize the $P2Y_{12}$ receptor. In fact, this approach has led to the discovery of a novel reversible short-acting antagonist, cangrelor (**1**),[6] and AZD6140 (**2**),[7] a reversible orally available antagonist discovered by AstraZeneca scientists (Fig. 1).

Although the ATP mimetic approach has been successful, several other reversible $P2Y_{12}$ antagonists have been described that do not mimic the ATP structural components necessary for inhibition (Fig. 1).[8,9] Work in our own laboratories led to the discovery of INS50589 (**3**), an adenosine monophosphate derivative with 2′,3′-cyclic acetal and *N*6-urea modifications (Fig. 2).[10]

Compound **3** inhibits platelet aggregation with an IC_{50} of 16 nM. This compound was evaluated in early clinical trials as an intravenously delivered drug for cardiovascular use.

This letter describes our initial efforts to discover non-phosphate 5′-substituents that would maintain the potency of our phosphate analogues and might also be useful for developing an orally active reversible antagonist.

Based on previous results in the phosphate series, we decided to focus on derivatives that contain the *trans*-acetal derived from the reaction of the 2′- and 3′-hydroxyl groups of adenosine with *trans*-cinnamaldehyde and an *N*6-ethyl urea. Our previous work demonstrated

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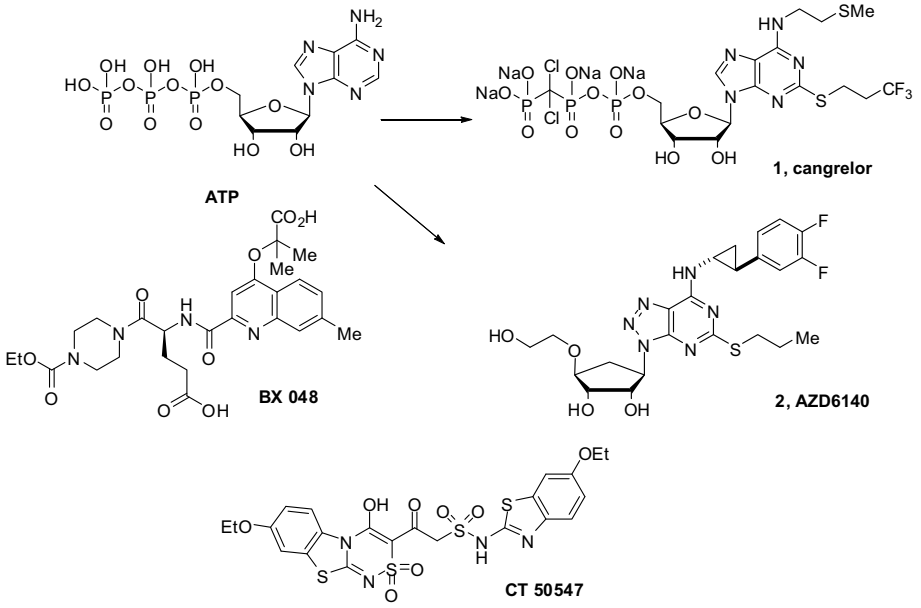


Figure 1. Potent $P2Y_{12}$ antagonists in preclinical and clinical development.

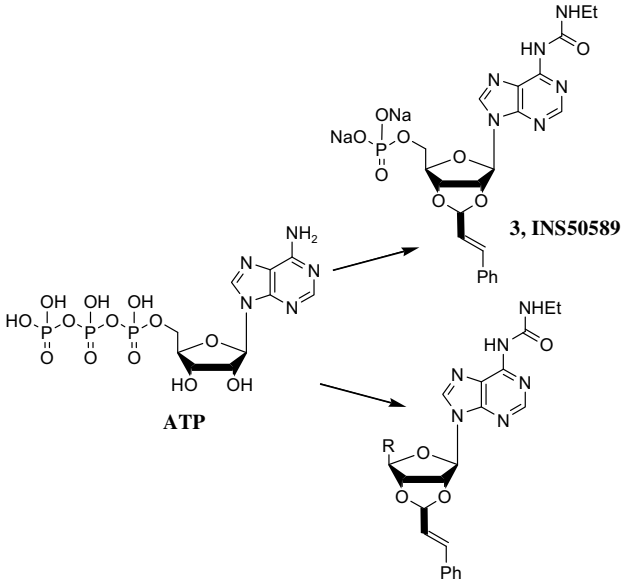
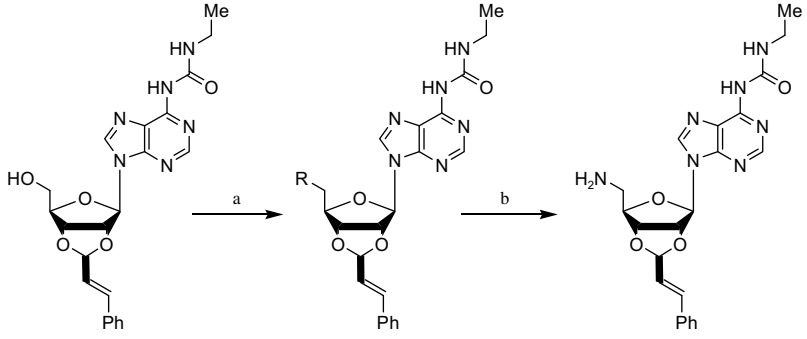


Figure 2. Simple ATP mimetics as $P2Y_{12}$ antagonists.

these functional groups were important for both $P2Y_{12}$ potency and selectivity. Conveniently, the acetal also provided the synthetic advantage of protecting the secondary alcohols of adenosine from interfering with *N*6- and 5′-alcohol modifications. The synthesis of starting material **4** has been described elsewhere.[11]

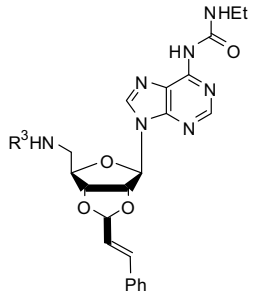
5′-Amides and amines. Alcohol **4** was reacted with *para*-toluenesulfonyl chloride in pyridine at 0 °C to afford the tosylate **5** in high yield (Scheme 1, 95%). Tosylate **5** was displaced with sodium azide to give azide **6**, which was then reduced using Staudinger's conditions to provide the 5′-amine **7** in 80% yield. Reductive aminations of **7** using an aldehyde and sodium triacetoxyborohydride in dichloroethane (DCE) afforded secondary and tertiary amines. In addition, amine **7** was used to construct several amides by treating the amine with commercially available acids in the presence of EDC, HOBt, and DMF (Table 1).

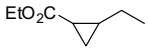
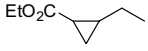
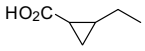
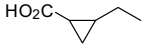
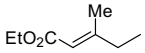
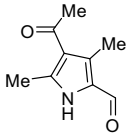
5′-Sulfides and sulfones. Tosylate **5** was also treated with several thiols under basic conditions to afford



Scheme 1. Reagents and conditions: (a) TsCl, pyridine, 0 °C then NaN_3, DMF, 100 °C, 85% for two steps; (b) PPh_3, THF, H_2O, 80%.

Table 1. Effect of selected amines and amides on the relative potency to inhibit platelet aggregation in a washed platelet assay



Compound	R^3	IC_{50} μM (n = 3)	% Inhibition at 30 μM
7	H	4.73 ± 1.94	—
13		4.33 ± 1.40	—
14[a]		—	46 ± 4.2
15		2.70 ± 0.95	—
16[a]		0.58 ± 0.11	—
17		—	70 ± 7
18		2.55 ± 0.68	—
19[a]		—	NR
20		—	73 ± 12
21		—	70 ± 12
22		0.513 ± 0.16	—
23		2.95 ± 0.32	—
24		4.86 ± 0.91	—
25		2.23 ± 0.44	—
26		—	53 ± 7

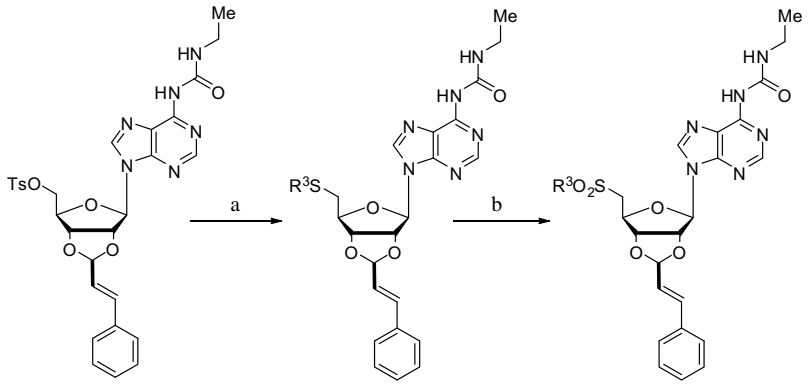
[a] Trisubstituted amine derivative.

sulfide derivatives **8** (Scheme 2). The sulfides were then oxidized to the corresponding sulfones **9** using potassium hydrogen persulfate (oxone®) at 0 °C in methanol and sodium bicarbonate (pH 5) (see Table 2).

5′-Ethers and acids. Alcohol **4** was also oxidized directly (Scheme 3) to either the aldehyde (**10**) or the corresponding acid (**11**). Aldehyde **10** was then homologated using either a stabilized or unstabilized Wittig reagent. Subsequent modifications provided chain extended carboxylic acids. For example, aldehyde **10** was reacted with the anion of $(EtO)_2POCH_2CO_2Me$ in THF, reduced and hydrolyzed to provide acid **12**. In addition, alcohol **4** was also alkylated with reactive electrophiles to provide a variety of alkyl and aryl ether derivatives (see Table 3). Experimental conditions for all derivatives reported in this letter are provided in the Supplemental materials section.

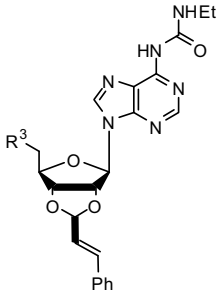
As part of the design of the acute use $P2Y_{12}$ receptor antagonist INS50589 (**3**), the phosphate group contributes to the high binding potency yet is readily cleaved in situ by endogenous ectonucleotidases to a 100-fold less potent metabolite (**4**, IC_{50} = 1.27. ± 0.41 μM). Our goal in this study was to determine what type of functional groups could regain this loss in potency and serve as potential leads for chronic orally active $P2Y_{12}$ antagonists. Initially we examined the potency of the 5′-alcohol (**4**), amine (**7**), and thiomethyl ether (**27**). None of these derivatives provided any gain in potency relative to **4**. Thiopropyl (**28**) and thioheptyl (**30**) derivatives were also found to be inactive. Substitution of the propyl chain with hydroxyl groups (**33** and **35**) proved to be better than the alkyl derivatives, and (for **35**) comparable in potency to alcohol **4**. Removal of the terminal methyl group of **33**, hydroxyl ethyl derivative **31**, led to a loss of potency. The corresponding sulfones of **28**, **31**, **33**, and **35** (**29**, **32**, **34**, and **36**, respectively) offered no improvement. The highly charged terminal sulfate (**37**) was prepared and found to be modestly potent. On the other hand, acetic acid derivative **38** and its respective sulfone derivative **39** were both found to be comparable in potency to **4**.

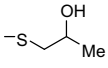
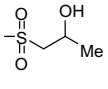
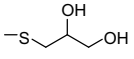
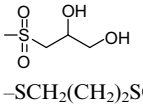
Based on these findings we focused our efforts on replacing the phosphate charge with a carboxylic acid moiety. Preparation of the one, two, and three carbon analogues, **11**, **40**, and **12**, respectively, resulted in improved potency when compared to **4** (1.27 μM vs 0.201, 0.663, and 0.495 μM, respectively). The nitrogen and oxygen versions of sulfide **38** (amine **22**, 0.513 μM and ether **41**, 0.243 μM) proved to be fairly potent, with the ether comparable in potency to the one carbon acid derivative **11** (0.243 vs 0.201 μM, respectively). The slightly more rigid α,β-cyclopropane amino acids (**15** and **16**), 5 atoms in length, were found to be close in potency to **4**. Interestingly, one of their corresponding esters (**13**) did not lose much in binding potency. In the amine series



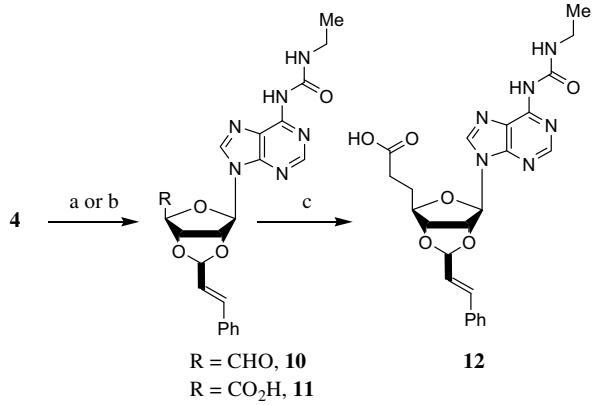
Scheme 2. Reagents and conditions: (a) R^3SH, *t*-BuOK, DMSO; (b) **8**, oxone, $NaHCO_3$, MeOH, 0 °C.

Table 2. Effect of selected 5′-sulfides and sulfones on the relative potency to inhibit platelet aggregation in a washed platelet assay



Compound	R^3	IC_{50} μM ($n = 3$)	% Inhibition at 30 μM
27	$-SCH_3$	—	63 ± 6
28	$-SCH_2CH_2CH_3$	—	52 ± 5
29	$-SO_2CH_2CH_2CH_3$	—	65 ± 7
30	$-SCH_2(CH_2)_5CH_3$	—	38 ± 9
31	$-SCH_2CH_2OH$	—	63 ± 2
32	$-SO_2CH_2CH_2OH$	—	57 ± 3
33		—	76 ± 4
34		2.49 ± 0.8	—
35		9.9 ± 2	—
36		—	14 ± 5
37	$-SCH_2(CH_2)_2SO_3H$	—	90 ± 6
38	$-SCH_2CO_2H$	3.8 ± 0.8	—
39	$-SO_2CH_2CO_2H$	2.48 ± 0.6	—

we prepared and examined the potency of a randomly selected list of benzyl/heteroaryl amines (**17**–**20**) and heteroaryl amides (**24**–**26**) without much success in improving potency but not completely eliminating it either. Amide **23** was found to be

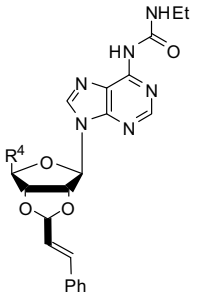


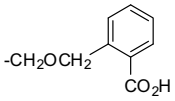
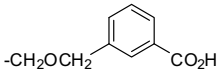
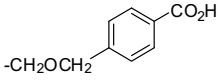
Scheme 3. Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2 for **10**; (b) $NaClO_2$, $NaHPO_4$, DMSO, H_2O, 0 °C for **11**; (c) $(EtO)_2POCH_2CO_2Me$, NaH, THF, then **10**; $CuSO_4$, $NaBH_4$, MeOH; LiOH, THF, H_2O, 22% for three steps.

modestly potent. Recognizing that the 5′-heteroatom of choice was oxygen, aryl groups were tolerated and acids provided some increase in potency (~6-fold) respective to alcohol **4**, we decided to examine 5′-benzyl ethers that differed in the location of a carboxylic acid on the ring. It was eventually found that in the series, *ortho* (**42**), *meta* (**43**), and *para* (**44**) the potency to inhibit platelet aggregation was measured to be 0.040, 0.616, and 1.31 μM, respectively. Benzoic acid **42** was only 2-fold less potent than our starting phosphate clinical candidate.

Using the acetal/urea ribose backbone established from INS50589, we have discovered non-phosphate 5′-modifications that have achieved similar low nanomolar activity. We were able to identify a 2-carboxybenzyl group as a bioisostere for a phosphate group. Additional studies are planned to evaluate the pharmacokinetic and pharmacodynamic effects of this new functional group in a series of antagonists as well as the effects of modifications of the acetal and urea substituents on $P2Y_{12}$ potency.

Table 3. Effect of 5′-acids and ethers on the relative potency to inhibit platelet aggregation in a washed platelet assay



Compound	R^4	IC_{50} μM (n = 3)
3	$-CH_2OPO(ONa)_2$	0.016 ± 0.013
4	$-CH_2OH$	1.27 ± 0.41
11	$-CO_2H$	0.201 ± 0.071
40	$-CH_2CO_2H$	0.663 ± 0.020
12	$-CH_2CH_2CO_2H$	0.495 ± 0.041
41	$-CH_2OCH_2CO_2H$	0.243 ± 0.06
42		0.040 ± 0.017
43		0.616 ± 0.214
44		1.31 ± 0.38

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.038.

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