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Novel Tricyclic Benzothiazolo[2,3-*c*]thiadiazine Antagonists of the Platelet ADP Receptor ($P2Y_{12}$)

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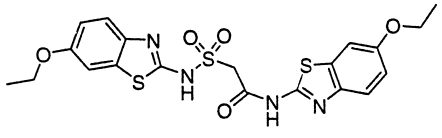
Abstract—Novel non-nucleoside tricyclic platelet ADP receptor ($P2Y_{12}$) antagonists have been discovered that bind reversibly and with high affinity to the platelet receptor. Condensation of various 2-aminobenzothiazoles with chlorosulfonylacetyl chloride affords these novel tricyclic heterocycles, which are novel and unpredicted products of this reaction.

Platelet aggregation plays an important role in arterial thrombosis thus making platelets the targets of several important classes of antithrombotic agents such as aspirin,[1] the glycoprotein IIb-IIIa antagonists,[2] and the thienopyridines (ticlopidine and clopidogrel).[3] The mechanism of action of the thienopyridines is not fully understood but appears to be principally mediated through blockade of specific platelet ADP receptors. ADP-dependent platelet aggregation requires activation of at least two distinct G protein-coupled ADP receptors, the $P2Y_1$ receptor and the recently cloned $P2Y_{12}$ receptor (variously called $P2T_{AC}$, $P2Y_{ADP}$, $P2Y_{AC}$ or $P2Y_{cyc}$), which appears to be the target of the thienopyridines.[4,5] Interestingly, the thienopyridines do not directly block ADP receptors but require a metabolic conversion in vivo to highly unstable 'active metabolites' that are thought to covalently modify the receptors.[6] Because of the importance of ADP-mediated signaling in initiating platelet activation and in stabilizing large platelet aggregates induced by other platelet agonists, we and others have initiated efforts designed to identify direct-acting and reversible antagonists of the platelet $P2Y_{12}$ receptor.[7] We now report the discovery of a novel class of non-nucleoside-containing tricyclic antagonists of the platelet $P2Y_{12}$ receptor, and some preliminary structure–activity relationships within this series.

Screening of the COR compound library using a 96-well platelet ADP receptor binding assay employing the agonist radioligand [^{3}H]2-MeS-ADP (1 nM)[4,8] with human platelets identified a novel, high affinity antagonist, which was initially assigned the bis-benzothiazole structure **1**. The antagonist displayed an $IC_{50} = 0.17\ \mu M$ in the binding assay and was found to inhibit ADP-induced aggregation of washed human platelets (as determined 5 min after addition of 1 μM ADP in 96-well assay) with an $IC_{50} = 0.18\ \mu M$. Further characterization of the compound sample revealed that the assigned structure **1** was inconsistent with the observed mass (HR-MS for $C_{20}H_{20}N_4O_5S_3$; M + H expected = 493; found M + H = 597.0248). Based on the observed molecular weight, synthetic considerations and additional spectral and chemical characterization, we have assigned structure **2** (CT50547) to the antagonist.[9]



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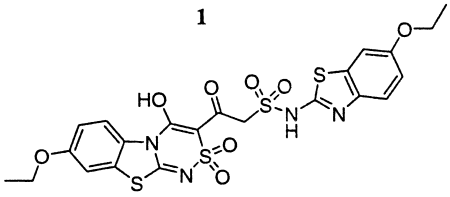

2 CT50547

The synthesis of the antagonist **2** was achieved by the reaction of one equivalent of chlorosulfonylacetyl chloride with two equivalents of 6-ethoxy-2-aminobenzothiazole

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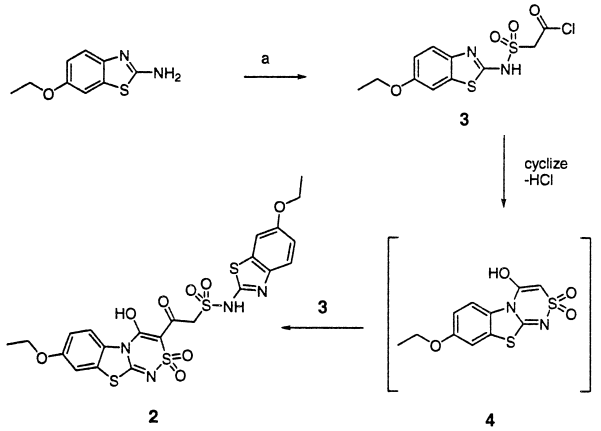
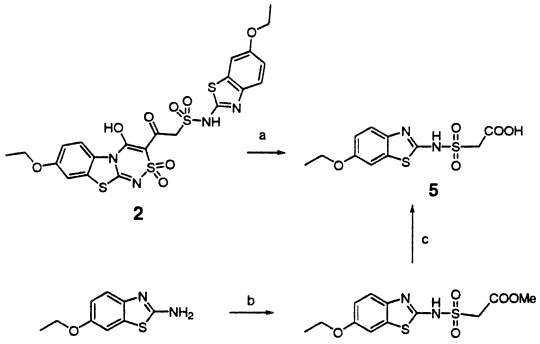
catalyzed by triethylamine and was thus originally believed to be structure **1**.[9] We propose the following reaction mechanism, whereby an initially formed sulfonamido acid chloride intermediate **3** can cyclize to form the benzothiazolo[2,3-*c*]thiadiazine ring system **4** that can subsequently undergo further acylation with the acid chloride **3** to form the product antagonist **2**. The benzothiazolo[2,3-*c*]thiadiazine ring system has not been heretofore described in the chemical literature. Interestingly, we did not isolate under any conditions the proposed unsubstituted tricyclic intermediate **4**, suggesting that **4** is extremely reactive toward another molecule of acid chloride **3** (Scheme 1). When the stoichiometry of the reaction was modified to using one equivalent of 2-aminobenzothiazole and chlorosulfonylacetyl chloride, the yield and purity of **2** was slightly improved.

In order to confirm the proposed structure **2**, much effort was expended in an attempt to obtain a crystalline sample amenable to X-ray diffraction analysis, but without success. Thus, the structural assignment of compound **2** rests mainly on the spectroscopic evidence presented,[9] as well as on the results of limited chemical degradation studies. From the ^{1}H and ^{13}C NMR spectra, the two benzothiazole rings were clearly differentiated (ethoxy groups and aromatic resonances noncoincidental), and from gentle acid hydrolysis, the connectivity of the sulfonyl chloride with the amino group of the aminobenzothiazole was established (Scheme 2). The terminal product in this reaction was **5**, the structure of which was confirmed by independent synthesis (Scheme 2). The main structural issue then became the nature of the connectivity of the two halves of the molecule, with removal of the elements of hydrogen chloride. Two structures came immediately to mind, structure **2** and the isomeric enol ester **6**.[10] On the basis of the infrared spectrum, we believe that the absorption at 1588 cm^{-1} is strongly indicative of an enolized β-dicarbonyl moiety. Correspondingly, the lack of an ester carbonyl stretch near 1750 cm^{-1} would argue against structure **6**.[10] Also, there appears no peak in the vinylic region of the ^{1}H NMR spectrum which would correspond to the proton on the thiadiazine ring. A ^{1}H–^{13}C HETCOR experiment was consistent with the proposed structure, and NMR experiments with the other inhibitors listed in Table 1 provided corroborative results.



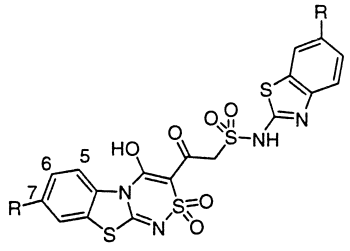
Scheme 1. Synthesis of benzothiazolo[2,3-*c*]thiadiazine **2**. (a) Chlorosulfonylacetyl chloride/triethylamine/THF.

With the assignment of structure **2** for the antagonist established, we initiated a synthetic program to elucidate the structural requirements for $P2Y_{12}$ receptor antagonism by other novel heterocyclic analogues of **2**. Reaction of a variety of 6-substituted 2-amino-benzothiazoles with chlorosulfonylacetyl chloride afforded in all cases poor yields (4–8% following HPLC purification) of analogues of **2** (Table 1).[11] However, only the parent tricyclic ring analogue **18** (Table 2) was formed from 6-trifluoromethoxy-2-aminobenzothiazole, with no acylated product being obtained. Surprisingly, when analogue synthesis was attempted with certain 4-substituted 2-aminobenzothiazoles, the reactions did not yield the acylated benzothiazolo[2,3-*c*]thiadiazines. Thus, 4-chloro-2-aminobenzothiazole or 4-methyl-2-aminobenzothiazole only yielded analogues of **1**. However, 4-methoxy-2-aminobenzothiazole afforded the



Scheme 2. (a) 3 N HCl; (b) methyl (chlorosulfonyl)acetate/triethylamine/THF; (c) $LiOH/H_2O$/THF.

Table 1. Benzothiazolo[2,3-*c*]thiadiazine analogues **2** and **7**–**17**



Analogues	R	[^{3}H]2-MeS-ADP binding inhibition IC_{50} (μM)[a]	Aggregation inhibition IC_{50} (μM)[a]
2	CH_3CH_2O	0.17 (±0.12)	0.18 (±0.08)
7	CH_3O	0.80 (±0.3)	1.5 (±0.9)
8	CO_2Et	1.8 (±0.1)	0.8 (±0.5)
9	$MeSO_2$	1.1 (±0.3)	2.9 (±1.0)
10	H	21 (±9)	26 (±12)
11	CH_3	22 (±6)	9.5 (±3.2)
12	Cl	35 (±21)	64 (±23)
13	NO_2	35 (±0.5)	50 (±44)
14	F	37 (±0.8)	>120
15	Ph	>50	>24
16	PhO	>50	>24
17	c-Hexyl	>120	>120

[a]Values are means of 2–8 experiments performed in duplicate, standard deviation is given in parentheses.

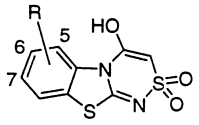
parent unacylated tricyclic ring analogue **19** (Table 2). It is not readily apparent from this limited set of analogues what factors may be influencing the reactivity of the 2-aminobenzothiazole with chlorosulfonylacetyl chloride or what promotes further acylation of the tricyclic ring system **4**.

Analogues of **2** exhibit a very stringent structure–activity relationship (Table 1). Only analogues with small substitutents on the benzo ring of the tricyclic nucleus that have the ability to participate in hydrogen bonding retain good receptor affinity (analogues **2**, **7**, and **9**). Other electron withdrawing or bulky substituents attached to the benzo ring greatly reduce the $P2Y_{12}$ receptor binding affinity of analogues. One of the two unacylated benzothiazolo[2,3-*c*]thiadiazines prepared retained little functional activity (analogue **19**, Table 2), and displayed extremely weak inhibition in the binding assay. Thus, from this limited set of analogues, activity in this series of $P2Y_{12}$ antagonists optimally requires a relatively small substituent R in the benzo ring that is capable of forming a potential H-bond with the receptor. Additionally, a 2-oxo-ethanesulfonamide group attached to the tricyclic nucleus is required to retain high affinity for the $P2Y_{12}$ platelet ADP receptor (**2** and **7**–**17** versus **18** and **19**).

To assess the selectivity of these series for the $P2Y_{12}$ receptor, the most potent compound, CT50547, was evaluated for inhibition of the cloned $P2Y_{12}$ receptor and the other recently described platelet ADP receptor, $P2Y_1$. CT50547 (also referred to as C1330-7[9]) blocked the human $P2Y_{12}$ receptor expressed in *Xenopus* oocytes with an IC_{50} value of 40 nM.[5] Washed human platelets or Jurkat cells transfected with the cloned human $P2Y_1$ receptor were loaded with Fura-2 and peak elevations of intracellular calcium ions in response to $P2Y_1$ receptor stimulation were determined by fluorescence spectroscopy.[4b] The IC_{50} value for CT50547 in each of these assays was $>100\,\mu M$, indicating a 1000-fold selectivity for $P2Y_{12}$ over $P2Y_1$ (data not shown).

While CT50547 is a potent and novel antagonist of the platelet $P2Y_{12}$ receptor, the stringent requirements for activity in this series limits the useful SAR and the potential to further manipulate these compounds into drug candidates. However, CT50547 and analogues are the first reversible, purinergic receptor-specific, non-nucleoside based antagonists of the $P2Y_{12}$ receptor and make them useful reagents to study this newly characterized and important platelet ADP receptor.

Table 2. Benzothiazolo[2,3-*c*]thiadiazine analogues **18**–**19**



Analogues	R	[^{3}H]2-MeS-ADP binding inhibition IC_{50} (μM)[a]	Aggregation inhibition IC_{50} (μM)[a]
18	7-CF_3O	>120	>50
19	5-CH_3O	77 (±4.7)	>120

[a]Values are means of 2–3 experiments performed in duplicate, standard deviation is given in parentheses.

Acknowledgements

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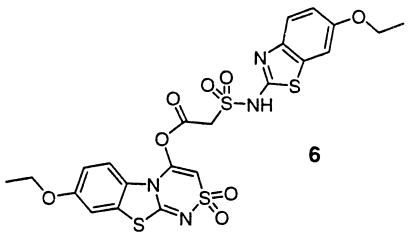
9. Characterization of **2** (also known as C1330-7, see ref 5) HRMS for $C_{22}H_{20}N_4O_8S_4$: M + H expected 597.0242; M + H obtained: 597.0248. ^{1}H NMR (DMSO-d_6) δ 7.95–7.97 (d, J = 8.8 Hz, 1H, aromatic H), 7.33 (d, J = 2.4 Hz, 1H, aromatic H), 7.27 (d, J = 2.4 Hz, 1H, aromatic H), 6.93–6.95 (d, J = 9.0, 1H, aromatic H), 6.81–6.83 (dd, J = 2.4, 9.0 Hz, 1H, aromatic H), 6.49–6.51 (dd, J = 2.6, 9.2 Hz, 1H, aromatic H), 4.65 (s, 2H, CH_2), 3.93–4.01 (q×2, J = 7.0, 7.0 Hz, 4H, ethyl CH_2), 1.27–1.31 (t×2, J = 7.0, 7.0 Hz, 6H, ethyl CH_3). ^{13}C NMR (DMSO-d_6) δ 189.84, 167.82, 159.13, 158.95, 156.04, 155.46, 131.09, 130.43, 126.84, 122.83, 119.52, 114.99, 113.53, 113.38, 107.89, 107.74, 101.41, 64.16, 63.93, 63.11, 15.12, 15.06. IR 1588/1553 cm^{-1}.
10. Alternate possible structure:



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11. The final compounds were analytically pure as determined by HPLC, ^{1}H NMR and MS. Representative synthesis: **2**, ***N*^{1}-(6-Ethoxy-1,3-benzothiazol-2-yl-2-(7-ethoxy-4-hydroxy-2,2-dioxo-2*H*-2]^{6}benzo[4,5][1,3]thiazolo[2,3-*c*][1,2,4] thiadiazin-3-yl)-2-oxo-**

1-ethanesulfonamide). To a solution of 2.58 g (13.1 mmol) of 6-ethoxy-2-aminobenzothiazole dissolved in 50 mL of anhydrous THF under argon at room temperature was added 3 mL (21.5 mmol) of triethylamine followed by addition of a solution of 1.0 g (5.65 mmol) of chlorosulfonylacetyl chloride in 10 mL of THF. A heavy precipitate formed immediately, and stirring was continued at ambient temperature for 48 h. The reaction was quenched by the addition of water, poured into ethyl acetate and washed with 10% HCl, twice with brine and dried over magnesium sulfate. Concentration in vacuo afforded a brownish yellow solid. The title compound was isolated by C_{18} reversed-phase HPLC, which eluted at 50–58% acetonitrile/0.1% TFA. The appropriate fractions were combined and lyophilized to afford 64 mg (4%) of **2** as a yellow solid. The isolated yields of all analogues listed in Table 1 were uniformly low (4–8%).