Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 1917–1923

BIOORGANIC & MEDICINAL CHEMISTRY

A Preliminary Investigation of Mesoionic Xanthine Analogues as Inhibitors of Platelet Aggregation

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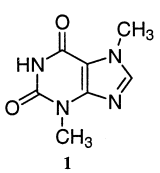
Received 15 February 2000; accepted 13 April 2000

Abstract—A series of mesoionic xanthines (e.g. mesoionic thiazolopyrimidines, **3**, and thiadiazolopyrimidines, **5**) and related analogues were examined as inhibitors of human platelet aggregation. Appropriately substituted compounds were found to fully inhibit platelet aggregation, and anhydro-(6-ethyl-8-isopentyl-7-oxo-5-hydroxy-1,3,4-thiadiazolo[3,2-*a*]pyrimidinium hydroxide) (**5b**) was 40 times more potent than the structurally related xanthine theophylline (**1**). Gel filtration studies suggest that compound **5b** irreversibly inhibits aggregation and this might be due to its ability to act as a latent acylation agent.

Introduction

The serious cardiovascular disorders of myocardial infarction and unstable angina are associated with the activation and aggregation of platelets. Ischemic stroke resulting from the transient or permanent reduction in cerebral blood is in most cases caused by the occlusion of a cerebral artery by either embolism or local thrombosis. The utility of anti-platelet agents in the treatment of cardiovascular disorders and stroke has recently been reviewed.[1]

A large number of agents such as papaverine[2] and the methyl xanthine derivatives theophylline (**1**) and 1-isobutyl-3-methylxanthine (IBMX; **2**) inhibit platelet aggregation through their activity as phosphodiesterase inhibitors.[3,4] In a number of reports Glennon and co-workers have shown that a series of mesoionic derivatives have activity as inhibitors of phosphodiesterase.[5–9] The possibility hat these compounds might also, then, inhibit platelet aggregation led to their evaluation as platelet aggregation inhibitors. Several previously published mesoionic compounds were evaluated, and a few novel mesoionic compounds were prepared to further develop the structure–activity relationships associated with the activity of these compounds as inhibitors of platelet aggregation.



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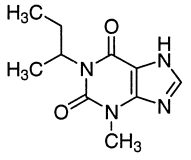

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As predicted by molecular orbital calculations, mesoionic thiazolo[3,2-*a*]pyrimidines and 1,3,4-thiadiazolo[3,2-a] pyrimidines undergo nucleophilic attack by amines at the 5-positions.[9–12] This is shown in Figure 1. The possibility exists, then, that these compounds might behave as latent acylating agents. However the use of these potential acylating agents to thereby irreversibly inhibit a biological response has not yet been demonstrated. Gel filtration of platelet rich plasma provides a method that results in the rapid and complete separation of human platelets from plasma. This procedure is useful in determining the reversibility of drug effects by removing drug from the platelet-containing suspending medium. The reversibility of the effects of beta lactam antibiotics[13] and an *N*-(2-chloroethyl) local anesthetic[14] on platelets has been assessed by this method. The possibility that the mesoionic xanthine derivatives might act by an irreversible mechanism as inhibitors of platelet aggregation was evaluated using the gel filtration technique.

Chemistry

The mesoionic xanthine analogues were prepared by the methods of Coburn et al.[10–12,16] and Glennon and

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PII: S0968-0896(00)00123-1

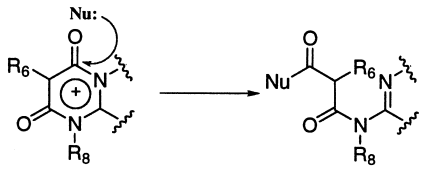


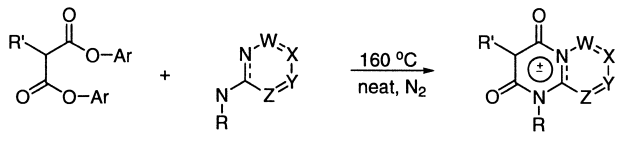
Figure 1. Attack of a mesoionic xanthine analogue by a nucleophile to afford a ring-opened compound.[9]

co-workers.[5–9,15,17] Briefly, the target compounds were prepared by the thermal condensation of an alkylaminoheterocycle and a bis(2,4,6-trichlorophenyl)-2-alkylmalonate ester under a continuous stream of nitrogen.[7,15,18,19] See Scheme 1 for the general synthesis of the mesoionic derivatives.

Most of the alkylaminoheterocyles were prepared by the acylation of the aminoheterocycle with the appropriate acid chloride or anhydride followed by the reduction of the resulting amide using lithium aluminum hydride or sodium bis(methoxyethoxy) aluminum hydride. The 2-aminopyrimidine derivatives were synthesized by the direct alkylation of 2-aminopyrimidine (ethyl, *n*-pentyl derivatives) or by the two-step procedure of Kemal and Reese (benzyl derivative).[20] The 3-pyridazine and 4-aminopyrimidine derivatives were prepared by amination of and hydrogenolysis of either 3,6-dichloropyridazine or 4,6-dichloropyrimidine.[21,22] The substituted 4-(methylamino)-2-aminothiazole derivatives were prepared by the method of Silberg.[23]

Pharmacology

The mesoionic compounds were evaluated by the method of Born[24] and Puri[25] for their ability to inhibit ADP-induced platelet aggregation. ADP at a final concentration of 2.5 μM was used to stimulate platelet aggregation. At this concentration ADP caused a monophasic tracing denoting platelet aggregation. In this preliminary study designed to provide an initial characterization of the platelet aggregation inhibitory activity of the mesoionic xanthine analogues, a 100% blockade of ADP-induced aggregation (minimum concentration necessary to cause 100% inhibition of aggregation, MIC_{100}) was used as a reference point for two reasons. First, the responsiveness of the platelets to aggregating agents changes with time. Using less than a 100% blockade would necessitate running a control sample between each drug sample. Secondarily, it allows for a dependable measurement of the inhibition of primary aggregation since the response to ADP can be comprised of both primary and secondary aggregation.



Scheme 1.

The inhibition of platelet aggregation by the mesoionic inhibitors was time dependent. A twenty minute incubation was selected since this incubation time gave a maximum inhibitory effect while allowing for a reasonable number of evaluations within the two-hour period in which the platelets were viable.

The gel filtration studies were carried out by a modification of Tangen's procedure using a Sepharose 2B column.[14,26] Platelets passed rapidly through the column and were eluted in what was essentially the void volume of the column. Proteins and presumably other small molecular species have substantially longer retention times which allowed for the efficient separation of the platelets from the other constituents of the platelet rich plasma. Results are shown in Figure 2; a second run gave similar results.

Results and Discussion

The results of the platelet aggregation studies are shown in Tables 1 and 2. Members of the thiazolopyrimidine series (i.e., **3**) were examined first. The diethyl analogue **3a** ($MIC_{100} = 4$ mM) was as potent as theophylline (**1**) and 80-fold less potent than adenosine as an inhibitor of platelet aggregation. Replacement of the N_8-ethyl substituent

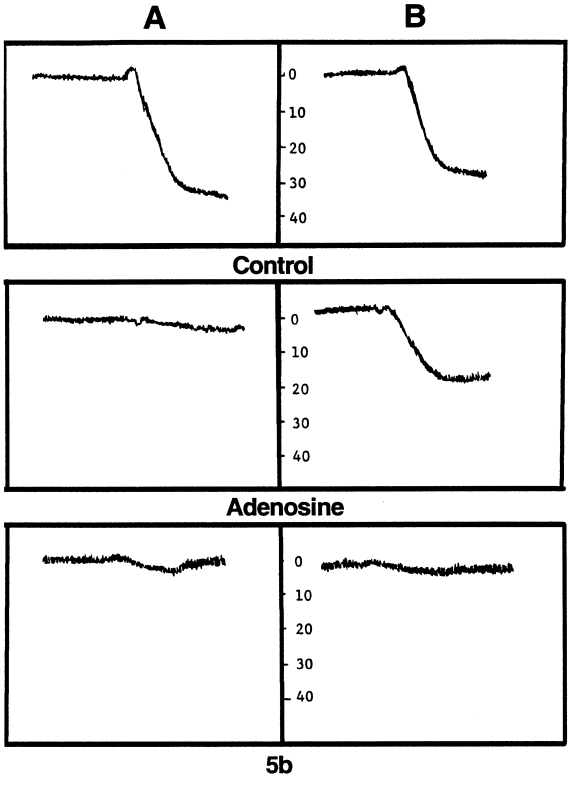
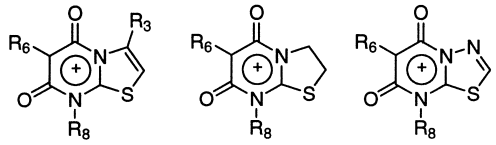


Figure 2. Platelet aggregation as measured by % change in light transmission before (A) and after (B) gel filtration. Platelet rich plasma was prepared and then incubated either with vehicle, adenosine (0.05 mM), or **5b** (0.8 mM) for 30 min and aggregation was stimulated by the addition of 2.5 μM ADP (Panel A). Platelet rich plasma was prepared and then incubated either with vehicle, adenosine (0.05 mM), or **5b** (0.8 mM) for 30 min then filtered through a Sepharose 2B column. Platelet poor plasma was added and aggregation was stimulated by the addition of 2.5 μM ADP (Panel B).

Table 1. Minimum 100% inhibitory concentrations (MIC_{100} values) for platelet aggregation by compounds **3**–**5**

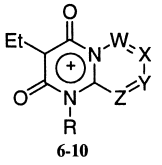


3 **4** **5**

	R_6	R_8	R_3	MIC_{100} (mM)[a]	Reference[b]
3a	Et	Et	H	4	7
3b	Et	Et	Me	1	8
3c	Et	Et	Phenyl	8	
3d	Et	*n*-Pentyl	H	2	
3e	Et	Phenyl	H	0.4	6
3f	Et	Benzyl	H	0.4	6
3g	Et	$CH_2CH_2N(Me)_2$	H	8	
3h	Et	Benzyl	$CH_2N(Me)_2$	6	
3i	Benzyl	Et	H	2	7
3j	Benzyl	$CH_2CH_2N(Me)_2$	H	6	
4	Et	Et	—	0.4	17
5a	Me	Et	—	0.2	7
5b	Me	*n*-Pentyl	—	0.1	6
5c	Et	Et	—	0.2	7
5d	*iso*Bu	Et	—	0.2	6
Adenosine				0.05	
Theophylline (**1**)				4	

[a]See Experimental for explanation of methods.
[b]Literature reference for preparation.

Table 2. Minimum 100% inhibitory concentrations (MIC_{100} values) for platelet aggregation by compounds **6**–**10**



6-10

	R	W	X	Y	Z	MIC_{100}, mM
6a	Et	CH	CH	CH	CH	2
6b	Et	CH-CH_3	CH	CH	CH	4
6c	*n*-Pentyl	CH	CH	CH	CH	4
6d	Benzyl	CH	CH	CH	CH	2
7	Et	N	CH	CH	CH	>8
8	Et	CH	N	CH	CH	2
9	Et	CH	CH	N	CH	4
10a	Et	CH	CH	CH	N	6
10b	*n*-Pentyl	CH	CH	CH	N	1
10c	Benzyl	CH	CH	CH	N	4

with larger or bulkier phenyl or benzyl substituents resulted in enhanced potency; N_8-substituent (followed by MIC_{100} values): phenyl (**3e**; 0.4 mM), benzyl (**3f**; 0.4 mM). Compounds **3e** and **3f** were ten times more potent than theophylline (**1**) and one-tenth as potent as adenosine. The N_8-pentyl substituent caused little change in potency (**3d**, 2 mM). Replacement of the C_6-ethyl substituent with the larger benzyl group (**3i**; $MIC_{100} = 2$ mM) resulted in little change in potency. Introduction at the 3-position of a methyl group (**3b**; $MIC_{100} = 1$ mM) enhanced potency by 4-fold whereas incorporation of a phenyl substituent (i.e. **3c**) decreased potency. Because many of the compounds display only limited aqueous solubility, an *N,N*-dimethylamino function was added to the alkyl groups of some of the derivatives (i.e. **3g**, **3h**, **3j**); this only served to reduce potency. Reduction of the C_2–C_3 double bond of **3a** provided **4** ($MIC_{100} = 0.4$ mM) and resulted in a 10-fold increase in potency (relative to **3a**). This feature was not further explored, however, because of the findings with **5c**.

Compound **5c** represents the 1,3,4-thiadiazole counterpart of the thiazolopyrimidine **3a**, and compound **5c** ($MIC_{100} = 0.2$ mM) was found to be 20 times more potent than **3a**. Shortening the C_6-ethyl group to a methyl group (i.e. **5a**) and lengthening it to an isobutyl group (i.e. **5d**) had no effect on potency. The C_6-methyl-N_8-*n*-pentyl derivative **5b** ($MIC_{100} = 0.1$ mM), however, was found to be the most potent analogue in the series with a potency 40 times that of theophylline and half that of adenosine.

Because the introduction of a limited amount of bulk at the 3-position (compare **3b** with **3a**), and incorporation of a ring nitrogen atom (compare **5c** with **3a**) was beneficial for activity, we prepared a series of ring-expanded analogues (i.e. **6**–**10**). Although nearly all of these compounds retained activity (with the exception of **7** which failed to produce 100% inhibition at the highest concentration evaluated), none was more potent than any members of the thiadiazolopyrimidine series **5**.

Gel filtration studies were conducted to provide evidence that the mesoionic xanthine derivatives might be irreversible inhibitors of platelet aggregation. Gel filtration of platelet rich plasma separates platelets from proteins and small molecular weight compounds. This procedure removes most reversibly acting platelet aggregation inhibitors. The mesoionic thiadiazole derivative **5b** was selected for these studies in part because it was the most

potent inhibitor of platelet aggregation, and in part because it has been demonstrated that the mesoionic thiadiazolo[3,2-*a*]pyrimidines are fairly reactive to nucleophilic attack.[9–12]

After a 30 min incubation at a concentration of 0.8 mM **5b** completely inhibited platelet aggregation stimulated by 2.5 μM ADP. Following gel filtration, platelet aggregation was still completely inhibited when the platelet-poor plasma was added to the filtered platelets and the platelets were stimulated with 2.5 μM ADP. A similar study using 0.05 mM of adenosine showed the expected complete inhibition of platelet aggregation prior to filtration and a complete reversal of platelet aggregation inhibition following gel filtration, the addition of platelet poor plasma and stimulation with 2.5 μM ADP. Concurrent control studies with untreated platelets showed that they retained their response to ADP following gel filtration and addition of platelet poor plasma.

The gel filtration studies clearly demonstrate that platelet aggregation by the mesoionic thiadiazolo[3,2-*a*]pyrimidine **5b** is irreversible. It is interesting to speculate that the irreversible inhibition of platelet aggregation may be the result of acylation of the receptor or active site by the mesoionic compound. This represents the first reported irreversible inhibition of a biological response by a mesoionic compound.

Conclusion

In summary, a series of mesoionic xanthine analogues demonstrated activity as inhibitors of platelet aggregation. In these preliminary studies, the thiadiazolo[3,2-*a*]pyrimidine (i.e. **5**) series was clearly more potent than the corresponding thiazolo[3,2-*a*]pyrimidines **3**, pyrido[1,2-*a*]pyrimidines **6**, pyrimido[1,2-*a*]pyrimidine **8**, pyrimido[1,6-*a*] pyrimidine **9**, and pyrazine[1,2-*a*]pyrimidines **10**. The pyridazo[1,2-*b*]pyrimidine **7** was only a partial inhibitor at 8 mM, the highest concentration tested. Further studies are needed to completely characterize the potency of these compounds as inhibitors of platelet aggregation.

The most potent compound anhydro-(6-methyl-7-oxo-8-pentyl-5-hydroxythiadiazolo[3,2-*a*]pyrimidinium hydroxide) (**5b**) was demonstrated to be an irreversible inhibitor of platelet aggregation as demonstrated by gel filtration studies. This represents the first reported irreversible inhibition of a biological response by this series of mesoionic xanthines. The potential utility of the mesoionic compounds as irreversible inhibitors in other biological systems remains to be explored.

Experimental

^{1}H NMR spectra were recorded on a Perkin–Elmer R-24 high resolution spectrophotometer and chemical shifts were reported relative to tetramethylsilane. IR spectra were obtained on a Beckman Acculab 8 grating spectrophotometer. Elemental analysis were performed by Atlantic Microlab Inc., Norcross GA and are within 0.4% of theoretical values. Melting points were determined using a Thomas-Hoover or Mel-Temp melting point apparatus. All melting points are uncorrected. Solvents were purified using the methods reported by Gordon and Ford.[27]

Anhydro-(6,8-diethyl-3-phenyl-7-oxo-5-hydroxythiazole [3,2-*a*]pyrimidinium hydroxide) (3c). A mixture of 4-phenyl-2-(ethylamino)thiazole (0.7 g, 3.6 mmol) and bis (2,4,6-trichlorophenyl) ethylmalonate (1.8 g, 3.6 mmol) was warmed at 160 °C under a stream of nitrogen for 4 min. The cooled melt was triturated with anhydrous ether to produce a tan solid. Recrystallization from 2-propanol yielded 0.6 g (56%) of a white solid, mp 212–214 °C. IR (KBr) 3050, 2960 (C−H), 1690, 1620 (C=O), 1590 cm^{-1} (C=C, C=N). ^{1}H NMR ($CDCl_3$) δ 7.40 (s, 5H, Ph), 6.80 (s,1, thiazole H), 4.20 (q, 2, $\underline{CH_2}$-N), 2.50 (q, 2, $\underline{CH_2}$-C), 1.45 (t, 3, $\underline{CH_3}$-CH_2-N), 1.05 (t, 3, $\underline{CH_3}$-CH_2-CH_2-C). Anal. ($C_{16}H_{16}N_2O_2S$) C, H, N.

Anhydro-(6-ethyl-7-oxo-8-pentyl-5-hydroxythiazolo[3,2-*a*] pyridinium hydroxide) (3d). The procedure described for **3c** was used with 2-(pentylamino)thiazole (0.6 g, 3.6 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (1.8 g, 3.6 mmol) to give **3d**. Recrystallization from 2-propanol yielded 0.6 g (63%) as a white solid, mp 132–134 °C. IR (KBr) 2950 (C−H), 1680, 1640 (C=O), 1580 cm^{-1} (C=C, C=N). ^{1}H NMR ($CDCl_3$) δ 8.25 (d, 1, thiazole H), 7.45 (d, 1, thiazole H), 4.15 (t, 2, $\underline{CH_2}$-N), 2.55 (q, 2, CH_3-$\underline{CH_2}$-C), 2.00–0.80 (m, 12, $(\underline{CH_2})_3\underline{CH_3}$, C-$CH_2$-$\underline{CH_3}$). Anal. ($C_{13}H_{18}N_2O_2S$) C, H, N.

Anhydro-(6-ethyl-7-oxo-8-benzyl-5-hydroxythiazole[3,2-*a*] pyrimidinium hydroxide (3f). The procedure described for **3c** was used with 2-(benzylamino)thiazole (0.68 g, 3.6 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (1.8 g, 3.6 mmol) to give **3f**. Recrystallization from toluene yielded 0.58 g, (56%) of **3f** as an off-white solid, mp 144–145 °C. IR (KBr) 2990 (C−H), 1690, 1660 (C=O), 1570 cm^{-1} (C=C, C=N). ^{1}H NMR ($CDCl_3$) δ 8.10 (d, 1, thiazole H), 7.40 (s, 5, Ph), 7.10 (d, 1, thiazole H), 5.40 (s, 2, $\underline{CH_2}$-Ph), 2.65 (q, 2, $\underline{CH_2}$-CH_3), 1.20 (t, 3, CH_3). Anal. ($C_{16}H_{14}N_2O_2S$) C, H, N.

Anhydro-(6-ethyl-7-oxo-8-(2-dimethylaminoethyl)-5-hydroxythiazolo[3,2 - *a*]pyrimidinium hydroxide) HCl (3g). The procedure described for **3c** was used with 2-[2-dimethylamino)ethylamino]thiazole (0.62 g, 3.6 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (1.8 g, 3.6 mmol) to give a white solid following column chromatography (chloroform:methanol, 9:1). Recrystallization from tetrahydrofuran/hexane yielded 0.53 g of a white solid, mp 123–124 °C. To form the hydrochloride salt, the free base was dissolved in anhydrous tetrahydrofuran and HCl gas was bubbled through the solution. The white solid that formed was collected by filtration and recrystallized from methanol yielding 0.57 g (62%) of **3g** as a white solid, mp 250–251 °C. IR (free base, KBr) 2950 (C−H), 2950 (C−H), 1665, 1620 (C=O) 1565 cm^{-1} (C=C, C=N). ^{1}H NMR (free base, $CDCl_3$) δ 8.15 (d, 1, thiazole H), 7.15 (d, 1 thiazole), 4.15 (t, 2, $\underline{CH_2}$-N-$(CH_3)_2$), 2.60–2.30 (m, 8, CH_3-$\underline{CH_2}$-, $(CH_3)_2$N-), 1.15 (t, 3, CH_2-$\underline{CH_3}$). Anal. ($C_{12}H_{17}N_3O_2S$ HCl) C, H, N.

Anhydro-[3-dimethylaminomethyl)-6-ethyl-7-oxo-8-benzyl-5-hydrothiazolo[3,2-*a*]pyridinium hydroxide) HCl] (3h). The procedure described for **3c** was used with 2-(benzylamino)-4-(dimethylaminomethyl)thiazole (0.8 g, 3.6 mmol) and bis(2,4,6-trichlorophenyl) benzylmalonate (1.8 g, 3.6 mmol) to give a clear oil following column chromatography (chloroform) which solidified on standing. The solid was dissolved in anhydrous tetrahydrofuran and HCl gas was bubbled through the solution. The white solid that formed was collected by filtration and recrystallized from absolute ethanol yielding 0.62 g (52%) of a white solid, mp 251–253 °C. IR (free base, KBr) 3100, 2940 (C−H), 2950 (C−H), 1685, 1640 (C=O), 1600 cm^{-1} (C−H). ^1H NMR (free base, $CDCl_3$) δ 7.40 (s, 5, Ph), 7.10 (s, 1 thiazole H), 5.30 (s, 2, $\underline{CH_2}$-Ph), 4.20 (s, $\underline{CH_2}$-N$(CH_3)_2$), 2.80–2.30 (m, 8, CH_3-$\underline{CH_2}$-, $\underline{(CH_3)_2}$-N-), 1.20 (t, 3, $\underline{CH_3}$-CH_2). Anal. ($C_{18}H_{28}N_3O_2S$·HCl) C, H, N.

Anhydro-(6-benzyl-7-oxo-8-ethyl-5-hydrothiazolo[3,2-*a*]pyrimidinium hydroxide) (3i). The procedure described for **3c** was used with 2-(ethylamino)thiazole (0.45 g, 3.6 mmol) and bis(2,4,6-trichlorophenyl) benzylmalonate (2.0 g, 3.6 mmol) to give **3i** after column chromatography (chloroform:methanol, 9:1). Recrystallization from toluene yielded 0.39 g (38%) of **3i** mp 167–168.5 °C. IR (KBr) 3100 (C−H), 1685, 1620 (C=O), 1600 cm^{-1} (C=C, C=N). ^1H NMR ($CDCl_3$) δ 7.95 (d, 1, thiazole H), 7.50–7.10 (m, 5, Ph), 6.95 (d, 1, thiazole-H), 4.10–3.80 (m, 4, N-CH_2, Ph-CH_2), 1.25 (t, 3, CH_3). Anal. ($C_{15}H_{14}N_2O_2S$) C, H, N.

Anhdro-[6-benzyl-7-oxo-8-(2-dimethylaminoethyl)-5-hydroxythiazolo[3,2-*a*]pyrimidinium hydroxide] HCl (3j). The procedure described for **3c** was used with 2-[2-dimethylamino)ethylamino]thiazole (1.2 g, 7.2 mmol) and bis (2,4,6-trichlorophenyl) benzylmalonate (1.2 g, 7.2 mmol) to give a clear oil following column chromatography (ethyl acetate:hexane, 20:1), which solidified upon trituration with anhydrous ether. Recrystallization from 2-propanol yielded 1.3 g of a white solid, mp 123–124 °C. To form the hydrochloride salt the free base was dissolved in anhydrous tetrahydrofuran and HCl gas was bubbled through the solution. The white solid that formed was collected by filtration and recrystallized from methanol yielding 0.95 g (37%) of a white solid, mp 254–256 °C. IR (free base, KBr) 3100, 2940 (C−H), 2950 (C−H), 1680, 1640 (C=O) cm^{-1}. ^1H NMR (free base, $CDCl_3$) δ 8.00 (d, 1, thiazole H), 7.50–7.10 (m, 5, Ph), 6.90 (d, 1 thiazole), 4.10 (t, 2, $\underline{CH_2}$-CH_2-N-$(CH_3)_2$), 3.85 (s, 2, PH-$\underline{CH_2}$-), 2.70 (t, 2, CH_2-$\underline{CH_2}$-N-$(CH_3)_2$), 2.30 (s, 6, $(CH_3)_2$N-). Anal. ($C_{17}H_{19}N_3O_2S$·HCl·H_2O) C, H, N.

Anhydro-(1,3-diethyl-4-oxo-2-hydroxypyrido[1,2-*a*]pyrimidinium hydroxide) (6a). The procedure described for **3c** was used with 2-(ethylamino)pyridine (0.25 g, 2.1 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (1.1 g, 2.1 mmol) to give **6a**. Recrystallization from absolute ethanol yielded 0.24 g (52%) of a yellow solid, mp 178.5–179.5 °C. IR (KBr) 3080, 2950 (C−H), 1685, 1640 (C=O), 1555 cm^{-1} (C=C, C=N). ^1H NMR ($CDCl_3$) δ 9.50 (d, 1, pyridine H), 8.15 (t, 1, pyridine H), 7.70–7.20 (m, 2, pyridine H), 4.40 (t, 2, $\underline{CH_2}$-N), 2.70 (q, 2, $\underline{CH_2}$-C), 1.60–1.00 (m, 6, $\underline{CH_3}$-CH_2-N, $\underline{CH_3}$-CH_2-C). Anal. ($C_{11}H_{13}N_2O_2$) C, H, N.

Anhydro-(1,3-diethyl-4-oxo-7-methyl-2-hydroxypyrido[1,2-*a*]pyridinium hydroxide) (6b). The procedure described for **3c** was used with 2-(ethylamino)-5-methylpyridine (0.80 g, 5.9 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (2.9 g, 4.9 mmol) to give **6b**. Recrystallization from 2-propanol yielded 0.85 g (62%) of a green–yellow solid, mp 233–235 °C. IR (KBr) 2960 (C−H), 1690, 1620 (C=O), 1570 cm^{-1} (C=C, C=N). ^1H NMR ($CDCl_3$) δ 9.20 (s, 1, pyridine H), 8.00 (d, 1, pyridine H), 7.45 (d, 1, pyridine), 4.40 (q, 2, $\underline{CH_2}$-N), 2.80 (q, 2, $\underline{CH_2}$-C), 1.40 (t, 3, $\underline{CH_3}$-CH_2-N), 1.20 (t, 3, $\underline{CH_3}$-CH_2-C). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

Anhydro-(1-pentyl-3-ethyl-4-oxo-2-hydroypyrido[1,2-*a*]pyrimidinium hydroxide) (6c). The procedure described for **3c** was used with 2-(pentylamino)pyridine (0.60 g, 3.6 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (1.80 g, 3.6 mmol) to give a yellow oil following column chromatography (chloroform:methanol, 95:5). Trituration with anhydrous ether produced a yellow solid, which was recrystallized from 2-propanol to yield 0.45 g (48%) of **6c** as a yellow solid, mp 85–87 °C. IR (free base, KBr) 2970 (C−H), 1690, 1650 (C=O), 1555 cm^{-1} (C=C, C=N). ^1H NMR (free base, $CDCl_3$) δ 9.50 (d, 1, pyridine H), 7.80–7.30 (m, 2, pyridine H), 4.35 (t, 2, $\underline{CH_2}$-N), 2.60 (q, 2, $\underline{CH_2}$-C), 1.80–0.85 (m, 12, $\underline{CH_3}$-$\underline{(CH_2)_3}$-, $\underline{CH_3}$-CH_2-C). Anal. ($C_{15}H_{20}N_2O_2$) C, H, N.

Anhydro-(1-benzyl-3-ethyl-4-oxo-2-hydroxypyrido[1,2-*a*]pyrimidinium hydroxide) (6d). The procedure described for **3c** was used with 2-(benzylamino)pyridine (0.70 g, 3.8 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (1.90 g, 3.8 mmol) to give **6d**. Recrystallization from absolute ethanol yielded 0.70 g (70%) of a white solid, mp 181–183 °C. IR (free base, KBr) 3150, 2970 (C−H), 1700, 1650 (C=O), 1620, 1580 cm^{-1} (C=C, C=N). ^1H NMR (free base, $CDCl_3$) δ 9.50 (d, 1, pyridine H), 7.95 (t, 1, pyridine H), 7.50–7.10 (m, 6, Ph H, pyridine H), 5.55 (s, 2, $\underline{CH_2}$-Ph), 2.75 (q, 2, $\underline{CH_2}$-CH_3), 1.30 (t, 2, $\underline{CH_3}$). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

Anhydro-(1,3-diethyl-4-oxo-2-hydroxypyridazino[1,2-*b*]pyrimidinium hydroxide) (7). The procedure described for **3c** was used with 2-(ethylamino)pyridazine (0.60 g, 4.9 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (2.4 g, 4.9 mmol) to give **7**. Recrystallization from 2-propanol yielded 0.65 g (61%) of a yellow solid, mp 226–228 °C. IR (KBr) 2960 (C−H), 1660, 1630 (C=O), 1600 cm^{-1} (C=C, C=N). ^1H NMR ($CDCl_3$) δ 8.90 (d, 1, pyridazine H), 8.20–8.00 (m, 2, pyridazine H), 4.50 (q,2, $\underline{CH_2}$-N), 2.70 (q, 2, $\underline{CH_2}$-C), 1.40 (t, 3, $\underline{CH_3}$-CH_2-N), 1.15 (t, 3, $\underline{CH_3}$-CH_2-C). Anal. ($C_{11}H_{13}N_3O_2$) C, H, N.

Anhydro-(1,3-diethyl-4-oxo-2-hydroxypyrimido[1,6-*a*]pyrimidinium hydroxide) (8). The procedure described for **3c** was used with 4-(ethylamino)pyridine (0.60 g, 4.9 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (2.4 g, 4.9 mmol) to give **8**. Recrystallization from 2-propanol yielded 0.92 g (85%) of a yellow solid, mp 165–167 °C. IR (KBr) 2980 (C−H), 1690, 1640 (C=O), 1605 cm^{-1} (C=C, C=N). ^1H NMR ($CDCl_3$) δ 10.0 (s, 1, pyrimidine H), 8.20 (d, 1, pyrimidine H), 7.40 (d, 1, pyrimidine H), 4.40 (q, 2, $\underline{CH_2}$-N), 2.70 (q, 2, $\underline{CH_2}$-C),

1.40 (t, 3, $\underline{CH_3}$-CH_2-N), 1.20 (t, 3, $\underline{CH_3}$-CH_2-C). Anal. ($C_{11}H_{13}N_3O_2$) C, H, N.

Anhydro-(1,3-diethyl-4-oxo-2-hydroxypyrazino[1,2-*a*]pyrimidinium hydroxide) (9). The procedure described for **3c** was used with 2-(ethylamino)pyrazine (0.51 g, 4.2 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (2.0 g, 4.2 mmol) to give **9**. Recrystallization from 2-propanol yielded 0.59 g (64%) of an orange solid, mp 198–99.5 °C. IR (KBr) 2970 (C−H), 1676, 1645 (C=O), 1550 cm^{-1} (C=C, C=N). ^1H NMR (free base, $CDCl_3$) δ 9.30 (s imposed on d, 2, pyrazine H), 8.60 (d, 1, pyrazine H), 4.60 (q, 2, $\underline{CH_2}$-N), 2.70 (q, 2, $\underline{CH_2}$-C), 1.80 (t, 3, $\underline{CH_3}$-CH_2-N), 1.20 (t, 3, $\underline{CH_3}$-CH_2-C). Anal. ($C_{11}H_{13}N_3O_2$) C, H, N.

Anhydro-(1,3-diethyl-4-oxo-2-hydroxypyrimido[1,2-*a*]pyrimidinium hydroxide) (10a). The procedure described for **3c** was used with 2-(ethylamino)pyrimidine (0.60 g, 4.9 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (2.2 g, 4.9 mmol) to give **10a**. Recrystallization from 2-propanol yielded 0.74 g (69%) of an orange solid, mp 169–171 °C. IR (KBr) 2950 (C−H), 1700, 1690 (C=O), 1600, 1580 cm^{-1} (C=C, C=N). ^1H NMR (free base, $CDCl_3$) δ 9.70 (d, 1, pyrimidine H), 9.15 (dd, 1, pyrimidine H), 7.55 (dd, 1, pyrimidine H), 4.60 (q, 2, $\underline{CH_2}$-N), 3.70 (q, 2, $\underline{CH_2}$-C), 1.40 (t, 3, $\underline{CH_3}$-CH_2-N), 1.20 (t, 3, $\underline{CH_3}$-CH_2-C). Anal. ($C_{11}H_{13}N_3O_2$) C, H, N.

Anhydro-(3-ethyl-4-oxo-1-pentyl-2-hydroxypyrimido[1,2-*a*]-pyrimidinium hydroxide) (10b). The procedure described for **3c** was used with 2-(pentylamino)pyridine (0.69 g, 4.2 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (2.1 g, 4.2 mmol) to give **10b**. Recrystallization from ethyl acetate yielded 0.77 g (62%) of a yellow green solid, mp 114.5–115.5 °C. IR (KBr) 2960 (C−H), 1690, 1650 (C=O), 1600 cm^{-1} (C=C, C=N). ^1H NMR ($CDCl_3$) δ 9.60 (dd, 1, pyrimidine H), 9.05 (dd, 1, pyrimidine H), 7.40 (t, 1, pyrimidine H), 4.50 (q, 2, $\underline{CH_2}$-N), 2.70 (q, 2, $\underline{CH_2}$-C), 2.30–0.85 (m, 12, $\underline{(CH_2)_3CH_3}$, $\underline{CH_3}$-CH_2-C). Anal. ($C_{14}H_{19}N_3O_2$) C, H, N.

Anhydro-(1-benzyl-3-ethyl-4-oxo-2-hydroxypyrimido[1,2-*a*]pyrimidinium hydroxide) (10c). The process described for **3c** was used with 2-(benzylamino)pyridine (0.40 g, 4.2 mmol) and bis(2,4,6-trichlorophenyl) ethylmalonate (2.1 g, 4.2 mmol) to give **10c**. Recrystallization from ethyl acetate yielded 0.77 g (62%) of a orange solid, mp 120–22 °C. IR (KBr) 2960 (C−H); 1690, 1640 (C=O); 1610 cm^{-1} (C=C, C=N). ^1H NMR ($CDCl_3$) δ 9.65 (dd, 1, pyrimidine H), 9.10 (dd, 1, pyrimidine H), 7.40 (m, 6, Ph, pyrimidine H), 6.70 (s,2, $\underline{CH_2}$-Ph), 2.70 (q, 2, $\underline{CH_2}$-C), 1.15 (t, 3, CH_3). Anal. ($C_{14}H_{19}N_3O_2$) C, H, N.

Preparation of platelet suspensions

Venous blood was obtained from normal male volunteers who had not ingested any known platelet inhibitory drugs for at least 72 h. The blood (9 mL) was added to 3.9% sodium citrate (1 mL) in a plastic tube. The blood was mixed and centrifuged at 150×*g* for 20 min, and the supernatant was removed and was designated as platelet-rich plasma. The remainder of the blood was centrifuged at 2000×*g* for 20 min to obtain platelet poor plasma.

Measurement of aggregation

Aggregation was determined by the turbidometric method of Born[24] using a Bio/Data Corporation Platelet Aggregation Profiler PAP/2A. platelet-rich plasma (400 μL) and barbital buffered saline (pH 7.4, 100 μL) or baribtal buffered saline solution of test drug (pH 7.4, 100 μL) were incubated for 20 min at 37 °C. Ten microliters of a barbital buffered saline solution of ADP (the final concentration of ADP was 2.5 μL) was added to the stirred sample and the change in optical density was recorded. An initial minimum 100% inhibitory concentration was determined by finding a concentration at which the inhibitor gave a complete blockade of ADP-induced aggregation. A series of dilutions was prepared and evaluated in subsequent tests until the agent no longer gave 100% blockade (data not shown). The initial minimum 100% inhibitory concentration was the lowest concentration of agent that still produced 100% inhibition of aggregation. The actual value is bounded by the minimum 100% inhibitory concentration and the next lowest concentration employed. Values for the initial minimum 100% inhibitory concentration are within a factor of two as the next lowest concentration examined differed by a factor of two. To confirm the final minimum 100% inhibitory concentration (MIC_{100}), tests were repeated using the minimum 100% inhibitory concentration and the next lowest concentration. The MIC_{100} values reported in Tables 1 and 2 represent the smaller of the two concentrations used to produce the effect.

Gel filtration-column preparation

A cylinder of a 50 mL plastic syringe (Becton-Dickson) with a 3.0 cm internal diameter was used as a chromatography column. Sepharose 2B (Pharmacia Fine Chemicals) was prepared and stored in calcium-free Tyrodes buffer (267). The column was prepared by inserting a porous plastic sleeve (Fenwal Lab). The gel slurry was added, allowed to pack to the 50 mL mark on the syringe and stored at 4 °C between uses. Prior to use the column was allowed to warm to room temperature and to equilibrate with modified calcium-free Tyrodes buffer which was used as an elution buffer (267).

Gel filtration of platelets

Platelet-rich plasma (6.5 mL) was treated with either barbital buffered saline (pH 7.4, 0.5 mL) or drug dissolved in barbital buffered saline (0.5 mL). The treated platelet-rich plasma was allowed to incubate for 30 min at room temperature. After the incubation period, duplicate samples of platelet-rich plasma (400 μL of platelet-rich plasma, 100 μL of platelet poor plasma) and treated platelet-rich plasma (400 μL of platelet-rich plasma, 100 μL of platelet poor plasma) were tested for platelet aggregation stimulated by 2.5 μM ADP. The remaining treated plasma was gel-filtered. The gel filtered platelets were collected in 3 mL fractions in plastic tubes. The appearance of gel-filtered platelets was detected visually and platelet counts were done to insure that the platelet count was within the bounds required by the platelet aggregation profiler. Duplicate samples of gel-filtered

(400 μL of platelet-rich plasma, 100 μL of platelet poor plasma) and the corresponding controls (400 μL of platelet-rich plasma, 100 μL of platelet poor plasma) were tested for platelet aggregation when stimulated by 2.5 μM ADP. The study was performed in duplicate.

References and Notes

1. White, H. D. *Am. Heart J.* **1999**, *138*, S570.
2. Markwardt, F.; Hoffman, A. *Biochem. Pharmacol.* **1970**, *19*, 2519.
3. Varagaftig, B. B.; Chignard, M.; Benveniste, J. *Biochem. Pharmacol.* **1981**, *30*, 263.
4. Holmsen, H. *Ser. Haematol* **1975**, *8*, 50.
5. Glennon, R. A.; Rogers, M. E.; Bass, R. G.; Ryan, S. B. *J. Pharm. Sci.* **1978**, *67*, 1762.
6. Rogers, M. E.; Glennon, R. A.; Smith, J. D.; Boots, M. R.; Nanavati, N.; Maconaughey, J. E.; Aud, D.; Mbagwu, G. *J. Med. Chem.* **1981**, *24*, 1284.
7. Glennon, R. A.; Rogers, M. E.; Smith, J. D.; El-Said, M. K.; Egle, J. L. *J. Med. Chem.* **1981**, *24*, 658.
8. Glennon, R. A.; Gaines, J. J.; Rogers, M. E. *J. Med. Chem.* **1981**, *24*, 766.
9. Mbagwu, G. O.; Bass, R. G.; Glennon, R. A. *J. Heterocycl. Chem.* **1985**, *22*, 465.
10. Coburn, R. A.; Glennon, R. A. *J. Heterocycl. Chem.* **1973**, *10*, 487.
11. Coburn, R. A.; Taylor, M. D.; Wright, W. L. *J. Pharm. Sci.* **1981**, *70*, 1322.
12. Coburn, R. A.; Bhooshan, B. *J. Org. Chem.* **1975**, *38*, 3868.
13. Flethcher, C.; Pearson, C.; Choi, S. C.; Duma, R. J.; Evans, H. J.; Qureshi, G. D. *J. Lab. Clin. Med.* **1986**, *108*, 217.
14. Stubbins, J. F.; Pearson, C.; Qureshi, G. D. *Thrombosis Res.* **1988**, *51*, 417.
15. Glennon, R. A.; Rogers, M. E.; El-Said, M. K. *J. Heterocycl. Chem.* **1980**, *17*, 337.
16. Coburn, R. A.; Glennon, R. A.; Chmielewicz, Z. *J. Med. Chem.* **1974**, *17*, 1025.
17. Glennon, R. A.; Bass, R. G.; Schubert, E. *J. Heterocycl. Chem.* **1976**, *16*, 903.
18. Kappe, T. *Monatsch. Chem.* **1967**, *98*, 874.
19. Kappe, T.; Lube, W. *Monatsch. Chem.* **1971**, *102*, 781.
20. Kemal, O.; Reese, C. B. *J. Chem. Soc. Perkins Trans. 1* **1981**, 1569.
21. Steck, E. A.; Fletcher, L. T. *J. Heterocycl. Chem.* **1974**, *11*, 1077.
22. Brown, D. J. *The Pyrimidines*; Interscience: NY, 1962, p 162.
23. Silberg, A.; Frenkel, A. *Bull. Chem. Soc. Fr* **1967**, *6*, 2235.
24. Born, G. V. R. *Nature* **1962**, *194*, 927.
25. Puri, R. N. *Biochem. Pharmacol.* **1999**, *57*, 851.
26. Tangen, O.; Berman, H. J.; Marfay, P. *Thrombosis. Diathes. Haemorrh* **1971**, *21*, 268–278.
27. Gordon, A. J., Ford, R. A. *The Chemist's Companion*, Wiley-Interscience: NY, 1972.