

Antiplatelet Agents Based on Cyclooxygenase Inhibition without Ulcerogenesis. Evaluation and Synthesis of 4,5-Bis(4-methoxyphenyl)-2-substituted-thiazoles

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The syntheses, biological evaluations, and structure-activity relationships of a series of 4,5-bis(4-methoxyphenyl)-2-substituted-thiazoles as potent antiplatelet agents with vasodilatory activity are described. 2-Guanidino-4,5-bis(4-methoxyphenyl)thiazole (**3**), designed from two parent compounds (itazigrel and timegadine), showed inhibitory activity of malondialdehyde (MDA, $IC_{50} = 31 \mu M$) production which is formed from the cyclooxygenase (CO)-catalyzed oxygenation of arachidonic acid in the synthesis of prostanoids in platelets, with vasodilatory activity ($ED_{50} = 2.0 \mu M$). Further structure-activity relationship studies on **3** culminated in the preparation of 4,5-bis(4-methoxyphenyl)-2-[(1-methylpiperazin-4-yl)carbonyl]thiazole (**10a**, FR122047) which exhibited potent inhibitory activity on MDA synthesis in vitro ($IC_{50} = 0.088 \mu M$) and platelet aggregation in guinea pigs *ex vivo* (100% inhibition even 6 h after 1.0 mg/kg administration) with vasodilatory activity in vitro ($ED_{50} = 6.2 \mu M$). Moreover, **10a** demonstrated no ulcerogenesis effect in rats even at 100 mg/kg dosage (safety margin in rats is more than 70 while that of aspirin is only 1.2) in spite of its potent CO inhibition ($IC_{50} = 0.43 \mu M^{14}$), while the use of aspirin, a CO inhibitor and the most popular thromboembolic drug, is restricted by the side effect.⁸ Pharmacokinetic studies on **10a** have revealed that **10a** is detectable in platelet-rich plasma but not in platelet-poor plasma 1 day after oral administration, which indicates that **10a** tends to be localized in platelets. This property could be responsible for its low toxicity and reduction of side effects in clinical studies.

Introduction

Medicinal research has significantly advanced clinical treatment of thromboembolic diseases. Nevertheless, such diseases still remain the leading cause of human morbidity and mortality.¹ Many compounds based on specific mediators such as thromboxane A_2 (TXA₂),² prostacyclin,³ phosphodiesterase (PDE),⁴ and thrombin,⁵ have been synthesized and tested in clinical trials to verify their effectiveness in the treatment of thromboembolic diseases.

The most popular thromboembolic drug, aspirin, which inhibits cyclooxygenase irreversibly,⁶ has shown its effectiveness in clinical trials.⁷ Aspirin, however, prevents not only the synthesis of TXA₂ in platelets but also that of prostaglandin I₂ in vascular endothelial cells. As a result, it induces stomach ulcers ("aspirin dilemma"),⁸ restricting its clinical use. Development of a new aspirin-like compound, based on cyclooxygenase inhibition and free from the side effects, is a major goal of thromboembolic research. To accomplish this purpose, some compounds which inhibit cyclooxygenase reversibly, such as itazigrel,⁹ KBT-3022,¹⁰ and E-5510¹¹ have been synthesized and clinically tested (other activities of these compounds have been also reported. For example, phospholipase C and/or A₂ are inhibited by E-5510¹¹).

We have reported¹² a new type of antiplatelet agent, 5-alkyl-2-(substituted aryl)-4-pyridylimidazoles, which exhibited potent antiplatelet activity with vasodilation activity without the gastric side effect. We think that vasodilatory activity could be beneficial in treatment of thromboembolic diseases and are continuing our studies aimed at the development of potent aspirin-like antiplatelet agents with vasodilatory activities without gastrointestinal side effect.

Structural analyses of itazigrel, E-5510, and KBT-3022

have shown that the bis(4-methoxyphenyl) moiety is essential for potent cyclooxygenase inhibition (Figure 1). We have also observed that a guanidino derivative, timegadine,¹³ shows antiplatelet and vasodilatory properties (Table 1). On the basis of this information, we synthesized a fused compound, 2-guanidino-4,5-bis(4-methoxyphenyl)thiazole (**3**, Figure 1). Compound **3**, our lead compound in this study, showed the desired properties: inhibitory activity of MDA production ($IC_{50} = 31 \mu M$) and vasodilatory activity ($ED_{50} = 2.0 \mu M$).

We describe here the syntheses and structure-activity relationships of 4,5-bis(4-methoxyphenyl)-2-substituted-thiazoles and their pharmacological effects on platelet aggregation and vasodilation. Further pharmacological results of the most potent derivative, 4,5-bis(4-methoxyphenyl)-2-[(1-methylpiperazin-4-yl)carbonyl]thiazole (**10a**, FR122047¹⁴), are also described.

Chemistry

4,5-Bis(4-methoxyphenyl)-2-(substituted guanidino)-thiazoles (**3**, **7a-i**) were prepared as shown in Scheme 1. Conversion of the hydroxy group of anisoin (**1**) into a chloro group by thionyl chloride gave 2-chloro-1,2-bis(4-methoxyphenyl)-1-ethanone (**2**). Condensation of **2** with guanidinothioamide or thiourea provided 2-guanidino- (**3**) or 2-amino-4,5-bis(4-methoxyphenyl)thiazole (**4**), respectively. The synthetic approach to N-substituted-guanidino derivatives (**7a-e,g-i**) was to convert **4** into 4,5-bis(4-methoxyphenyl)-2-thioureidothiazole (**5a**) via 2-(3-benzoylthioureido)-4,5-bis(4-methoxyphenyl)thiazole (**6**), followed by methylation of the thiourea moiety with methyl iodide and subsequently substitution of the methylthio group with corresponding amines. Condensation of **4** with methyl isothiocyanate afforded 4,5-bis(4-methoxyphenyl)-3-(methylthioureido)thiazole (**5b**), which was converted into N,N'-dimethylguanidino derivative (**7f**).

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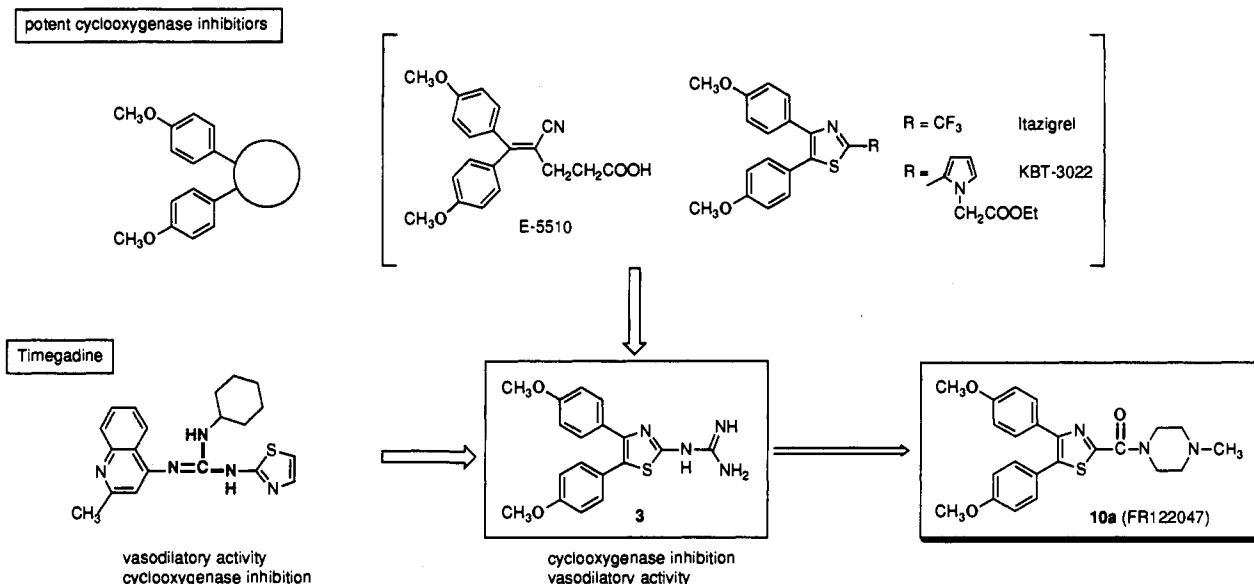


Figure 1. Design of **3** from two kind of parent compounds and evolution of 4,5-bis(4-methoxyphenyl)-2-[(4-methylpiperazin-1-yl)-carbonyl]thiazole (**10a**, FR122047).

Table 1. Product Characterization and in Vitro Activity: 4,5-Bis(4-methoxyphenyl)-2-(substituted guanidino)thiazole Derivatives

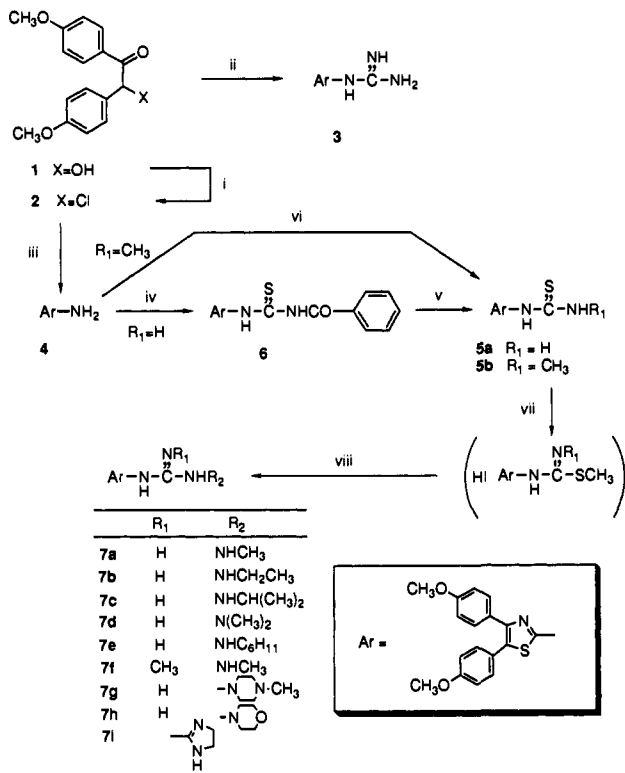
compd	R ₁	R ₂	R ₃	yield, %	mp, °C	formula	anal. ^a	antiplatelet ^b MDA ^c IC ₅₀ , μM	vasodilation ^b rat aorta, in vitro ED ₅₀ , μM
3	H	H	H	46.2	167–8	C ₁₈ H ₁₈ N ₄ O ₂ S	C, H, N	31	2.0
7a	H	H	CH ₃	58.1	155–6	C ₁₉ H ₂₀ N ₄ O ₂ S	C, H, N	0.062	1.2
7b	H	H	Et	59.4	183–5	C ₂₀ H ₂₂ N ₄ O ₂ S	C, H, N	>0.1	NT ^d
7c	H	H	<i>i</i> -Pr	84.5	195–7	C ₂₁ H ₂₄ N ₄ O ₂ S·CH ₃ SO ₃ H	C, H, N	>0.1	9.1
7d	H	CH ₃	CH ₃	30.3	233–5	C ₂₀ H ₂₂ N ₄ O ₂ S·HCl	C, H, N	0.037	4.8
7e	H	H	<i>c</i> -hex	74.5	162–4	C ₂₄ H ₂₈ N ₄ O ₂ S	C, H, N	>1.0	>100
7f	CH ₃	H	CH ₃	32.1	231–3	C ₁₈ H ₁₈ N ₄ O ₂ S·CH ₃ SO ₃ H	C, H, N	0.97	2.4
7g	H	<i>N</i> -Me-piperidine		21.9	161–2	C ₂₃ H ₂₇ N ₆ O ₂ S	C, H, N	>1.0	21
7h	H	morpholine		50.9	172–3	C ₂₂ H ₂₄ N ₄ O ₃ S	C, H, N	<1.0 ^e	66
7i	H	imidazoline		44.9	211–4	C ₂₀ H ₂₀ N ₄ O ₂ S	C, H, N	>0.1	NT
aspirin								16	>600
itazigrel								0.0056	>300
timegadin								0.031	2.5

^a Analytical results were within ±0.4% of the theoretical value. ^b The evaluation methods are described in Experimental Section. ^c MDA: inhibitory activity of malondialdehyde production induced by AA. ^d NT: not tested. ^e Inhibitory activity of **7h** on the MDA production at 1 μM was 79.9%.

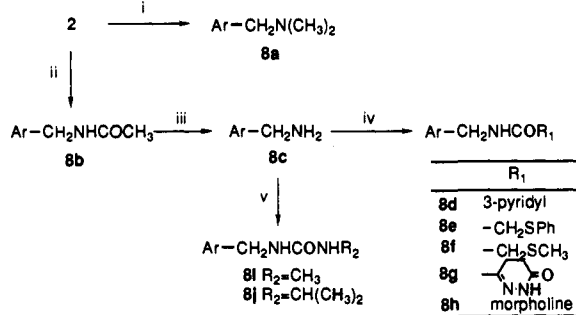
The synthesis of 2-((substituted amino)methyl)thiazoles (**8a–j**) is shown in Scheme 2. Condensation of **2** with ((dimethylamino)methyl)thioamide or ((acetylamino)-methyl)thioamide provided 4,5-bis(4-methoxyphenyl)-2-((dimethylamino)methyl)thiazole (**8a**) or 2-((acetylamino)-methyl)-4,5-bis(4-methoxyphenyl)thiazole (**8b**), respectively. Hydrolysis of **8b** with 35% HCl provided 2-(aminomethyl)thiazole (**8c**). Condensations of **8c** with acyl chlorides, carboxylic acids, or isocyanates gave 2-((acylamino)methyl)- (**8d–h**) and 2-ureidothiazoles (**8i–j**), respectively.

2-(Substituted amido)thiazoles (**10a–m**) were synthesized as shown in Scheme 3. Condensation of (ethoxycarbonyl)thiourea with **2** provided 2-(ethoxycarbonyl)-4,5-bis(4-methoxyphenyl)thiazole (**9**), an oily compound, which was used for the next reactions without purification. Aminolysis of **9** with various amines gave 2-(substituted amido)thiazoles (**10a–l**). Reaction of **10g** with isopropyl isocyanate afforded 2-[4-((isopropylamino)carbonyl)piperazin-1-yl]thiazole (**10m**).

Reduction of the ester **9** with LiAlH₄ and subsequent oxidation with activated MnO₂ gave 2-formyl-4,5-bis(4-methoxyphenyl)thiazole (**11**) (Scheme 4). The Knoevenagel condensation of **11** with ethyl cyanoacetate afforded ethyl 2-cyano-3-[4,5-bis(4-methoxyphenyl)thiazolyl]propenoate (**12**). The Knoevenagel condensation of **11** with ethyl acetoacetate and subsequent condensation with ethyl 3-aminocrotonate provided 2-(1,4-dihydro-3,5-bis(ethoxycarbonyl)-2,6-dimethylpyridin-4-yl)-4,5-bis(4-methoxyphenyl)thiazole (**13**). The Wittig reaction of **11** with ethyl (triphenylphosphoranylidene)acetate gave ethyl 3-[4,5-bis(4-methoxyphenyl)thiazolyl]-(*E*)-propenoate (**14**). The configuration of the ethenyl moiety was determined by coupling constants from ¹H-NMR analysis. Hydrolysis of **14** gave 3-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]-(*E*)-propenoic acid (**15**), which was coupled with *N*-methylpiperazine to yield 3-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]propenoyl-*N*-methylpiperazine (**16**). Catalytic hydrogenation of **15** over 10% Pd-C at atmospheric pressure afforded 3-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]pro-

Scheme 1^a

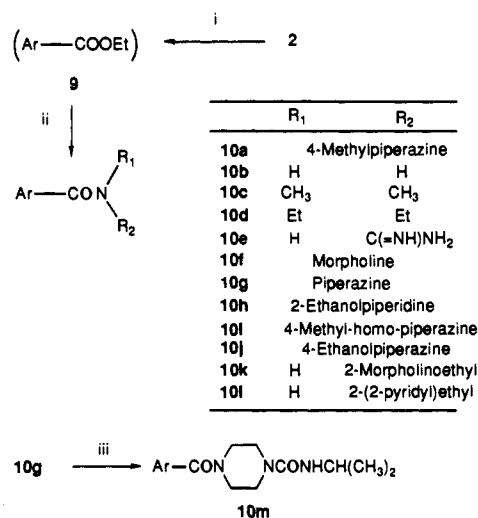
^a Reagents: (i) SOCl₂, rt, 1 h; (ii) H₂NC(=S)NHC(=NH)NH₂/EtOH, reflux, 2 h; (iii) H₂NC(=S)NH₂/CH₃CN, reflux, 3 h; (iv) PhCONCS, 0 °C, 1 h; (v) NaOH/MeOH, 60 °C, 2 h; (vi) CH₃NCS/DMF, 90 °C, 4 h; (vii) CH₃I/MeOH, CHCl₃, reflux, 1 h; (viii) R₂NH₂/EtOH, reflux.

Scheme 2^{a,b}

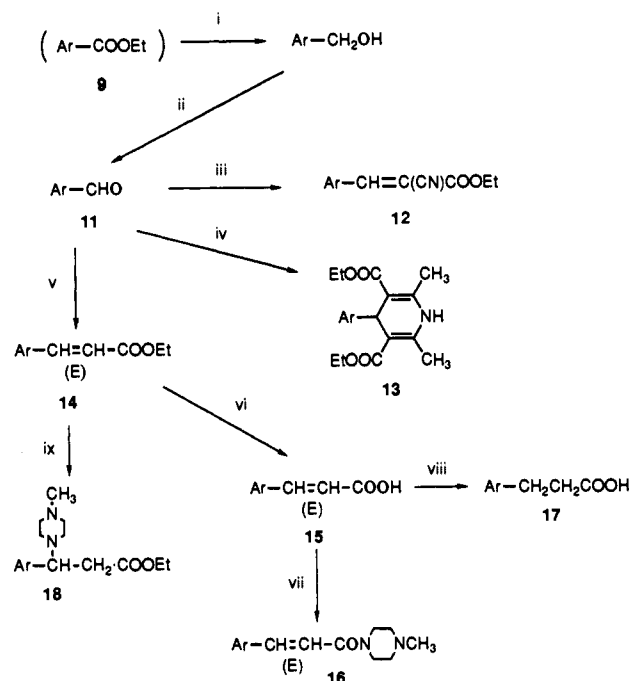
^a Reagents: (i) H₂NC(=S)CH₂N(CH₃)₂/EtOH, reflux, 3 h; (ii) H₂NC(=S)CH₂NHCOCH₃ in EtOH, reflux, 1 h; (iii) 35% HCl, rt, 0.5 h; (iv) (a) R₁COOH, EDC/DMF, rt, 3 h (for 8e-g), (b) R₁COCl, NEt₃/CH₂Cl₂, reflux, 1 h (for 8d and 8h); (v) R₂NCO/THF, MeOH, rt, 1.5 h. ^b Ar = 4,5-bis(4-methoxyphenyl)thiazol-2-yl.

panoic acid (17). Reaction of 14 with *N*-methylpiperazine provided ethyl 3-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]-3-(4-methylpiperazin-1-yl)propionate (18).

The synthesis of other derivatives (19–22) is shown in Scheme 5. 2,3,4,5-Tetrahydro-6-thiocarbamoyl-3-pyridazinone was condensed with 2 to give 4,5-bis(4-methoxyphenyl)-2-(2,3,4,5-tetrahydro-3-oxopyridazin-6-yl)thiazole (19). Reaction of 4-thiocarbamoylpyridine with 2 afforded 2-(4-pyridyl)thiazole (20). *N*-Methylation of 20 by methyl iodide and subsequent reduction with NaBH₄ provided 4,5-bis(4-methoxyphenyl)-2-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)thiazole (21). Reaction of 2-chloro-4,5-bis(4-methoxyphenyl)thiazole with *N*-methylpiperazine afforded the 2-(4-methylpiperazin-1-yl)thiazole derivative (22).

Scheme 3^{a,b}

^a Reagents: (i) EtOCOSNH₂/EtOH, reflux, 4 h; (ii) aminolysis by HNR₁R₂; (iii) *i*-C₃H₇-NCO/THF, MeOH, rt, 2 h. ^b Ar = 4,5-bis(4-methoxyphenyl)thiazol-2-yl.

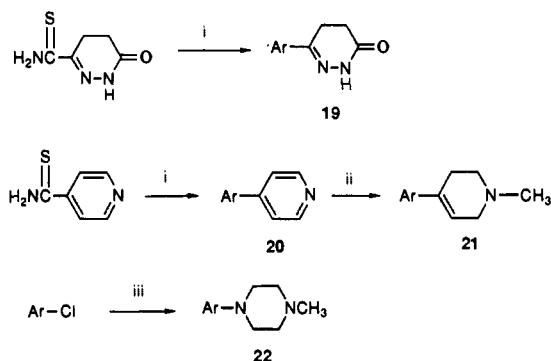
Scheme 4^{a,b}

^a Reagents: (i) LiAlH₄/THF, 0 °C, 30 min; (ii) MnO₂/AcOEt, rt, 5.5 h; (iii) NCCH₂COOEt, AcOH, AcONH₄/C₆H₆, reflux, 7.5 h; (iv) (a) CH₃COCH₂COOEt, AcOH, morpholine/C₆H₆, reflux, (b) H₂N-(H₃C)CCHCOOEt/EtOH, reflux, overnight; (v) (C₆H₅)₃P=CHCOOEt/CH₂Cl₂, reflux, 4 h; (vi) 0.1 N NaOH, reflux, overnight; (vii) Me-piperazine, EDC, NEt₃/DMF, rt, 5 h; (viii) H₂/Pd(OH)₂, rt, 1 atm, 4 h; (ix) Me-piperazine, 80–90 °C, 6 h. ^b Ar = 4,5-bis(4-methoxyphenyl)thiazol-2-yl.

Biological Results and Discussion

Inhibitory activities on malondialdehyde (MDA) synthesis and KCl-induced contraction were measured to evaluate the cyclooxygenase (CO) inhibition and vasodilation activity of the novel thiazoles in this study. MDA is formed from the CO-catalyzed oxygenation of arachidonic acid (AA) in the synthesis of prostanoids so that inhibitory activity on MDA synthesis is considered to be consistent with CO inhibition activity. The reason for evaluation of vasodilation activity in this study is that we consider that it may be beneficial in the treatment of

Scheme 5a,b



^a Reagents: (i) 2/EtOH, reflux, 2 h; (ii) (a) CH₃I, rt, 10 h, (b) NaBH₄/MeOH, rt, 1 h; (iii) *N*-methylpiperazine, 80–90 °C, 6 h.^b Ar = 4,5-bis(4-methoxyphenyl)thiazol-2-yl.

thrombotic disease. Inhibitors active in both tests, at or below 0.1 μM on the MDA and 10 μM on the vasodilation assay, were tested for their *ex vivo* abilities to prevent platelet aggregation induced by AA or collagen 1 or 6 h after oral administration (3.2 and/or 1.0 mg/kg) in guinea pigs.

2-(Substituted guanidino)-4,5-bis(4-methoxyphenyl)thiazoles. Aspirin and itazigrel, CO inhibitors, showed inhibition of MDA synthesis but no effect in the vasodilation assay (Table 1). Timegadine, one of our reference compounds, showed inhibition of MDA production (IC₅₀ = 0.031 μM) and vasodilation activities (ED₅₀ = 2.5 μM). *Ex vivo* potency of timegadine, however, is much less than that of itazigrel (Table 5).

Fusion of these two kinds of compounds (Figure 1) has been attempted to obtain a potent platelet aggregation inhibition based on CO inhibition with vasodilatory activity and provided 2-guanidino-4,5-bis(4-methoxyphenyl)thiazole (3). Compound 3 showed inhibitory activity of MDA synthesis (IC₅₀ = 31 μM) with vasodilatory activity (ED₅₀ = 2.0 μM) as expected. The vasodilatory activity of 3 was equipotent to that of timegadine, but the MDA-inhibitory activity was much weaker than that of itazigrel (IC₅₀ = 0.0056 μM). Our initial efforts focused on increasing the potency of MDA-inhibition activity while retaining the vasodilation activity.

Modification of guanidino moiety of 3 (7a–i) was carried out (Table 1). Introduction of a methyl group onto the guanidino moiety provided 7a (IC₅₀ = 0.062 μM) and 7d (IC₅₀ = 0.037 μM) with an increase of the MDA-inhibition activity of 500 and 1000 times, respectively, compared with 3. Compounds 7a and 7d also showed comparable vasodilatory activity to 3. The *N,N'*-dimethylated guanidine (7f) was less potent than the singly methylated derivative (7a). Substitution with morpholine (7h) showed potent inhibition of MDA synthesis (79.9% inhibition at 1.0 μM) with weak vasodilation activity (ED₅₀ = 66 μM).

Compounds 7a and 7d were subsequent evaluated *ex vivo* (Table 5) since they were active in the two tests. Compound 7a inhibited platelet aggregation induced by AA 1 h after oral administration of 1.0 mg/kg. Compound 7a, however, did not completely prevent collagen-induced aggregation at the same dose while itazigrel did. Compound 7d was slightly more potent than 7a. These studies showed that the novel thiazoles were much stronger than aspirin and timegadine in an *ex vivo* study, which indicated that these thiazoles were suitable as lead compounds for further study. Our next efforts focused on increasing *ex*

vivo potency while retaining vasodilatory activity since their *ex vivo* activities were much less potent than that of itazigrel. In order to accomplish this purpose, various 4,5-bis(4-methoxyphenyl)-2-substituted-thiazoles were synthesized and tested.

2-((Substituted amino)methyl)thiazoles. 2-((Substituted amino)methyl)-4,5-bis(4-methoxyphenyl)thiazoles (8a–j) were synthesized and tested, and their results are shown in Table 2.

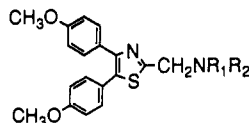
2-(Aminomethyl)-4,5-bis(4-methoxyphenyl)thiazole (8c) showed vasodilation activity (ED₅₀ = 7.7 μM) with little inhibition of MDA synthesis (IC₅₀ > 0.1 μM). Dimethylation of the amino moiety (8a) increased the inhibitory activity on MDA synthesis (IC₅₀ = 0.096 μM) while retaining vasodilatory activity (ED₅₀ = 8.4 μM). Since acetylation of 8c produced potent MDA synthesis inhibition (IC₅₀ = 0.015 μM) while retaining vasodilatory activity (ED₅₀ = 8.1 μM), several (substituted amino)-methyl derivatives (8d–j) were tested. 2,3,4,5-Tetrahydro-3-pyridazinone ring (8g), the nucleus of potent phosphodiesterase inhibitors,¹⁵ was introduced onto the amino group in an attempt to increase vasodilation activity. Compound 8g, however, showed only similar vasodilation activity (ED₅₀ = 7.3 μM) to that of 8c. Substitution of the 2,3,4,5-tetrahydro-3-pyridazinone ring with 3-pyridyl (8d), phenyl thioether (8e), and methyl thioether (8f) also exhibited potent anti-MDA synthesis activity while retaining vasodilation activity. 4,5-Bis(4-methoxyphenyl)-2-((substituted ureido)methyl)thiazoles (8h–j) were also synthesized and tested. Among them, *N*-(isopropylureido) derivative (8j) showed potent activities in both assays (MDA, IC₅₀ = 0.022 μM; vasodilation, ED₅₀ = 5.7 μM).

Active compounds from the above aminomethyl derivatives, 8a–b,d–g,j, were then assessed in an *ex vivo* study alongside itazigrel (Table 5). Among them, 8g and 8j exhibited potent *ex vivo* activity, that is, complete inhibition of platelet aggregation for 6 h at a low dose (1.0 mg/kg). These *ex vivo* potencies are much higher than that of the guanidino derivatives and equal to that of itazigrel. Further pharmacological studies on these compounds are now underway.

2-(Substituted amido)thiazoles. 2-(Substituted amido)thiazoles (10a–m, Table 3) and derivatives (13, 21–22, Table 4) were also synthesized and evaluated.

N,N-Dialkylamido derivatives (10c, 10d) showed potent inhibition of MDA synthesis (IC₅₀ < 0.1 μM and IC₅₀ < 0.01 μM, respectively) while that of the unsubstituted amido derivative (10b) was very weak (IC₅₀ > 1 μM). Compound 10d, however, was precluded from further studies due to its weak vasodilatory activity (ED₅₀ = 14 μM). Since compounds with a basic functional group tend to show vasodilation activity,¹² introduction of a basic group onto the amido moiety (10a, FR122047) was carried out. Compound 10a exhibited potent MDA-synthesis inhibition (IC₅₀ = 0.088 μM) with vasodilation activity (ED₅₀ = 6.2 μM); therefore, derivatives of 10a (10f–m, 13, 21–22) were prepared. Replacement of the *N*-methylpiperazine of 10a by morpholine (10f) gave much stronger inhibitory activity of MDA synthesis (IC₅₀ < 0.01 μM) while diminishing the vasodilation effect (ED₅₀ > 100 μM). (2-Hydroxyethyl)piperidine derivative (10h) showed inhibitory activity of MDA synthesis (IC₅₀ = 0.045 μM) with vasodilation activity (ED₅₀ = 6.8 μM) while other modifications of the *N*-methylpiperazine moiety (10g,i–k) resulted in decreasing anti-MDA production activity.

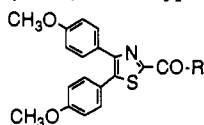
Table 2. Product Characterization and in Vitro Activity: 4,5-Bis(4-methoxyphenyl)-2-((substituted aminomethyl)thiazole Derivatives



compd	R ₁	R ₂	yield, %	mp, °C	formula	anal. ^a	antiplatelet ^b MDA ^c IC ₅₀ , μM	vasodilation ^b rat aorta, in vitro ED ₅₀ , μM
8a	CH ₃	CH ₃	12.3	204-6	C ₂₀ H ₂₂ N ₂ O ₂ S·HCl	C, H, N	0.096	8.4
8b	COCH ₃	H	33.2	138-41	C ₂₀ H ₂₀ N ₂ O ₃ S	C, H, N	0.015	8.1
8c	H	H	86.3	146-8	C ₁₈ H ₁₈ N ₂ O ₂ S·HCl	C, H, N, S	>0.1	7.7
8d	CO-3-C ₆ H ₄ N	H	49.7	135-44	C ₂₄ H ₂₁ N ₄ O ₂ S·HCl	C, H, N	0.025	8.2
8e	COCH ₂ S-C ₆ H ₅	H	56.6	132-3	C ₂₆ H ₂₄ N ₂ O ₃ S ₂	C, H, N, S	<0.1 ^d	4.2
8f	COCH ₂ SCH ₃	H	28.1	74-5	C ₂₁ H ₂₂ N ₂ O ₃ S	C, H, N, S	<0.1 ^d	6.0
8g		H	40.1	174-5	C ₂₃ H ₂₂ N ₄ O ₄ S	C, H, N	0.055	7.3
8h	CO-morpholino	H	82.4	111-6	C ₂₃ H ₂₆ N ₃ O ₄ S·H ₂ O	C, H, N, S	0.66	12
8i	CONHCH ₃	H	84.1	115-8	C ₂₀ H ₂₁ N ₃ O ₃ S	C, H, N, S	0.022	15
8j	CONH- <i>i</i> -C ₃ H ₇	H	91.6	146-9	C ₂₂ H ₂₆ N ₃ O ₃ S	C, H, N, S	0.042	5.7

^{a-c} See Table 1. ^d Inhibitory activities of 8e and 8f on the MDA production at 0.1 μM were 73.6 and 73.3%, respectively.

Table 3. Product Characterization and in Vitro Activity: 4,5-Bis(4-methoxyphenyl)-2-(substituted amido)thiazole Derivatives



compd	R	yield, %	mp, °C	formula	anal. ^a	antiplatelet ^b MDA ^c IC ₅₀ , μM	vasodilation ^b rat aorta, in vitro ED ₅₀ , μM
10a (FR122047)	4-CH ₃ -piperazine	46.5	254-6	C ₂₃ H ₂₆ N ₃ O ₃ S·HCl	C, H, N	0.088	6.2
10b	NH ₂	28.9	syrup	C ₁₈ H ₁₆ N ₂ O ₃ S	C, H, N	>1	NT ^d
10c	N(CH ₃) ₂	24.1	syrup	C ₂₀ H ₂₀ N ₂ O ₃ S	C, H, N ^e	<0.1 ^f	3.0
10d	NEt ₂	8.3	syrup	C ₂₂ H ₂₄ N ₂ O ₃ S	C, H, N	<0.01 ^f	14
10e	NHC(NH)NH ₂	56.0	253-5	C ₁₉ H ₁₈ N ₄ O ₃ S	C, H, N	>0.1	>100
10f	morpholine	25.2	118-22	C ₂₂ H ₂₂ N ₂ O ₄ S	C, H, N	<0.01 ^f	>100
10g	piperidine	22.6	109-14	C ₂₂ H ₂₃ N ₃ O ₃ S·HCl·H ₂ O	C, H, N	>1	11
10h	2-ethanolpiperidine	42.1	syrup	C ₂₅ H ₂₈ N ₂ O ₄ S	C, H, N	0.045	6.8
10i	4-CH ₃ -homopiperazine	74.0	106-16	C ₂₄ H ₂₇ N ₃ O ₃ S·HCl·2H ₂ O	C, H, N	>0.1	4.3
10j	4-(2-hydroxyethyl)piperazine	20.6	198-201	C ₂₄ H ₂₇ N ₃ O ₄ S·HCl·1/5H ₂ O	C, H, N	>1	9.5
10k	NHCH ₂ CH ₂ -morpholine	58.1	249-51	C ₂₄ H ₂₇ N ₃ O ₄ S·HCl·1/2H ₂ O	C, H, N	>1	8.7
10l	NHCH ₂ CH ₂ -3-pyridyl	29.1	125-7	C ₂₅ H ₂₃ N ₃ O ₃ S	C < H, N	<0.1 ^f	20
10m	-piperazino-CONH- <i>i</i> -Pr	65.0	157-9	C ₂₆ H ₃₀ N ₄ O ₄ S·1/5H ₂ O	C, H, N	>1	15

^{a-d} See Table 1. ^e C: calcd, 65.20; found, 65.76. ^f Inhibitory activities of 10c, 10d, 10f, and 10l on the MDA production at 0.1 μM (for 10c, 10l) or 0.01 μM (for 10d, 10f) were 51.4, 65.0, 74.8, and 56.6%, respectively.

Deletion of the carbonyl moiety of 10a (22) and its derivative (21) resulted in similar potencies in the MDA and vasodilation assays, compared to those of 10a. Introduction of a 1,4-dihydropyridine ring, the nucleus of the Ca²⁺ antagonists,¹⁶ to the 2-position of the thiazole ring in an attempt to increase vasodilatory activity provided 13 with disappointing poor vasodilation activity (ED₅₀ > 3.2 μM).

The active amido derivatives in the both tests (10a, c, h, 21, 22) were subjected to ex vivo evaluation (Table 5) to assess their in vivo potency. Compound 10h inhibited platelet aggregation, even 6 h after oral administration of 3.2 mg/kg. Compound 10h, however, was excluded from additional studies due to its physical form (oil). Compound 10a demonstrated potent ex vivo potency, that is, 10a completely prevented the platelet aggregation induced by AA and showed 83.1% (79% for itazigrel) inhibition induced by collagen after 6 h at 1.0 mg/kg. These results exhibited that 10a has potent and long-lasting antiplatelet activity that is much more potent than that of aspirin and timegadin and a little more potent than that of itazigrel. Other amido derivatives (10c, 21-22) were less potent than

10a in ex vivo studies. Therefore, 10a was subjected to further pharmacological tests as shown below.

Other 4,5-Bis(4-methoxyphenyl)thiazoles. Some compounds with a carboxylic acid or ester at the 2-position of the thiazole ring (12, 14, 15, 17, and 18) were synthesized to compare with the above nonacidic compounds since CO inhibitors are often classified into acidic compounds, for example, aspirin, indomethacin, E-5510, and KBT-3022. The results are shown in Table 4. The acidic compounds showed potent MDA-production inhibition and little vasodilation activity. Consequently, these compounds were excluded from our further studies.

Pharmacokinetic Studies on 10a (FR122047). Because 10a showed potent and long-lasting antiplatelet activity as shown above, pharmacokinetic studies were carried out in guinea pigs to determine the reason for its long duration of activity.

Interestingly, 10a remains in PRP for more than 48 h, but it is almost undetectable after 6 h and completely undetectable after 24 and 48 h in PPP as shown in Figure 2. The ex vivo activity of 10a correlated with the concentration in PRP but not that in PPP. These results

Table 4. Product Characterization and in Vitro Activity: 4,5-Bis(4-methoxyphenyl)-2-substituted-thiazole Derivatives

compd	R	yield, %	mp, °C	formula	anal. ^a	antiplatelet ^b MDA ^c IC ₅₀ , μM	vasodilation ^b rat aorta, in vitro ED ₅₀ , μM
12	CH=C(CN)COOEt	35.4	138–40	C ₂₃ H ₂₀ N ₂ O ₆ S	C, H, N	<0.01 ^e	>100
13		10.2	177–8	C ₃₀ H ₃₂ N ₂ O ₆ S	C, H, N	<0.1 ^e	>32
14	CH=CHCOOEt	70.6	94–5	C ₂₂ H ₂₁ NO ₄ S	C, H, N	0.020	>100
15	CH=CHCOOH	34.6	170–81	C ₂₀ H ₁₇ NO ₄ S	C, H, N	>0.1	55
16		27.1	83–93	C ₂₅ H ₂₇ N ₃ O ₃ S·6H ₂ O	C, H, N	>1	20
17	CH ₂ CH ₂ COOH	31.6	218–25	C ₂₀ H ₂₅ NO ₄ S	C, H, N	<0.1 ^e	>100
18		34.3	157–61	C ₂₇ H ₃₃ N ₃ O ₆ S·HCl	C, H, N	>1	2.6
19		34.1	242–7	C ₂₁ H ₁₈ N ₄ O ₃ S	C, H, N	>1	NT ^d
21		8.6	131–2	C ₂₃ H ₂₄ N ₂ O ₂ S	C, H, N	<0.01 ^e	5.9
22	4-CH ₃ -piperazine	50.2	135–6	C ₂₂ H ₂₅ N ₃ O ₂ S	C, H, N	0.039	5.2

^{a-d} See Table 1. ^e Inhibitory activities of 12, 13, 17, and 21 on the MDA production at 0.1 μM (for 13, 17) or 0.01 μM (for 12, 21) were 66.2, 64.7, 72.1, and 64.7%, respectively.

Table 5. Inhibitory Effects of Selected Compounds 1 and/or 6 h after Oral Administration on Platelet Aggregation Induced by Arachidonic Acid (AA) and Collagen in Guinea Pigs *ex Vivo*^a

	AA, dose, mg/kg				collagen dose, mg/kg			
	3.2		1.0		3.2		1.0	
	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h
aspirin	64	– ^b	–	–	9	–	–	–
itazigrel	–	–	100	100	–	–	100	79
timegadine	–	–	–	–	52.4	–	14.5	–
7a	100	–	100	–	76.2	–	9	–
7d	100	100	48.5	–	100	98	33.1	–
8a	100	0	–	–	100	0	–	–
8b	–	–	–	60.9	–	–	–	26.6
8d	–	40.3	–	–	–	37.4	–	–
8e	–	–	–	16.5	–	–	–	–2.6
8f	–	–	–	–3.9	–	–	–	–13.3
8g	–	100	–	100	–	94.1	–	84.3
8j	–	100	–	100	–	100	–	100
10a	–	100	–	100	–	100	–	83.1
10c	–	89	–	–	–	4.1	–	–
10h	–	100	–	–	–	100	–	–
21	–	100	–	–	–	69.3	–	–
22	–	–	–	60.9	–	–	–	65.4

^a The evaluation methods are described in the Experimental Section. ^b –: not tested.

indicated that 10a is localized in platelets after oral administration, inhibiting platelet aggregation. We believe that the reason for the localization is that 10a is easily metabolized¹⁷ and excreted after oral administration except that which binds to CO in platelets with a low K_{off} value.

As we mentioned in the introduction section, the use of aspirin, the most widely-used antiplatelet drug, for treatment of thromboembolic disease is limited by its ulcerogenic properties. Development of a CO inhibitor without the ulcerogenesis is a major goal in thromboembolic

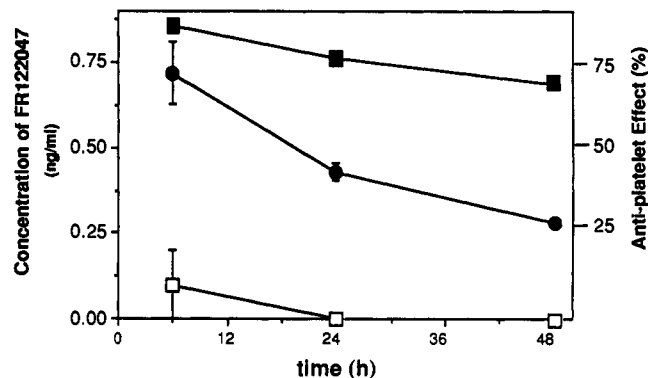


Figure 2. The time dependence of the concentration of FR122047 (● in PRP, □ in PPP) and antiplatelet effect (■) induced by collagen after oral administration (1 mg/kg) in guinea pigs. Each concentration value is the mean ± SE for three animals. Antiplatelet value is the mean for three animals.

research. We evaluated ulcerogenic activity of 10a in rats (Table 6) according to our two presumptions: (1) compounds with a basic function like 10a are less likely to cause gastric damage because they exist predominantly in an ionized, lipid-insoluble form, at the low pH levels characteristic of the stomach and (2) 10a is localized in platelets after oral administration as shown above so that inhibition of PGI₂ synthesis in vascular endothelial cells is short-lived compared with that in platelets, that is, 10a may be free from the "aspirin dilemma".¹⁸ As expected, 10a demonstrated no ulcerogenic activity even at 100 mg/kg, which is 70 times higher than the effective dose (ED₅₀ = 1.4 mg/kg in rats), while aspirin damaged the stomachs at its effective doses (the safety margin of aspirin in rats is only 1.2). Itazigrel showed a little ulceration in a dose-dependent manner (32 and 100 mg/kg, Table 6).

Table 6. Induction of Acute Stomach Lesion and Inhibition of Collagen-Induced Platelet Aggregation in Rats by 10a, Itazigrel, and Aspirin

compd	dose, mg/kg	ulcer index ^a	no. of rats with ulceration	ex vivo ^b ED ₅₀ , mg/kg
10a	100	0.0 ± 0.0	0/5	1.4
aspirin	10	0.6 ± 0.6	1/5	8.4
	32	2.0 ± 0.8	3/5	
	100	2.2 ± 0.6	4/5	
	320	3.8 ± 0.2	5/5	
itazigrel	32	0.4 ^c	1/5	
	100	1.4	2/5	

^a Values are means ± SE for five animals. ^b ED₅₀ values for collagen-induced platelet aggregation in rat. ^c Values are means for three animals.

Conclusion

2-Guanidino-4,5-bis(4-methoxyphenyl)thiazole (3), our lead compound in this study, was designed from two parent compounds (itazigrel and timegadin) to aim at development of compounds which show potent antiplatelet activity based on CO inhibition in vivo with vasodilatory activity. Structure-activity relationship studies on 3 culminated in the synthesis of 4,5-bis(4-methoxyphenyl)-2-[(1-methylpiperazin-4-yl)carbonyl]thiazole (10a, FR122047). Compound 10 demonstrated potent inhibitory activity on MDA synthesis (IC₅₀ = 0.088 μM) with vasodilatory activity (ED₅₀ = 6.2 μM) in in vitro studies. Compound 10a completely inhibited platelet aggregation induced by AA or collagen 6 h after oral administration of 1.0 mg/kg in guinea pigs ex vivo. Moreover, 10a has no ulcerogenesis in rats even at 100 mg/kg dose in spite of its potent cyclooxygenase inhibition (IC₅₀ = 0.43 μM¹⁴), while the use of aspirin, the most widely-used antiplatelet drug, is limited by its ulcerogenesis (the safety margin of aspirin is only 1.2 while that of 10a is more than 70). Pharmacokinetic studies revealed that 10a is detectable in PRP after 48 h but not in PPP after 6 h, which indicates that 10a is localized in platelets. We think that the reason for the lack of the ulcerogenesis are that (1) compounds with a basic function like 10a exist predominantly in an ionized, lipid-insoluble form, at the low pH levels characteristic of the stomach, and (2) 10a is localized in platelets after oral administration as shown above so that inhibition of PGI₂ synthesis in vascular endothelial cells is short-lived compared with that in platelets.¹⁸ We also believe that the localization in platelets after oral administration could help prevent side effects and reduce its toxicity in clinical studies of 10a. Preclinical studies on 10a are now underway to evaluate the effectiveness of its potent antiplatelet and vasodilatory activities.

Experimental Section

Melting point determinations were performed in a capillary melting point apparatus (Thomas-Hoover). All melting points are uncorrected. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F-254 plate and Merck aluminum oxide 60 F-254 (type E). For normal chromatography, Merck silica gel type 60 (size 70–230 mesh) and Wako activated alumina (size 300 mesh) were used. All evaporations were performed with a rotary evaporator under water aspirator. The structures of all compounds were confirmed by their infrared (IR, Hitachi 260-10), mass (MASS, Finiganat TSQ70 and an Hitachi M-80 mass spectrometer), and 60-, 90-, and 200-MHz proton nuclear magnetic resonance (¹H-NMR, JEOL PMX-60SI, Varian EM-390, and Bruker AC200P) spectra. The chemical shift values are reported in parts per million on the δ scale from internal standard tetramethylsilane. No attempt was made to maximize

the yields. All in vitro values are means for three experiments. Ex vivo values are means for five animals.

2-Chloro-1,2-bis(4-methoxyphenyl)-1-ethanone (2). A mixture of anisoin (1, 27.2 g, 10 mmol) and SOCl₂ (15.5 g, 13 mmol) in CH₂Cl₂ was stirred at room temperature for 1 h. After evaporation, the resulting precipitate was recrystallized from diethyl ether (Et₂O)-isopropyl ether (IPE) to give 2 (16.4 g, 55.6%): mp 80–4 °C; IR (Nujol) 1665, 1600, 1560, 1500 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 3.90 (s, 6 H), 7.18 (d, 4 H, *J* = 8 Hz), 7.92 (d, 4 H, *J* = 8 Hz); mass spectrum *m/e* 290 (M⁺).

2-Guanidino-4,5-bis(4-methoxyphenyl)thiazole (3). A mixture of 2 (8.00 g, 27.5 mmol) and 2-guanidinothioamide (4.88 g, 41.3 mmol) in ethanol (EtOH, 160 mL) was stirred and refluxed for 2 h. After cooling, the resulting precipitate was removed through filtration. The filtrate was evaporated, and then the resulting residue was purified by chromatography over Al₂O₃ [chloroform (CHCl₃)-methanol (MeOH) as eluent] and subsequently recrystallized from EtOH to give 3 (4.50 g, 46.2%): mp 167–168 °C; IR (Nujol) 3450, 3370, 3300, 3200, 1625, 1610, 1580, 1540, 1510, 1490 cm⁻¹; ¹H-NMR (90 MHz, DMSO-*d*₆) δ 3.75 (s, 3 H, OCH₃), 3.80 (s, 3 H, OCH₃), 6.88 (d, 2H, *J* = 8.8 Hz), 6.94 (d, 2 H, *J* = 8.8 Hz), 7.12 (d, 2 H, *J* = 8.8 Hz), 7.28 (d, 2 H, *J* = 8.8 Hz).

Compound 9 was obtained in a similar manner to 3. Compound 9 was used for the subsequent reactions without purification, and the yield of this condensation was assumed to be 100% for the next reaction.

2-Amino-4,5-bis(4-methoxyphenyl)thiazole Hydrochloride (4-HCl). A mixture of 2 (10.00 g, 34.4 mmol), thiourea (7.11 g, 55.1 mmol), and a small amount of NaI in acetonitrile (CH₃CN, 100 mL) was stirred and refluxed for 3 h. After cooling, the resulting precipitate was collected by filtration. The precipitate was washed with CH₃CN and subsequently recrystallized from CH₃CN-EtOH-Et₂O to give 4-HCl (10.00 g, 93.1%): mp 174–5 °C; IR (Nujol) 3250, 3150, 1625, 1600, 1560, 1500 cm⁻¹; ¹H-NMR (90 MHz, DMSO-*d*₆) δ 3.68 (s, 6 H), 6.7–7.4 (m, 8 H); mass spectrum, *m/e* 312 (M⁺, free).

2-(3-Benzoylthioureido)-4,5-bis(4-methoxyphenyl)thiazole (6). A mixture of NH₄SCN (12.2 g, 171 mmol) and benzoyl chloride (24.0 g, 171 mmol) in acetone (300 mL) was stirred and refluxed for 5 min. To a suspension of NaH (4.58 g, 114 mmol) in dimethylformamide (DMF, 60 mL) and toluene (60 mL) was added a suspension of 3 (35.7 g, 114 mmol) in DMF (60 mL) and toluene (60 mL) dropwise over 30 min at 0 °C and then was stirred at the same temperature for 30 min. To this reaction mixture was added the above mixture, including benzoyl isocyanate, at 0–5 °C. The whole mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. The reaction mixture was poured into a mixture of water and ice and was extracted with a mixture of ethyl acetate (AcOEt) and tetrahydrofuran (THF). The organic layer was washed with water and brine and dried over MgSO₄. After evaporation, the resulting precipitate was recrystallized from AcOEt to give 6 (41.86 g, 77.1%): mp 184–5 °C; IR (Nujol) 3220, 1660, 1605 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 3.75 (s, 3 H), 3.80 (s, 3 H), 6.8–8.2 (m, 13 H), 11.97 (s, 1 H); mass spectrum, *m/e* 475 (M⁺).

4,5-Bis(methoxyphenyl)-2-thioureidothiazole (5a). To a suspension of 6 (20.90 g, 43.9 mmol) in MeOH (110 mL) and water (2.2 mL) was added a mixture of NaOH (1.76 g, 43.9 mmol) in MeOH (20 mL) dropwise over 10 min at 55–60 °C. The reaction mixture was stirred at the same temperature for 2 h. After the mixture was concentrated to ca. 20 mL, 400 mL of water was added to the resulting mixture. The resulting precipitate was collected by filtration and washed with water to afford 5a (16.4 g, 100%): mp 229–231 °C; IR (Nujol) 1600, 1560, 1510, 1500 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 3.69 (s, 3 H), 3.73 (s, 3 H), 6.7–7.5 (m, 8 H), 11.70 (br s, 1 H); mass spectrum, *m/e* 371 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-(3-methylguanidino)thiazole (7a). A mixture of 5a (10.5 g, 28.3 mmol) and MeI (17.6 mL, 283 mmol) in MeOH (140 mL) and CHCl₃ (140 mL) was stirred and refluxed for 1 h. After evaporation, the resulting residue was dissolved with EtOH (70 mL). The solution was transferred into a sealed tube, and then methylamine hydrochloride (9.54 g, 141 mmol) and triethylamine (19.7 mL, 141 mmol) were added. After the tube was sealed, the mixture was heated at 100 °C for 12 h. After removal of solvents, the resulting residue was dissolved

with water and AcOEt. The organic layer was washed with water and brine and dried over MgSO₄. After evaporation, the resulting residue was purified by chromatography over silica gel (CHCl₃-AcOEt as eluent) and subsequently recrystallized from EtOH to give **7a** (6.06 g, 58.1%): mp 155–6 °C; IR (Nujol) 3400, 1660, 1610, 1590, 1505 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 2.72 and 2.77 (both s, 3 H, NHCH₃), 3.73 (s, 6 H), 6.7–7.6 (m, 11 H); mass spectrum, *m/e* 368 (M⁺).

Compounds **7b–e, g–i** were prepared in a similar manner to **7a**. Ethylamine hydrochloride (for **7b**, 59.4% yield), isopropylamine (for **7c**, 84.5% yield), dimethylamine hydrochloride (for **7d**, 30.3% yield), cyclohexylamine (for **7e**, 74.5% yield), *N*-methylpiperazine (for **7g**, 32.1% yield), morpholine (for **7h**, 50.9% yield), and 1,2-diaminoethane (for **7i**, 44.9% yield) were used in place of methylamine hydrochloride.

4,5-Bis(4-methoxyphenyl)-2-(3-methylthioureido)thiazole (5b). Compound **4** (1.00 g, 3.20 mmol) was added to a mixture of NaH (0.08 g, 3.20 mmol) in DMF (5 mL) dropwise over 15 min at room temperature. After the reaction mixture was stirred at room temperature for 20 min, CH₃NCS (2.19 mL, 32.0 mmol) was added. The whole mixture was stirred at room temperature for 30 min and then stirred at 80–90 °C for 5 h. After cooling, the mixture was poured into water (100 mL). The resulting precipitate was collected by filtration and washed with IPE to afford **5b** (0.81 g, 65.7%): mp 201–2 °C; IR (Nujol) 3380, 3170, 1610, 1590, 1570, 1510 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 3.20 (s, 3 H), 4.00 (s, 6 H), 6.9–7.7 (m, 8 H); mass spectrum, *m/e* 385 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-(*N,N*-dimethylguanidino)thiazole (7f). Compound **5b** (0.75 g, 1.95 mmol) and methylamine hydrochloride (0.66 g, 9.73 mmol), reacted as described for **7a**, yielded 32.1% (0.30 g) of **7f**: mp 231–3 °C; IR (Nujol) 3150, 1680, 1630, 1610, 1540, 1510, 1490 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 2.82 (s, 3 H, NCH₃), 2.90 (s, 3 H, NCH₃), 3.69 (s, 6 H, 2 × OCH₃), 6.7–7.4 (m, 8 H), 8.7 (br s, 3 H); mass spectrum, *m/e* 382 (M⁺).

4,5-Bis(methoxyphenyl)-2-((dimethylamino)methyl)thiazole (8a). A mixture of **2** (2.00 g, 6.88 mmol) and ((dimethylamino)methyl)thioformamide (1.22 g, 10.3 mmol) in EtOH (20 mL) was stirred and refluxed for 3.5 h. After cooling, the mixture was poured into a mixture of saturated NaHCO₃ solution and AcOEt. The organic layer was washed with saturated NaHCO₃ solution, water, and brine and dried over MgSO₄. After evaporation, the resulting residue was purified by chromatography over Al₂O₃ (benzene and AcOEt as eluent). The fractions containing the desired compound were combined and concentrated to one-tenth volume, and HCl/EtOH was added to the solution. The resulting precipitate was washed with Et₂O to give **8a**·HCl (0.30 g, 12.3%): mp 204–6 °C; IR (Nujol) 2560, 2530, 2470, 1610, 1510 cm⁻¹; ¹H-NMR (60 MHz, D₂O) δ 3.30 (s, 6 H, 2 × NH₃), 3.85 (s, 6 H), 4.91 (s, 2 H, CH₂), 6.7–7.8 (m, 8 H); mass spectrum, *m/e* 354 (M⁺).

Compound **8b** (33.2% yield) was prepared in a manner similar to **8a**.

2-(Aminomethyl)-4,5-bis(4-methoxyphenyl)thiazole (8c). A mixture of **8b** (6.43 g, 17.5 mmol) in 35% HCl (35 mL) was stirred at room temperature for 30 min and then stirred and refluxed for 30 min. After cooling, the reaction mixture was poured into water. The resulting precipitate was collected by filtration and washed with water to afford **8c**·HCl (5.48 g, 86.3%): mp 146–8 °C; IR (Nujol) 3350, 1600, 1540, 1505 cm⁻¹; ¹H-NMR (90 MHz, DMSO-*d*₆) δ 3.69 (s, 3 H), 3.73 (s, 3 H), 4.38 (s, 2 H), 6.7–7.5 (m, 8 H), 8.85 (br s, 3 H); mass spectrum, *m/e* 326 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-((nicotinylamino)methyl)thiazole (8d). A mixture of **8b** (1.00 g, 2.71 mmol) and 35% HCl (7 mL) was stirred and refluxed for 3 h. After cooling, the reaction mixture was poured into a mixture of water and AcOEt. After the aqueous layer was alkalinized with saturated K₂CO₃ solution, the separated organic layer was washed with water and brine and dried over MgSO₄. The organic layer was evaporated. The resulting syrup (compound **8c**) was dissolved in dichloromethane (CH₂Cl₂, 10 mL), and triethylamine (0.33 mL, 3.25 mmol) was added. To the reaction mixture was added dropwise over 10 min at room temperature a suspension of nicotinyl chloride hydrochloride (0.58 g, 3.25 mmol) in CH₂Cl₂, and then the mixture was

refluxed for 1.5 h. After cooling, the reaction mixture was poured into water. The organic layer was washed with saturated NaHCO₃ solution, water, and brine and dried over MgSO₄. After evaporation, to the resulting residue was added HCl/EtOH with water cooling. The resulting precipitate was recrystallized from CH₂Cl₂-Et₂O to give **8d** (0.63 g, 49.7%): mp 135–44 °C; IR (Nujol) 3400, 3200, 1680, 1610, 1520 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 3.75 (s, 3 H), 3.77 (s, 3 H), 4.83 (d, 2 H, *J* = 5.6 Hz, CH₂), 6.88 (d, 2 H, *J* = 8.5 Hz), 6.94 (d, 2 H, *J* = 8.5 Hz), 7.12 (d, 2 H, *J* = 8.5 Hz), 7.30 (d, 2 H, *J* = 8.5 Hz), 8.15 (dd, 1 H, *J* = 8.1, 5.6 Hz), 9.0–9.2 (m, 2 H), 10.42 (t, 1 H, *J* = 5.6 Hz); mass spectrum, *m/e* 431 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-[[*N*-(2-phenylthioacetyl)amino]methyl]thiazole (8e). A suspension of **8c**·HCl (4.00 g, 11.0 mmol) in CH₂Cl₂ and water was neutralized with saturated NaHCO₃ solution. The organic layer was washed with brine and dried over MgSO₄. After evaporation, the resulting residue was dissolved in DMF (60 mL). To the mixture were added 2-(phenylthio)acetic acid (1.85 g, 11.0 mmol) and 1-ethyl-3-((dimethylamino)propyl)carbodiimide hydrochloride (EDC·HCl, 2.34 g, 11.0 mmol), and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was poured into water and then extracted with AcOEt. The organic layer was washed with saturated NaHCO₃ solution, 1 N HCl, water, and brine and dried over MgSO₄. After evaporation, the resulting residue was recrystallized from AcOEt-Et₂O to give **8e** (2.77 g, 56.6%): mp 132–3 °C; IR (Nujol) 3320, 1660, 1610, 1550, 1520 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 3.7–3.8 (m, 8 H, 2 × OCH₃ and CH₂S), 4.56 (d, 2 H, *J* = 6.0 Hz, CH₂NH), 6.8–7.0 (m, 4 H), 7.3–7.4 (m, 4 H), 9.05 (t, 1 H, *J* = 6.0 Hz, NHCO); mass spectrum, *m/e* 476 (M⁺).

Compounds **8f, g** (28.1 and 40.1% yield, respectively) were prepared in a manner similar to **8e**.

4,5-Bis(4-methoxyphenyl)-2-((3-methylureido)methyl)thiazole (8i). A suspension of **8c**·HCl (1.00 g, 2.76 mmol) in CH₂Cl₂ and water was neutralized with saturated NaHCO₃ solution, and the separated organic layer was washed with brine and dried over MgSO₄. After evaporation, the resulting mixture was dissolved in THF (20 mL) and MeOH (7 mL). To the mixture was added CH₃NCO (0.23 mL, 3.86 mmol), and the mixture was stirred for 4.5 h. After evaporation, the resulting precipitate was recrystallized from a mixture of THF, MeOH, and AcOEt to give **8i** (0.89 g, 84.1%): mp 115–8 °C; IR (Nujol) 3300, 1620, 1600, 1500 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 2.60 (s, 3 H), 3.74 (s, 3 H), 3.76 (s, 3 H), 4.48 (s, 2 H), 6.86 (d, 2 H, *J* = 8.8 Hz), 6.93 (d, 2 H, *J* = 8.8 Hz), 7.23 (d, 2 H, *J* = 8.8 Hz), 7.36 (d, 2 H, *J* = 8.8 Hz); mass spectrum, *m/e* 383 (M⁺).

Compound **8j** (91.6% yield) was obtained in a manner similar to that of **8i**.

4,5-Bis(4-methoxyphenyl)-2-((morpholinocarbonyl)amino)methylthiazole (8h). A suspension of **8c**·HCl (1.00 g, 2.76 mmol) in CH₂Cl₂ and water was neutralized with saturated NaHCO₃ solution, and the separated organic layer was washed with brine and dried over MgSO₄. After evaporation, the resulting residue was dissolved in THF (20 mL), and pyridine (0.50 mL, 6.18 mmol) was added. A solution of morpholinocarbonyl chloride (0.7 mL, 6.00 mmol) in THF (10 mL) was added to the reaction mixture at room temperature, and the resultant mixture was stirred overnight. The reaction mixture was poured into water and then extracted with AcOEt. The organic layer was washed with saturated NaHCO₃ solution, water, and brine and dried over MgSO₄. After evaporation, the resulting residue was purified by chromatography over silica gel (acetone-CHCl₃ as eluent) to give **8h** (1.00 g, 82.4%): mp 111–6 °C; IR (Nujol) 3300, 1630, 1600, 1540, 1515 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 3.33 (s, 8 H, morpholino), 3.74 (s, 3 H), 3.77 (s, 3 H), 4.50 (d, 2 H, *J* = 6.0 Hz), 6.86 (d, 2 H, *J* = 8.8 Hz), 6.95 (d, 2 H, *J* = 8.8 Hz), 7.23 (d, 2 H, *J* = 8.8 Hz), 7.36 (d, 2 H, *J* = 8.8 Hz), 7.54 (t, 1 H, *J* = 6.0 Hz); mass spectrum, *m/e* 439 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-[(4-methylpiperazin-1-yl)carbonyl]thiazole (10a). A mixture of **9** (1.00 g, 2.71 mmol) and *N*-methylpiperazine (1.80 mL, 16.2 mmol) was stirred at 80–90 °C for 14 h. After cooling, the mixture was poured into a mixture of water and AcOEt. The organic layer was washed with water and brine, dried over MgSO₄, and evaporated. The resulting residue was dissolved in EtOH and Et₂O, and then HCl/

EtOH was added to give white precipitate. The precipitate was washed with Et₂O and dried to afford **10a** (0.58 g, 46.5%): mp 254–6 °C; IR (Nujol) 3400, 2430, 1625, 1610, 1560, 1535, 1510 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 2.75 (s, 3 H, NCH₃), 3.0–3.6 (m, 8 H, piperidino), 3.72 (s, 3 H), 3.83 (s, 3 H), 6.89 (d, 2 H, *J* = 9 Hz), 6.92 (d, 2 H, *J* = 9 Hz), 7.28 (d, 2 H, *J* = 9 Hz), 7.36 (d, 2 H, *J* = 9 Hz); mass spectrum, *m/e* 423 (M⁺).

Ammonia bubbling to a solution of **9** in MeOH afforded **10b** (28.9% yield) in a similar manner to **10a**.

The aminolysis for preparation of **10c–d** (24.1 and 8.3% yields, respectively) was carried out in a sealed tube in a similar manner to **10a**.

Compounds **10f–l** (25.2, 22.6, 42.1, 74.0, 20.6, 58.1, and 29.1% yields, respectively) were obtained in a similar manner to **10a**.

2-(Guanidinocarbonyl)-4,5-bis(4-methoxyphenyl)thiazole (10e). Guanidine hydrochloride (1.42 g, 14.9 mmol) was added to a mixture of 28% sodium methoxide (2.61 mL, 13.5 mmol) in MeOH (5 mL) at room temperature, and the mixture was stirred at the same temperature for 15 min. After removal of the resulting precipitate, the filtrate was added dropwise to a mixture of **9** in MeOH (10 mL) at room temperature and was stirred at room temperature for 2 h. The resulting precipitate was collected by filtration and was recrystallized from EtOH and Et₂O to give **10e** (0.58 g, 56.0%): mp 253–5 °C; IR (Nujol) 3420, 3180, 1660, 1630, 1610, 1530 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 3.76 (s, 3 H), 3.78 (s, 3 H), 6.90 (d, 2 H, *J* = 9.0 Hz), 6.95 (d, 2 H, *J* = 9.0 Hz), 7.28 (d, 2 H, *J* = 9.0 Hz), 7.38 (d, 2 H, *J* = 9.0 Hz); mass spectrum, *m/e* 382 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-[4-((isopropylamino)carbonyl)piperazin-1-yl]thiazole (10m). A mixture of **10g** (1.00 g, 2.25 mmol) in CH₂Cl₂ and water was neutralized with saturated NaHCO₃ solution, and the organic layer was washed with water and brine, dried over MgSO₄, and evaporated. The resulting residue was dissolved with THF (20 mL) and MeOH (7 mL), and isopropyl isocyanate (0.32 mL, 3.15 mmol) was added. The mixture was stirred at room temperature for 2 h. After removal of the solvents, the resulting precipitate was recrystallized from IPE and EtOH to give **10m** (0.72 g, 65.0%): mp 157–9 °C; IR (Nujol) 3260, 1610, 1530, 1510 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 1.07 (d, 6 H, *J* = 6.6 Hz, CH₃), 3.4–4.4 (m, 9 H, piperidino and CH), 3.76 (s, 3 H), 3.79 (s, 3 H), 6.28 (d, 1 H, *J* = 7.4 Hz, NHCH), 6.92 (d, 2 H, *J* = 8.6 Hz), 6.97 (d, 2 H, *J* = 8.6 Hz), 7.32 (d, 2 H, *J* = 8.6 Hz), 7.39 (d, 2 H, *J* = 8.6 Hz); mass spectrum, *m/e* 494 (M⁺).

2-Formyl-4,5-bis(4-methoxyphenyl)thiazole (11). A mixture of **9** (19.66 g, 53.2 mmol) and THF (25 mL) was added to a suspension of LiAlH₄ (2.22 g, 58.5 mmol) in THF (20 mL) at 0 °C and was stirred at room temperature for 30 min. After quenching and removal of the resulting precipitates, the filtrate was evaporated. The resulting residue was purified by chromatography over silica gel (AcOEt and benzene as eluent) to give **2-(hydroxymethyl)-4,5-bis(4-methoxyphenyl)thiazole** (5.22 g, 30.0%), an oily compound: IR (Nujol) 3200, 1600, 1570, 1500 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 3.74 (s, 3 H), 3.78 (s, 3 H), 4.75 (d, 2 H, *J* = 5.8 Hz, CH₂), 6.10 (d, 1 H, *J* = 5.8 Hz, OH), 6.82 (d, 2 H, *J* = 8.8 Hz), 6.92 (d, 2 H, *J* = 8.8 Hz), 7.23 (d, 2 H, *J* = 8.8 Hz), 7.38 (d, 2 H, *J* = 8.8 Hz); mass spectrum, *m/e* 327 (M⁺). A mixture of the above alcohol (5.04 g, 15.4 mmol) and activated MnO₂ (25.2 g, 5 w/w) in AcOEt (250 mL) was stirred at room temperature for 5.5 h. After removal of the insoluble material, the filtrate was evaporated. The resulting precipitate was recrystallized from AcOEt and *n*-hexane to give **11** (4.10 g, 81.9%): mp 82–5 °C; IR (Nujol) 1690, 1605, 1515 cm⁻¹; ¹H-NMR (60 MHz, CDCl₃) δ 3.84 (s, 6 H), 6.8–6.9 (m, 4 H), 7.33 (d, 2 H, *J* = 9 Hz), 7.45 (d, 2 H, *J* = 9 Hz), 9.28 (s, 1 H, CHO); mass spectrum, *m/e* 325 (M⁺).

Ethyl 3-[4,5-Bis(4-methoxyphenyl)thiazol-2-yl]-2-cyano-propionate (12). A mixture of **11** (0.70 g, 2.15 mmol), ethyl cyanoacetate (0.23 mL, 2.15 mmol), ammonium acetate (0.03 g, 0.4 mmol), and AcOH (0.10 mL, 1.7 mmol) in benzene (20 mL) was stirred and refluxed for 7.5 h while the resulting water was removed using a Dean-Stark apparatus. After cooling, the mixture was poured into water and extracted with AcOEt. The organic layer was washed with saturated NaHCO₃ solution, water, and brine, dried over MgSO₄, and evaporated. The resulting residue was purified by chromatography over silica gel (benzene-

AcOEt as eluent) to afford **12** (0.32 g, 35.4%): mp 138–40 °C; IR (Nujol) 2200, 1720, 1590, 1505 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 1.33 (t, 3 H, *J* = 7.1 Hz, Et), 3.77 (s, 3 H), 3.81 (s, 3 H), 4.34 (q, 2 H, *J* = 7.1 Hz, Et), 6.93 (d, 2 H, *J* = 8.8 Hz), 7.01 (d, 2 H, *J* = 8.8 Hz), 7.36 (d, 2 H, *J* = 8.8 Hz), 7.46 (d, 2 H, *J* = 8.8 Hz), 8.45 (s, 1 H, CH); mass spectrum, *m/e* 418 (M⁺ - 2).

3,5-Bis(ethoxycarbonyl)-4-[4,5-bis(4-methoxyphenyl)thiazol-2-yl]-2,6-dimethyl-1,4-dihydropyridine (13). A mixture of **11** (0.992 g, 30.5 mmol), ethyl acetoacetate (0.43 mL, 3.36 mmol), morpholine (0.027 mL, 0.31 mmol), and AcOH (0.017 mL, 0.31 mmol) in benzene (10 mL) was stirred and refluxed for 30 min. After cooling, the mixture was poured into water and extracted with AcOEt. The organic layer was washed with water and brine, dried over MgSO₄, and evaporated. The resulting residue was dissolved with EtOH (10 mL) and ethyl 3-aminocrotonate (0.42 mL, 3.36 mmol), and the mixture was stirred and refluxed overnight. The mixture was evaporated, and to the resulting residue were added water and AcOEt. The separated organic layer was washed with 1 N HCl, water, and brine, dried over MgSO₄, and evaporated. The resulting residue was purified over Al₂O₃ (benzene-AcOEt as eluent) to give **13** (0.17 g, 10.2%): mp 177–8 °C; IR (Nujol) 1690, 1675, 1610, 1500 cm⁻¹; ¹H-NMR (90 MHz, DMSO-*d*₆) δ 1.27 (t, 6 H, *J* = 7 Hz, Et), 2.33 (s, 3 H, CH₃), 3.75 (s, 3 H), 3.78 (s, 3 H), 4.15 (q, 2 H, *J* = 7 Hz, Et), 5.36 (s, 1 H, CH), 6.81 (d, 2 H, *J* = 9 Hz), 6.87 (d, 2 H, *J* = 9 Hz), 7.18 (d, 2 H, *J* = 9 Hz), 7.28 (d, 2 H, *J* = 9 Hz), 9.05 (s, 1 H, NH); mass spectrum, *m/e* 547 (M⁺ - 1).

Ethyl 3-[4,5-Bis(4-methoxyphenyl)thiazol-2-yl]-(*E*)-propeonate (14). A mixture of **11** (0.14 g, 0.43 mmol) and (carbethoxymethylene)triphenylphosphorane (0.18 g, 0.52 mmol) in CH₂Cl₂ (5 mL) was stirred and refluxed for 4 h. After evaporation, the resulting residue was purified over silica gel (CH₂Cl₂ as eluent) to give **14** (0.12 g, 70.6%): mp 94–5 °C; IR (Nujol) 1703, 1620, 1600, 1500 cm⁻¹; ¹H-NMR (90 MHz, DMSO-*d*₆) δ 1.31 (t, 3 H, *J* = 6 Hz), 3.78 (s, 3 H), 3.80 (s, 3 H), 4.22 (q, 2 H, *J* = 6 Hz), 6.70 (d, 1 H, *J* = 15 Hz, (*E*)-CH=CH), 6.82 (d, 2 H, *J* = 9 Hz), 6.85 (d, 2 H, *J* = 9 Hz), 7.26 (d, 2 H, *J* = 9 Hz), 7.38 (d, 2 H, *J* = 9 Hz), 7.68 (d, 1 H, *J* = 15 Hz, (*E*)-CH=CH); mass spectrum, *m/e* 395 (M⁺).

3-[4,5-Bis(4-methoxyphenyl)thiazol-2-yl]-(*E*)-propeonic Acid (15). A mixture of **14** (0.50 g, 12.6 mmol) and 0.1 N NaOH (5 mL) in dioxane (5 mL) was stirred and refluxed overnight. The reaction mixture was acidified with 10% HCl and extracted with Et₂O. The organic layer was washed with water and brine, dried over MgSO₄, and evaporated. The resulting residue was purified over silica gel (CHCl₃-MeOH as eluent) to give **15** (0.16 g, 34.6%): mp 170–181 °C; IR (Nujol) 1660, 1620, 1600, 1560, 1500 cm⁻¹; ¹H-NMR (60 MHz, DMSO-*d*₆) δ 3.70 (s, 6 H), 6.65 (d, 1 H, *J* = 15 Hz), 6.80 (d, 2 H, *J* = 7 Hz), 6.85 (d, 2 H, *J* = 7 Hz), 7.15 (d, 2 H, *J* = 7 Hz), 7.30 (d, 2 H, *J* = 7 Hz), 7.51 (d, 1 H, *J* = 15 Hz); mass spectrum, *m/e* 367 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-[2-[4-methylpiperazin-1-yl]carbonyl]jethenyl]thiazole (16). A mixture of **15** (0.42 g, 1.14 mmol), *N*-methylpiperazine (0.13 mL, 1.14 mmol), EDC-HCl (0.22 g, 1.14 mmol), and NEt₃ (0.16 mL, 1.14 mmol) in DMF (10 mL) was stirred at room temperature for 7 h. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with water, saturated NaHCO₃ solution, and brine, dried over MgSO₄, and evaporated. The resulting residue was purified over silica gel (CHCl₃-MeOH as eluent). After concentration, HCl/EtOAc was added to give a white precipitate, which was washed with Et₂O to afford **16-HCl** (0.15 g, 27.1%): mp 83–93 °C; IR (Nujol) 1640, 1600, 1510 cm⁻¹; ¹H-NMR (200 MHz, DMSO-*d*₆) δ 2.76 (d, 3 H, *J* = 4.4 Hz), 3.4–3.8 (m, 2 H), 3.76 (s, 3 H), 3.79 (s, 3 H), 4.2–4.8 (m, 6 H), 6.91 (d, 2 H, *J* = 8.9 Hz), 6.97 (d, 2 H, *J* = 8.9 Hz), 7.29 (d, 2 H, *J* = 8.9 Hz), 7.40 (d, 2 H, *J* = 8.9 Hz), 7.42 (d, 1 H, *J* = 15.2 Hz), 7.62 (d, 1 H, *J* = 15.2 Hz), 11.48 (br s, 1 H); mass spectrum, *m/e* 449 (M⁺).

3-[4,5-Bis(4-methoxyphenyl)thiazol-2-yl]propionic Acid (17). Compound **15** (1.04 g, 2.83 mmol) was dissolved with CHCl₃ (10 mL) and MeOH (10 mL), and the reaction mixture was hydrogenated at room temperature and atmospheric pressure over 5% Pd(OH)₂-C (0.8 g) for 4 h. After filtration and evaporation of the filtrate, the resulting precipitate was recrystallized from EtOH and Et₂O to give **17** (0.35 g, 31.6%): mp 218–225 °C; IR (Nujol) 3300, 1600, 1560, 1505 cm⁻¹; ¹H-NMR (60

MHz, DMSO- d_6) δ 2.59 (br s, 2 H), 3.4 (br s, 2 H), 3.70 (s, 3 H), 3.72 (s, 3 H), 6.6–7.4 (m, 8 H); mass spectrum, m/e 369 (M^+).

Ethyl 3-[4,5-Bis(4-methoxyphenyl)thiazol-2-yl]-3-(4-methylpiperazin-1-yl)propionate (18). Compound 14 (0.50 g, 1.26 mmol) and *N*-methylpiperazine (5 mL), condensed as described for 10a, yielded 34.3% of 18-HCl: mp 157–61 °C; IR (Nujol) 2350, 1735, 1605, 1510 cm^{-1} ; 1H -NMR (90 MHz, DMSO- d_6) δ 1.16 (t, 3 H, $J = 7$ Hz, Et), 2.81 (br s, 3 H, NCH_3), 2.5–3.8 (m, 10 H, morpholino and CH_2), 3.75 (s, 3 H), 3.78 (s, 3 H), 4.11 (q, 2 H, $J = 7$ Hz, Et), 4.73 (m, 1 H, thiazole-CH), 6.87 (d, 2 H, $J = 9$ Hz), 6.96 (d, 2 H, $J = 9$ Hz), 7.26 (d, 2 H, $J = 9$ Hz), 7.38 (d, 2 H, $J = 9$ Hz), 11.01 (br s, 1 H, NH^+); mass spectrum, m/e 495 (M^+ of free).

2-(4,5-Dihydro-3-oxopyridazin-6-yl)-4,5-bis(4-methoxyphenyl)thiazole (19). Compound 2 (1.24 g, 4.27 mmol) and 4,5-dihydro-6-thiocarbamoyl-3-pyridazinone (0.61 g, 3.88 mmol), condensed as described for 10a, yielded 34.1% of 19: mp 242–7 °C; IR (Nujol) 3200, 3100, 1680, 1610, 1580, 1520 cm^{-1} ; 1H -NMR (200 MHz, DMSO- d_6) δ 2.51 (t, 2 H, $J = 8.4$ Hz, oxopyridazino), 3.27 (t, 2 H, $J = 8.4$ Hz, oxopyridazino), 3.76 (s, 3 H), 3.78 (s, 3 H), 6.90 (d, 2 H, $J = 8.8$ Hz), 6.97 (d, 2 H, $J = 8.8$ Hz), 7.29 (d, 2 H, $J = 8.8$ Hz), 7.39 (d, 2 H, $J = 8.8$ Hz), 11.17 (s, 1H); mass spectrum, m/e 393 (M^+).

4,5-Bis(4-methoxyphenyl)-2-(1-methyl-1,2,5,6-tetrahydropyridin-4-yl)thiazole (21). 2 (3.00 g, 10.3 mmol) and 4-thiocarbamoylpyridine (1.57 g, 11.3 mmol), condensed as described for 10a, yielded 50.6% of 4,5-bis(4-methoxyphenyl)-2-(4-pyridyl)thiazole (20): mp 113–7 °C; IR (Nujol) 3225, 1670, 1600, 1505 cm^{-1} ; 1H -NMR (200 MHz, DMSO- d_6) δ 3.77 (s, 3 H), 3.80 (s, 3 H), 6.83 (d, 2 H, $J = 8.9$ Hz), 6.95 (d, 2 H, $J = 8.9$ Hz), 7.35 (d, 2 H, $J = 8.9$ Hz), 7.43 (d, 2 H, $J = 8.9$ Hz), 8.6–8.8 (m, 4 H, 4-pyridyl); mass spectrum, m/e 374 (M^+). A mixture of the above thiazole (1.78 g, 4.75 mmol), methyl iodide (2.96 mL, 47.5 mmol), MeOH (8 mL), and $CHCl_3$ (20 mL) was stirred at room temperature for 10.5 h. After evaporation, the resulting precipitate was removed by filtration and washed with $CHCl_3$. To a solution of the combined filtrate were added dropwise MeOH (20 mL) and subsequently $NaBH_4$ (0.54 g, 14.3 mmol) at room temperature, and the mixture was stirred at the same temperature for 1 h. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated. The resulting residue was purified over silica gel ($CHCl_3$ -MeOH as eluent) to give 21 (0.16 g, 8.6%): mp 131–2 °C; IR (Nujol) 1603, 1505, 1485 cm^{-1} ; 1H -NMR (200 MHz, DMSO- d_6) δ 2.31–3.4 (m, 6 H, $CH_2CH_2NCH_2$), 2.60 (br s, 3 H, NCH_3), 3.67 (s, 3 H, OCH_3), 3.69 (s, 3 H, OCH_3), 6.55 (m, 1 H, =CH), 6.83 (d, 2 H, $J = 8$ Hz), 6.91 (d, 2 H, $J = 8$ Hz), 7.22 (d, 2 H, $J = 8$ Hz), 7.35 (d, 2 H, $J = 8$ Hz); mass spectrum, m/e 392 (M^+).

4,5-Bis(4-methoxyphenyl)-2-(4-methylpiperazin-1-yl)thiazole (22). 2-Chloro-4,5-bis(4-methoxyphenyl)thiazole (0.50 g, 1.51 mmol) and *N*-methylpiperazine (1.67 mL, 15.1 mmol), condensed as described for 10a, yielded 50.2% of 22: mp 135–6 °C; IR (Nujol) 1605, 1570, 1505 cm^{-1} ; 1H -NMR (200 MHz, DMSO- d_6) δ 2.23 (s, 3 H, NCH_3), 2.4–2.6 (br s, 4 H, 2 \times CH_2), 3.3–3.5 (br s, 4 H, CH_2NCH_2), 3.73 (s, 3 H), 3.75 (s, 3 H), 6.82 (d, 2 H, $J = 8.8$ Hz), 6.89 (d, 2 H, $J = 8.8$ Hz), 7.16 (d, 2 H, $J = 8.8$ Hz), 7.34 (d, 2 H, $J = 8.8$ Hz); mass spectrum, m/e 395 (M^+).

Pharmacological Tests. Blood from male Hartley guinea pigs was collected into plastic tubes containing 3.8% sodium citrate (1/10 volume of blood), and blood from male Sprague-Dawley rats was collected into tubes containing 2.2% sodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation of blood at 120g for 10 min, and platelet-poor plasma (PPP) was obtained by centrifugation of the remaining blood at 1500g for 15 min. Platelet aggregation was studied by the turbidimetric method of Born and Cross¹⁹ with an NKK HEMATRACER 1.

MDA Formation. PRP from rabbits was centrifuged at 150g for 15 min. The pellets were suspended in 0.002% saponin-1% ammonium oxalate solution (Technicon). After further centrifugation of the tubes for 10 min, the platelets were resuspended in phosphate-buffered saline (PBS, pH = 7.4) at a concentration of 10^9 platelets/mL. MDA was measured by the modified method of Placer et al.²⁰ Platelet suspension (0.9 mL) was preincubated with 0.1 mL of a solution of drug for 5 min at 37 °C, and the

reaction was started by the addition of 20 μ L of 2.5 mM AA; the incubation lasted 3 min. The reaction was terminated by addition of 1 mL of thiobarbituric acid reagent, followed by boiling for 10 min. After centrifugation of the test tubes at 1500g for 10 min, the absorption of supernatant solution was measured at 532 nm.

Vasodilatory Activity. Helical strips of rat thoracic aorta were suspended in an organ bath containing Tyrode solution gassed with 95% O_2 -5% CO_2 at 37 °C under 0.5 g load. Contraction was induced by addition of KCl solution (final concentration was 30 mM). After the tonus reached a plateau, drug solution (dissolved in DMSO) was added cumulatively and, finally, 10^{-4} M of papaverine was added to obtain maximum relaxation. Activities of the test compound were expressed as ED_{50} values, i.e., the dose required to relax the isolated rat aorta by 50%.

Ex Vivo Studies on Platelet Aggregation. Male Hartley guinea pigs weighing 200–300 g were used after a 24-h fast, and male Sprague-Dawley rats weighing about 200 g were used after an overnight fast. Blood was obtained from the abdominal aorta under ether anesthesia at scheduled times after oral administration of drugs. The final concentration of collagen was 0.5 μ g/mL for guinea pigs and 2.0 μ g/mL for rats. AA was used at 50 μ M in guinea pigs. The percent inhibition was calculated from the total aggregation.

Gastrointestinal Activity. Male Sprague-Dawley rats were used after a 24-h fast. Drugs were orally administered to groups of five rats 5 h before autopsy. The stomachs were macroscopically inspected and scored as follows: 0, no evidence of gastric lesions; 1, spotty submucosal hemorrhage; 2, some areas of submucosal hemorrhage or appearance of erosion; 3, widespread adherence of blood and large areas of submucosal hemorrhage or one to four small ulcers; 4, more than four small ulcers or one large ulcer (diameter: >3 mm); 5, numerous large ulcers.

Measurement of FR122047 Concentration in Plasma. PRP and PPP were obtained from guinea pigs after oral administration as above described. To 0.1 mL of PRP or PPP was added 0.1 mL of 50% EtOH, 1 mL of pH = 8 buffer solution (Merck), and 4 mL of AcOEt. The mixture was shaken for 10 min and centrifuged at 2500 rpm for 10 min. The organic phase was dried with nitrogen gas and dissolved in 2.3 mM Na_2PO_4 , 1.2 mM KH_2PO_4 , and 50% CH_3CN . Twenty-five microliters of the resulting solution was injected automatically into a HPLC (pump, Waters 6000A; detector, JASCO 821-FD; injector, Waters 710B) with a 15-cm stainless steel column (3.9-mm internal diameter) packed μ BONDASPHERE 5μ CN100A. The flow rate was 1 mL/min.

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Supplementary Material Available: Table listing IR, mass, and 1H -NMR data of compounds 3, 7a–i, 8a–j, 10a–m, 12–19, and 21–22 (4 pages). Ordering information is given on any current masthead page.

References

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