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 nM) 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 μM). 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 μM) 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 μM). 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 μM), while the use of aspirin, a CO inhibitor and the most popular thromboembolic drug, is restricted by the side effect. 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. Many compounds based on specific mediators such as thromboxane A2 (TXA2), prostacyclin, phosphodiesterase (PDE), and thrombin, 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, has shown its effectiveness in clinical trials. However, aspirin, 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”), 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, KBT-3022, and E-5510, 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-5510). We have reported a new type of antiplatelet agent, 5-alkyl-2-(substituted aryl)-4-pyridylimidazoles, which exhibited potent antiplatelet activity with vasodilatation 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, 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 μM) and vasodilatory activity (ED_{50} = 2.0 μM). 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 vasodilatation. Further pharmacological results of the most potent derivative, 4,5-bis(4-methoxyphenyl)-2-[(1-methylpiperazin-4-yl)carbonyl]thiazole (10a, FR122047), 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 into N,N'-dimethylguanidino derivative (7a-e,g-i) was to convert 4 into 4,5-bis(4-methoxyphenyl)-2-thioiudothiazole (5a) via 2-(3-benzoxythioeiredo)-4,5-bis(4-methoxyphenyl)thiazole (6), followed by methylation of the thiazole 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-(methylthioeiredo)thiazole (5b), which was converted into N,N'-dimethylguanidino derivative (7f).
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

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* Analytical results were within ±0.4% of the theoretical value. † The evaluation methods are described in Experimental Section. MDA: inhibitory activity of malondialdehyde production induced by AA. NT: not tested. " 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-((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-1). 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-amino-2-crotonate provided 2-(1,4-dihydro-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-
The synthesis of other derivatives (19-22) is shown in Scheme 5. 2,3,4,5-Tetraydroxy-6-thiobamamonyl-3-pyridazinone was condensed with 2 to give 4,5-bis(4-methoxyphenyl)-2-(2,3,4,5-tetraydroxy-3-oxopyridazin-6-yl)thiazole (19). Reaction of 4-thiobamamolpyridine 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-tetraydroxypridin-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).

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 (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-Tetraydroxy-6-thiobamamonyl-3-pyridazinone was condensed with 2 to give 4,5-bis(4-methoxyphenyl)-2-(2,3,4,5-tetraydroxy-3-oxopyridazin-6-yl)thiazole (19). Reaction of 4-thiobamamolpyridine 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-tetraydroxypridin-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).
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 = 0.0036 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 morpholino (7h) showed potent inhibition of MDA synthesis (79.9% inhibition at 1.0 μM) with weak vasodilatory activity (ED50 = 66 μM).

Compounds 7a and 7d were subsequently 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 aminomethyl) derivatives (8d–j) were tested. 2,3,4,5-Tetrahydro-3-pyridazinone ring (8g), the nucleus of potent phosphodiesterase inhibitors, was introduced onto the amino group in an attempt to increase vasodilatory 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)thiazole (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 vasodilatory activity, 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 vasodilatory 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, 1–k) resulted in decreasing anti-MDA production activity.
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 Ca\(^{2+}\) antagonists,\(^{16}\) to the 2-position of the thiazole ring in an attempt to increase vasodilatory activity provided 13 with disappointing poor vasodilation activity (ED\(_{50}\) > 3.2 \(\mu M\)).

The active amido derivatives in the both tests (10a,c,h, 21, 22) were subjected to ex vivo evaluation (Table 3) to compounds showed potent MDA-production inhibition for itazigrel inhibition 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 in PPP. These results
the ulcerogenesis is a major goal in thromboembolic
genic properties. Development of a CO inhibitor without
administration, inhibiting platelet aggregation. We believe
after Oral Administration on Platelet Aggregation Induced by

\( \text{Table 4. Product Characterization and in Vitro Activity: 4,5-Bia(4-methoxyphenyl)-2-substituted-thiazole Derivatives} \)

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<th>compd</th>
<th>R</th>
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<td>C&lt;sub&gt;29&lt;/sub&gt;H&lt;sub&gt;32&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;S</td>
<td>C, H, N</td>
<td>0.039</td>
<td>5.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Table 1. <sup>b</sup> 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 Vitro<sup>a</sup>

<table>
<thead>
<tr>
<th>AA, dose, mg/kg</th>
<th>collagen dose, mg/kg</th>
<th>3.2</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 h</td>
<td>6 h</td>
<td>1 h</td>
<td>6 h</td>
</tr>
<tr>
<td>aspirin</td>
<td>64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>itazigrel</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>timegaine</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7a</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>7d</td>
<td>100</td>
<td>48.5</td>
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<tr>
<td>8a</td>
<td>100</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>8b</td>
<td>40.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8d</td>
<td>43.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8e</td>
<td>16.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8f</td>
<td>13.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8g</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>8j</td>
<td>100</td>
<td>100</td>
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<td>10a</td>
<td>100</td>
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<tr>
<td>10c</td>
<td>89</td>
<td>-</td>
<td>-</td>
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<tr>
<td>10h</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>60.9</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> The evaluation methods are described in the Experimental Section. <sup>b</sup> <sup>c</sup>: 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
metabolized<sup>17</sup> and excreted after oral administration
except that which binds to CO in platelets with a low K<sub>eff</sub>
value.

As we mentioned in the introduction section, the use of
aspirin, the most widely-used antiplatelet drug, for treat-
ment of thromboembolic disease is limited by its ulceroge-
ic properties. Development of a CO inhibitor without
the ulcerogenesis is a major goal in thromboembolic
research. We evaluated ulcerogenic activity of 10a in rats
(Table 6) according to our two presumptions: (1) com-
ounds 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 PGI<sub>2</sub> synthesis in vascular endothelial cells
is short-lived compared with that in platelets, that is, 10a
may be free from the "aspirin dilemma". As expected,
10a demonstrated no ulcerogenic activity even at 100 mg/
kg, which is 70 times higher than the effective dose (ED<sub>90</sub>
= 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

<table>
<thead>
<tr>
<th>compd</th>
<th>dose, mg/kg</th>
<th>ulcer indexa</th>
<th>no. of rats with ulceration</th>
<th>ex vivoEXOd, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>100</td>
<td>0.0 ± 0.0</td>
<td>0/5</td>
<td>1.4</td>
</tr>
<tr>
<td>aspirin</td>
<td>10</td>
<td>0.6 ± 0.6</td>
<td>1/5</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>2.0 ± 0.8</td>
<td>3/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>2.2 ± 0.6</td>
<td>4/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>3.8 ± 0.2</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>itazigrel</td>
<td>32</td>
<td>0.44</td>
<td>1/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.4</td>
<td>2/5</td>
<td></td>
</tr>
</tbody>
</table>

a Values are means ± SE for five 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 timegadine) 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 in vascular endothelial cells is short-lived compared with that in platelets.1* We think that the reason for the lack of the ulcerogenesis are that (1) compounds with a lipid-insoluble form, at the low pH levels characteristic of the localization in platelets after oral administration could 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 evaporation 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, Finigan TSQ70 and an Hitachi M-80 mass spectrometer), and 60-, 90-, and 200-MHz proton nuclear magnetic resonance (1H-NMR, JEOL, PMX-60S1, Varian EM-390, and Bruker AC200P) spectra. The chemical shift values are reported in parts per million on the 6 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 (IFE) to get 2 (16.4 g, 55.6%): mp 80–84 °C; IR (Nujol) 1685, 1600, 1560, 1500 cm⁻¹; 1H-NMR (60 MHz, DMSO-d6) δ 3.90 (s, 6 H), 7.18 (d, 4 H, J = 8.8 Hz), 7.32 (d, 4 H, J = 8.8 Hz); mass spectrum m/e 250 (M+).

2-Guanidino-4,5-bis(4-methoxyphenyl)thiazole (3). A mixture of 2 (8.00 g, 27.5 mmol) and 2-guanidinomethioamide (4.88 g, 13.1 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 the resulting residue was purified by chromatography over Al2O3 (chloroform (CHCl3)–methanol (MeOH) as eluent) and subsequently recrystallized from EtOH to give 3 (4.80 g, 42.5%): mp 167–168 °C; IR (Nujol) 3500, 3470, 3000, 2920, 1652, 1610, 1580, 1540, 1510, 1490 cm⁻¹; 1H-NMR (90 MHz, DMSO-d6) δ 3.75 (s, 3 H, OCH3), 3.80 (s, 3 H, OCH3), 6.85 (d, 2H, J = 8.8 Hz), 6.94 (d, 2H, J = 8.8 Hz), 7.12 (d, 2H, J = 8.8 Hz), 7.28 (d, 2H, 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 174–175 °C; IR (Nujol) 3250, 3150, 1625, 1600, 1560 cm⁻¹; 1H-NMR (90 MHz, DMSO-d6) δ 3.68 (s, 6 H), 6.7–7.4 (m, 8 H); mass spectrum, m/e 415 (M+), free.

2-(3-Benzoylthioureido)-4,5-bis(4-methoxyphenyl)thiazole (5). A mixture of NH4SCN (12.2 g, 171 mmol) and benzoyl chloride (24.0 g, 171 mmol) in acetonitrile (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 5 (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 3 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, dried over MgSO4. After evaporation, the resulting precipitate was recrystallized from AcOEt to give 5 (41.86 g, 77.1%): mp 184–185 °C; IR (Nujol) 3320, 1600, 1506 cm⁻¹; 1H-NMR (60 MHz, DMSO-d6) δ 7.55 (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+).
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-d6) δ 2.72 and 2.77 (both 3 H, NCH3), 3.73 (3 H, OCH2), 6.7-7.6 (m, 11 H); mass spectrum, m/e 368 (M⁺).

Compounds 7b–g were prepared in a similar manner to 7a. Ethyl 1-ethoxy-2-naphthalene sulfonate (for 7b, 59.4% yield), isopropylamine (for 7c, 84.5% yield), dimethyl hydrochloride (for 7d, 30.4% yield), cyclohexylamine (for 7e, 74.5% yield), N-methylpiperazin-1-yl (for 7f, 32.1% yield), morpholine (for 7g, 68.2% yield), and 1,2-diaminoethane (for 7h, 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 N,N-dimethylformamide (15 mL) and ACN (30 mL) dropwise over 15 min at room temperature. After the reaction mixture was stirred for 4.5 h, the reaction mixture was poured into water (100 mL) and the mixture was filtered and washed with water to afford 5b (0.81 g, 65.7%): mp 201-2 °C; IR (Nujol) 3380, 3170, 1680, 1630, 1610, 1590, 1540, 1510 cm⁻¹; 1H-NMR (200 MHz, DMSO-d6) δ 2.41 (s, 3 H), 2.72 (s, 3 H), 3.74 (s, 6 H), 6.18-7.4 (m, 11 H); mass spectrum, m/e 476 (M⁺).

Compounds 8f,g were prepared in a manner similar to 8a.

4,5-Bis(4-methoxyphenyl)-2-[[N-(2-phenylthioacetetyl)amino]methyl]thiazole (8). 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 water and dried over MgSO4. After evaporation, the resulting residue was dissolved in DMP (60 mL). To the mixture was added 2-phenylthioacetic acid (1.58 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 5 h. The reaction mixture was poured into water and then extracted with AcOEt. The organic layer was washed with saturated NaHCO3 solution, 1N HCl, water, and brine and dried over MgSO4. After evaporation, the resulting residue was recrystallized from AcOEt-Et2O to give 8e (2.77 g, 66.6%): mp 192-3 °C; IR (Nujol) 3360, 1660, 1610, 1520 cm⁻¹; 1H-NMR (200 MHz, DMSO-d6) δ 3.7-3.8 (m, 8 H, 2 × OCH2 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-[[N-(2-ethylthioacetetyl)amino]methyl]thiazole (8f). 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 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 8e (0.81 g, 84.1%): mp 118-20 °C; IR (Nujol) 3300, 1650, 1570, 1540 cm⁻¹; 1H-NMR (60 MHz, DMSO-d6) δ 2.90 (s, 3 H), 2.72 and 2.77 (both s, 3 H), 3.73 and 3.77 (both s, 3 H), 6.7-7.7 (m, 8 H); mass spectrum, m/e 382 (M⁺).

4,5-Bis(4-methoxyphenyl)-2-(2-phenylthioacetetyl)thiazole (8a). A mixture of 2 (2.00 g, 6.88 mmol) and (dimethylamino)methylthiourea (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/EtOAc 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) 3400, 1650, 1510, 1450 cm⁻¹; 1H-NMR (60 MHz, DMSO-d6) δ 3.69 (3 H, NCH3), 6.9 (6 H, 2 × OCH2 and CH2S), 7.23 (5 H, J = 6.0 Hz, NHCO); 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 and AcOEt. The organic layer was washed with saturated K2CO3 solution and then washed with water. The reaction mixture was collected by filtration and washed with water to afford 8c·HCl (3.40 g, 91.6%): mp 192-3 °C; IR (Nujol) 3360, 1660, 1610, 1520 cm⁻¹; 1H-NMR (60 MHz, DMSO-d6) δ 5.69 (3 H, NCH3), 6.37 (3 H, 4.38 (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)methylthiazole (8d). A mixture of 8b (1.00 g, 2.71 mmol) and 55% 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 washed 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 (compounding 8e) 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–EtO 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-d6) δ 3.75 (3 H, 3.77 (3 H), 3.83 (2 H, 4.50 (br s, 4 H, 6.0 Hz), 8.68 (d, 2 H, J = 8.8 Hz), 8.95 (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 (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-methylpiperazin-1-yl (1.80 mL, 15.2 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 EtO and dried to afford 10a (0.58 g, 46.5%): mp 285–30°C; IR (Nujol): 1690, 1530 cm⁻¹; 1H-NMR (200 MHz, DMSO-d₆) δ 7.25 (s, 3 H, NCH₃), 3.0–3.6 (m, 5 H, piperidino), 3.78 (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). Ammonia bubbling to a solution of 9 in MeOH afforded 10b (28.9% yield) in a similar manner to 10a.

The anion 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–1 (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 EtO to give 10e (0.58 g, 56.0%): mp 255–5°C; IR (Nujol): 3420, 3180, 1660, 1630, 1530 cm⁻¹; 1H-NMR (200 MHz, DMSO-d₆) δ 3.76 (s, 3 H), 3.78 (s, 3 H), 6.90 (d, 2 H, J = 9 Hz), 6.95 (d, 2 H, J = 9 Hz), 7.28 (d, 2 H, J = 9 Hz), 7.38 (d, 2 H, J = 9 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 (0.52 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 PE and EtOH to give 10m (0.72 g, 65.0%): mp 157–9°C; IR (Nujol): 3260, 1610, 1510, 1510 cm⁻¹; 1H-NMR (200 MHz, DMSO-d₆) 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 (11l). 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/MeOH as eluent) to afford 11l (0.32 g, 31.6%): mp 187°C, mp 187–188°C; IR (Nujol): 3420, 3180, 1660, 1630, 1510 cm⁻¹; 1H-NMR (200 MHz, DMSO-d₆) δ 3.74 (s, 3 H), 3.78 (s, 3 H), 4.75 (d, 2 H, J = 5.8 Hz, CH₃), 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 (23.2 g, 5 w/w) in AcOEt (250 mL) was stirred at room temperature for 2 h. After removal of the solvents, the resulting precipitate was washed with CH₂Cl₂ and 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), 6.5–6.9 (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-Bis-[4-methoxyphenyl]thiazol-2-yl]-2-cyanoacetate (12l). A mixture of 11 (0.70 g, 2.15 mmol), ethyl cyanoacetate (0.23 mL, 3.15 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 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–
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 µL of 2.5 mM AA; the incubation lasted 5 min. The reaction was terminated by addition of 1 mL of thioarbituric 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% O₂-5% CO₂ at 37 °C under 0.5 g load. Contraction was induced by addition of KCl solution (final concentration was 50 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 ED₅₀ 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 times after oral administration of drugs. The final concentration of collagen was 0.5 µg/mL for guinea pigs and 2.0 µg/mL for rats. AA was used at 50 µM in guinea pigs. The percent inhibition was calculated from the total aggregation.

Gastroenterogenic 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 wide 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 FRI22047 Concentration in Plasma. PRP and PPP were obtained from guinea pigs after oral drug administration as described above. 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 mL NaNO₂, 1.2 mM KH₂PO₄, and 50 mM CH₃CN. 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 ABONDASPERE 5u-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–1, 5a–J, 10a–m, 12–19, and 21–22 (4 pages). Ordering information is given on any current masthead page.

References
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(17) FR122047 (10a) is easily metabolized in rats after oral administration of FR122047 to produce an N-oxidized compound (unpublished results).

(18) The production of TXB2 was inhibited in rats after oral administration of FR122047 (0.1 mg/kg, po) while no effect on that of 6-keto-PGF1α (unpublished results).
