Polycyclic N-Heterocyclic Compounds. Part 62\textsuperscript{1}): Reaction of N-(Quinazolin-4-yl)amidine Derivatives with Hydroxylamine Hydrochloride and Anti-platelet Aggregation Activity of the Products

Kensuke OKUDA,*a Ying-Xue ZHANG,b Hiromi OHTOMO,b Takashi HIROTA,b and Kenji SASAKIb

\textsuperscript{a} Gifu Pharmaceutical University; 1–25–4 Daigaku-nishi, Gifu 501–1196, Japan: and \textsuperscript{b} Faculty of Pharmaceutical Sciences, Okayama University; 1–1–1 Tsushima-naka, Kita-ku, Okayama 700–8530, Japan.

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The reactions of N-(5,6,7,8-tetrahydroquinazolin-4-yl)amidines and their amide oximes with hydroxylamine hydrochloride gave abnormal cyclization products via a ring cleavage of pyrimidine component accompanied with a ring closure of 1,2,4-oxadiazole to give N-[2-[[1,2,4]oxadiazol-5-yl]cyclohexen-1-yl]formamide oximes. Similarly, N-(quinazolin-4-yl)amidines reacted with hydroxylamine hydrochloride gave the same results. The evaluation of inhibitory activities against platelet aggregation \textit{in vitro} is also described to show one derivative has potent activity.

\textbf{Key words} rearrangement; 1,2,4-oxadiazole; anti-platelet aggregation; N-(quinazolin-4-yl)amidine; amide oxime; hydroxylamine hydrochloride

3,5-Disubstituted 1,2,4-oxadiazole is a well utilized scaffold for medicinal chemistry. A class of 3,5-diphenyl-1,2,4-oxadiazole based compounds have been identified as potent sphingosine-1-phosphate-1 (S1P1) receptor agonists with minimal affinity for the S1P2 and S1P3 receptor subtypes.\textsuperscript{2)\textsuperscript{2)} Another derivative, 5-(3-chlorothiophen-2-yl)-3-(5-chloropyridin-2-yl)-1,2,4-oxadiazole has been identified as a novel apoptosis inducer.\textsuperscript{3)\textsuperscript{3) These two examples demonstrate the potential of 3,5-disubstituted 1,2,4-oxadiazole use in the development of new pharmaceutics.

\textbf{1,2,4-Oxadiazoles}\textsuperscript{4)} are usually prepared by \textsuperscript{1) Paal–Knorr type ring closure of O-acylamide oximes, \textsuperscript{5) 2) reaction of imide equivalents with NH\textsubscript{2}OH, \textsuperscript{6) 3) ring closure of N-acyl N'-substituted amidines, \textsuperscript{7) or 4) 1,3-dipolar cycloaddition of nitriles and nitrile oxides. \textsuperscript{8) Additionally, we have reported the pyrimidine ring opening reaction accompanying the formation of the 1,2,4-oxadiazole ring to give \textit{N}-[2-[[1,2,4]oxadiazol-5-yl]cycloalken-1-yl]formamide oximes (1) by the reaction of tricyclic \textit{N}-(aliphatic ring-fused pyrimidin-4-yl)amidine (2) or its amide oxime with hydroxylamine hydrochloride (Fig. 1).\textsuperscript{9—12) In this paper, we applied this reaction to one of the bicyclic \textit{N}-(aliphatic (or aromatic) ring-fused pyrimidin-4-yl)amidines, i.e. \textit{N}-(5,6,7,8-tetrahydroquinazolin-4-yl)amidines (3) and \textit{N}-(quinazolin-4-yl)amidines (4). Since current antiplatelet drugs are known to have certain detrimental side effects and reduced efficacy, we tested these new analogues for anti-platelet aggregation activity.

\textbf{Results and Discussion}

\textbf{Chemistry} First, we dealt with the aliphatic ring-fused amidines (3). As shown in Chart 1, the requisite amidine 3 starting materials were synthesized by previously reported methods.\textsuperscript{10—12) Amidines 3a, b were prepared by the reaction of 4-amino-5,6,7,8-tetrahydroquinazoline (5) with commercially available \textit{N},\textit{N}-dimethylformamide (or acetamide) dimethyl acetal in refluxing toluene. Other amidines 3c—g were produced by the reaction of compound 5 with the Vilsmeier reagent prepared from the corresponding \textit{N},\textit{N}-dimethylamide and phosphoryl chloride. When a hydrogen is attached to the amidine moiety (R/H), the reaction of 3a with 1.2 eq of hydroxylamine hydrochloride in methanol at room temperature gave the amide oxime (6a, 80% yield). 6a was converted to the desired 1,2,4-oxadiazole derivative 7a (22% yield) by reaction with 6 eq of hydroxylamine hydrochloride in a refluxing methanol (Chart 1).

In the \textbf{1H-NMR} spectrum of 7a, a characteristic formamide oxime one-proton doublet (J/Hz) appeared at 7.47 ppm coupled with an adjacent NH proton (J/Hz) which was D\textsubscript{2}O exchangeable. The formamide oxime signal changed to a singlet in the presence of D\textsubscript{2}O.

* To whom correspondence should be addressed. e-mail: okuda@gifu-pu.ac.jp © 2010 Pharmaceutical Society of Japan

![Fig. 1. Substrates (2) and Their Rearranged Products (1)](image)

![Chart 1. Synthesis of 7](image)
one-proton singlet at H-2 (pyrimidine ring) disappeared. One 1,2,4-oxadiazole ring proton was observed at 8.99 ppm as a singlet. These results suggest that pyrimidine ring cleavage and 1,2,4-oxadiazole ring closure occurred when 6a reacted with hydroxylamine hydrochloride.

Reacting alkyl group substituted amidine moieties (R = Me and Et) 3b and 3c with 1.1—1.2 eq of hydroxylamine hydrochloride in methanol at room temperature gave alkyl amide oximes 6b (50%) and 6c (42%), respectively. In the case of 3b, we also obtained the minor product 7b (12%). 6b and 6c were also converted to the desired 1,2,4-oxadiazole derivative 7b (77%) and 7c (84%) by reaction with 1.5—2 eq of hydroxylamine hydrochloride in methanol at room temperature.

Aryl group substituted amidine moieties 4d—g needed an excess amount of hydroxylamine hydrochloride to consume starting materials completely. When 4.6—6 eq of hydroxylamine hydrochloride were used the reaction did not produce amide oximes (6d—g), but gave the desired oxadiazole 7d—g.

A tentative explanation for this reactivity difference is as follows. All amide oximes (6) are expected to dominantly consist of the less sterically hindered (E)-oximes (Fig. 2).13 Isomerization of (E)-oximes to (Z)-oximes must occur to allow ipso attack of the oxime hydroxy group on the pyrimidine ring to start this rearrangement reaction.10—12 In the case of hydrogen or alkyl substituted 6a—c, this (E)-oxime is rather stable. On the other hand, aryl substituents of 6d—g caused (Z)-oxime isomerization due to their steric repulsion creating the 7d—g.

After our work with aliphatic ring-fused amidines, we focused our attention on the aromatic ring-fused amidines (4). As shown in Chart 2, the requisite amidines 4 starting materials were synthesized by the same method as described above for amidines 3.

When a hydrogen is attached to the amidine moiety (R = H) the reaction of 4a with 1.2 eq of hydroxylamine hydrochloride at room temperature gave the 1,2,4-oxadiazole derivative 10a (36%) instead of the amide oxime 9a. This result was quite different compared to the case of N-(aliphatic ring-fused pyrimidin-4-yl)formamidines (2, 3).10—12 In those cases, the amide oximes were obtained when a hydrogen or alkyl group is attached to the amidine moiety. Therefore, in the present study the fused benzene moiety of quinazoline affected the reactivity by facilitating the nucleophilic attack of amide oxime (9) to quinazoline ring. This increased nucleophilic attack lead to a ring-cleavage and ring-closure reaction to give the 1,2,4-oxadiazole derivatives (10a). This assumption is supported by lowest unoccupied molecular orbital (LUMO) energy level comparison of 6a and 9a. Calculated LUMO energy of 6a is −0.6795 eV, whilst that of 9a is −1.5788 eV.13 Therefore, it is reasonable that 9a is more susceptible for nucleophilic attack of amide oxime, because 9a is too unstable to isolate.

Amine with alkyl groups substituted in the amidine moiety 4b, c also gave the desired oxadiazole 10b, c in 65% and 76%, respectively, when reacted with 1.5—2 eq of hydroxylamine hydrochloride. Finally, aryl group substituted amides 4d—g also gave the desired oxadiazole 10d—g when reacted with the 5—10 eq of hydroxylamine hydrochloride.

**Biology** Atherothrombosis is characterized by a quick and unexpected burst of an atherosclerotic plaque and following occlusive thrombus formation. It is the main cause of acute ischemic syndromes such as acute coronary syndrome or ischemic stroke, which are the major cause of death in the developed world. The most critical step of these diseases is platelet activation and aggregation, therefore antiplatelet drugs are used to protect and treat acute ischemic syndromes. Because current antiplatelet drugs can have detrimental side effects and have low efficacy, we have been exploring antiplatelet aggregation agents as possible new drugs.14—21 The inhibitory activities of compounds 7a—g and 10a—g against platelet aggregation induced by arachidonic acid was assayed to demonstrate the possible therapeutic nature of these compounds.

The inhibitory assay was executed by a turbidimetric method developed by Born and Cross22) using an aggregometry. As shown in Table 1, comparison of the inhibition rate of 7a—g at a final concentration of 30 μM with that of Cilostazol23) revealed that the 4-chlorophenyl derivative 7e (75.2%) had potency comparable to Cilostazol (96.5%). Interestingly, neither phenyl derivative 7d nor 4-fluorophenyl derivative 7f showed any significant inhibitory activity against platelet aggregation. In addition, none of compounds 10a—g at a final concentration of 30 μM showed any significant inhibition of platelet aggregation.

In summary, we have developed a method for synthetically

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<th>Compound</th>
<th>% inhibition</th>
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<td>10a</td>
<td>13.9±3.3</td>
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<td>10d</td>
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<td>10e</td>
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<tr>
<td>Cilostazol</td>
<td>96.5±1.3*</td>
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Data represent % inhibition of the vehicle control group (mean±S.E. of 3 experiments). * Means significantly different from the vehicle control group at p<0.01 (Dunnett’s multiple range test).
producing various N-[2-(3-alkyl(or aryl)[1,2,4]oxadiazol-5-yl)cyclohexen-1-yl] (or phenyl)formamide oximes (7, 10) through the reaction of N-[5,6,7,8-tetrahydroquinazolin-4-yl]amidines (3) or N-(quinazolin-4-yl)amidines (4) with hydroxylamine hydrochloride via a ring cleavage of a pyrimidine component accompanied with a ring closure of 1,2,4-oxadiazole. Compound 7e showed considerable inhibitory activity against platelet aggregation comparable to clinically used Cilostazol. We are currently exploring their structure–activity relationships for further elucidation of anti-platelet aggregation compounds.

**Experimental**

All melting points were determined on a Yanagimoto micro-melting point apparatus, and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. The FAB-MS and EI-MS values in "Experimental" were measured on a Japan Spectroscopic FT/IR-200 spectrophotometer with KBr and frequency are expressed in cm⁻¹. The "1H-NMR spectra were recorded on a Varian VX-200 instrument operating at 200 MHz with tetramethylsilane as an internal standard. Chemical shifts are given in ppm (δ) and J values in Hz. Peaks assigned as follows: singlet: δ; doublet: δ, J; triplet: q; quartet: br, broad; m, multiplet. Column chromatography was performed on silica gel (IR-60-63-210-W, Daiso) or aluminum oxide active neutral (Merck). TLC was carried out on Kieselgel 60F254. Column chromatography and/or recrystallization to give 3d (82.8 mg, 24%) as colorless fine crystals, mp 119—120 °C. 1H-NMR (CDCl₃) δ: 1.79 (4H, m, H-6, 7), 2.55 (2H, m, H-5), 2.78 (2H, m, H-8), 3.10 (6H, br s, NMe₆), 7.15—7.35 (5H, m, Ph), 8.26 (1H, s, H-2). FAB-MS m/z: 281 (MH⁺).

3d. To a dry CHCl₃ solution of 3d (300 mg, 2.01 mmol) were added N,N-dimethyl-4-fluorobenzamide (442 mg, 2.41 mmol), POCl₃ (0.28 mm, 3.00 mmol), and triethylamine (1.00 ml, 7.17 mmol) sequentially, and the reaction mixture was refluxed for 24 h. After the workup procedure described above, the residue was purified by silica gel column chromatography (ethyl acetate/n-hexane, 1:1) to give 3d (207 mg, 72%) as colorless needles, mp 187—191 °C. 1H-NMR (CDCl₃) δ: 1.78 (4H, m, H-6, 7), 2.33 (3H, s, Me, 10.0 Hz, changed to 10.0 Hz, 2.32 (3H, s, Me, 10.0 Hz, 2.45 (2H, m, H-5), 2.67 (2H, m, H-8), 7.95 (1H, m, D₂O exchangeable, OH), 8.43 (1H, s, H-2), 10.05 (1H, s, D₂O exchangeable, OH), IR (KBr) cm⁻¹: 3380, 3130 (NH or OH). FAB-MS m/z: 207 (MH⁺).

To a solution of 5a (12 mg, 0.078 mmol) in dry methanol (6.0 ml) was added NH₂OH·HCl (58.0 mg, 0.84 mmol), and the reaction mixture was stirred at room temperature for 4 h. Water was added, and then it was made basic with sat. NaHCO₃ aq. The precipitate was filtered, washed with water then recrystallized with methanol. mp 106—103.6 mg, 10.0 Hz, 100°C. 1H-NMR (CDCl₃) δ: 1.77 (4H, m, H-6, 7), 2.27 (3H, br s, Me), 2.51 (2H, m, H-5), 2.67 (2H, m, H-8), 6.02 (1H, s, H-2). FAB-MS m/z: 295 (MH⁺). Anal. Calc. for C₂₂H₂₂N₂O: C, 73.44; H, 6.75; N, 19.03. Found: C, 73.67; H, 6.74; N, 19.17.

1H-NMR (CDCl₃) δ: 1.78 (4H, m, H-6, 7), 2.33 (3H, s, Me), 2.67 (2H, m, H-8), 7.95 (1H, m, D₂O exchangeable, OH), 8.43 (1H, s, H-2), 10.05 (1H, s, D₂O exchangeable, OH), IR (KBr) cm⁻¹: 3380, 3130 (NH or OH). FAB-MS m/z: 207 (MH⁺).

**Note**

To a solution of 3b (160 mg, 0.733 mmol) in dry methanol (6.0 ml) was added NH₂OH·HCl (56.0 mg, 0.806 mmol), and the reaction mixture was stirred at room temperature for 14 h. Water was added, and then it was made basic with sat. NaHCO₃ aq. The precipitate was filtered, washed with water then recrystallized from methanol to give 7a (250 mg, 1.68 mmol). 1H-NMR (CDCl₃) δ: 1.78 (4H, m, H-6, 7), 2.27 (3H, br s, Me), 2.51 (2H, m, H-5), 2.67 (2H, m, H-8), 6.02 (1H, s, H-2). FAB-MS m/z: 295 (MH⁺). Anal. Calc. for C₂₂H₂₂N₂O: C, 73.44; H, 6.75; N, 19.03. Found: C, 73.67; H, 6.74; N, 19.17.
N-[5,6,7,8-Tetrahydroquinazolin-4-yl]propionamide Oxime (6c) To a solution of 3c (279 mg, 1.20 mmol) in dry methanol (10 ml) was added NH$_2$OH/HCl (100 mg, 1.44 mmol), and the reaction mixture was stirred at room temperature for 20 h. Water was added, and then it was made basic with sat. NaHCO$_3$ aq. The precipitate was filtered, washed with water then recrystallized from methanol to give 6c (110.3 mg, 42%) as colorless fine crystals, mp 200—203 °C. 1H-NMR (DMSO-$d_6$) δ: 1.06 (3H, t, $J=7.6$ Hz, Me), 1.78 (4H, m, H-6, 7), 2.50 (2H, H-2, 5), 2.67 (2H, H-2, H-8), 2.87 (2H, q, $J=7.4$ Hz, CH$_2$Me), 7.89 (1H, d, $J=10.6$ Hz, D$_2$O exchangeable, NH), 8.43 (1H, s, H-2), 10.60 (1H, s, D$_2$O exchangeable, OH). IR (KBr) cm$^{-1}$: 3380, 3130 (NH or OH). FAB-MS m/z: 221 (MH$^+$). Anal. Calcd for C$_3$H$_3$N$_3$O: C, 59.98; H, 7.32; N, 25.44. Found: C, 59.99; H, 7.17; N, 25.18.

N-[2-(1,2,4)Oxadiazol-5-yl]cyclohexen-1-yl]formamide Oxime (7a) To a solution of 6a (360 mg, 1.87 mmol) in dry methanol (50 ml) was added NH$_2$OH.HCl (782 mg, 11.3 mmol), and the reaction mixture was refluxed for 24 h. Water was added, and then it was made basic with sat. NaHCO$_3$ aq., then extracted with CH$_2$Cl$_2$, and the reaction mixture was stirred at room temperature for 3 h. Water was added, and then it was made basic with sat. NaHCO$_3$ aq., and then extracted with CH$_2$Cl$_2$. Organic phase was washed with sat. brine, dried over Na$_2$SO$_4$, then evaporated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/n-hexane, 1:2), then recrystallized from methanol to give 7a (87.1 mg, 27.8%, 75%) as colorless needles, mp 201—203 °C. 1H-NMR (CDCl$_3$) δ: 1.14 (3H, t, $J=7.6$ Hz, Me), 1.69 (4H, m, H-4, 5), 2.45, 2.58 (each 2H, each 2H, each H-4, 5, 6), 2.73 (2H, q, $J=7.5$ Hz, CH$_2$Me), 7.45 (3H, d, $J=10.0$ Hz, changed to singlet after addition of D$_2$O, D$_2$O exchangeable, NH), 10.74 (1H, s, D$_2$O exchangeable, OH), 11.60 (1H, d, $J=10.0$ Hz, D$_2$O exchangeable, NH). IR (KBr) cm$^{-1}$: 3200, 3150 (br, NH or OH). FAB-MS m/z: 237 (MH$^+$). Anal. Calcd for C$_8$H$_6$N$_4$O: C, 55.92; H, 5.75; N, 26.19. Found: C, 55.92; H, 5.75; N, 26.20.

General Procedure for the Reaction of 3d with NH$_2$OH/HCl to Give 7d—g To a solution of 3d (400 mg, 2.76 mmol) and N,N-dimethylformamide dimethyl acetate (350 mg, 2.72 mmol) in dry toluene (30 ml) was refluxed for 12 h. After evaporation of reaction mixture, the residue was recrystallized from n-hexane to give 4a (410 mg, 74%) as colorless fine crystals, mp 75—76 °C (lit.$^{26}$ 69 °C). 1H-NMR (CDCl$_3$) δ: 2.37 (3H, s, NMe, 2), 3.18 (6H, s, NMe$_2$), 7.48—7.59 (1H, m, H-6), 7.75—7.87 (1H, m, H-7), 7.92 (1H, d, $J=7.8$ Hz, H-5), 8.12 (1H, d, $J=8.4$, 1.4 Hz, H-8), 8.80 (1H, s, H-2), 8.90 (1H, s, CH$_2$NMe). FAB-MS m/z: 501 (MH$^+$). Anal. Calcd for C$_{12}$H$_{14}$N$_4$: C, 67.27; H, 6.04; N, 25.98. Found: C, 67.21; H, 6.52; N, 26.08.

N,N-Dimethyl-N-(quinazolin-4-yl)acetaamide (4b) 8 (400 mg, 2.76 mmol) and N,N-dimethylacetamide dimethyl acetate (550 mg, 4.13 mmol) in dry toluene (30 ml) was refluxed for 12 h. After evaporation of reaction mixture, the residue was purified by silica gel column chromatography (ethyl acetate/n-hexane, 1:2) to give 4b (320 mg, 54%) as colorless fine crystals, mp 76—78 °C (lit.$^{26}$ 75—77 °C). 1H-NMR (CDCl$_3$) δ: 2.52 (6H, s, NMe$_2$), 7.44—7.54 (1H, m, H-6, H-7), 7.72—7.83 (1H, m, H-7), 7.90 (1H, d, $J=8.2$ Hz, H-5), 8.21 (1H, dd, $J=8.2$, 1.4 Hz, H-8), 8.80 (1H, s, H-2). FAB-MS m/z: 215 (MH$^+$). Anal. Calcd for C$_7$H$_7$N$_2$: C, 75.98; H, 6.04; N, 25.98. Found: C, 75.76; H, 6.07; N, 25.98.

N,N-Dimethyl-N-(quinazolin-4-yl)propionamide (4c) To a solution of 8 (220 g, 15.2 mmol) in dry CHCl$_3$ (50 ml) were added N,N-diethylpropionamide (1.69 g, 16.7 mmol), POCl$_3$ (2.15 ml, 23.1 mmol), and triethylamine (6.40 ml, 45.9 mmol) sequentially, and the reaction mixture was refluxed for 30 h. Water was added, and then it was made basic with sat. NaHCO$_3$ aq., and then extracted with CH$_2$Cl$_2$. Organic phase was washed with sat. brine, dried over Na$_2$SO$_4$, then evaporated in vacuo. The residue was purified by silica gel column chromatography (acetone/n-hexane, 4:1) and recrystallized from acetonitrile to give 4c. 1H-NMR (CDCl$_3$) δ: 1.14 (3H, t, $J=7.6$ Hz, Me), 2.68 (2H, q, $J=7.6$ Hz, CH$_2$Me), 3.21 (6H, s, NMe$_2$), 7.42—7.52 (1H, m, H-6, H-7), 7.71—7.81 (1H, m, H-7), 7.84 (1H, d, $J=8.3$ Hz, H-5), 8.17 (1H, dd, $J=8.3$, 1.4 Hz, H-8), 8.83 (1H, s, H-2). FAB-MS m/z: 229 (MH$^+$).
A N-Dimethyl-N2-(quinazolin-4-yl)-4-fluorobenzamidine (4f) To a solution of 8 (2.20 g, 15.2 mmol) in dry DC1Cl6 (60 ml) were added N2-Dimethyl-4-fluorobenzamidine (2.79 g, 16.7 mmol), POCl 3 (2.15 ml, 23.1 mmol), and triethylamine (6.40 ml, 45.9 mmol) sequentially, and the reaction mixture was refluxed for 24 h. After the workup procedure described above, the residue was purified by silica gel column chromatography (ethyl acetate/n-hexane, 1:1) to give 4f (350 mg, 10%) as a colorless oil. 1H-NMR (CDCl3): 0.14 (3H, s, Me), 2.00 (3H, s, NMe), 2.33 (3H, s, NMe), 6.91—7.12 (2H, m, H-4, 5), 7.56—7.73 (2H, m, H-5, 6), 8.01 (1H, d, J = 9.9 Hz, changed to singlet after addition of D2O, NH). IR (KBr) cm⁻¹: 3210, 3130 (br) (NH or OH). FAB-MS m/z: 295 (M⁺+H). Anal. Calcld for C17H15FN4 · 0.25AcOEt: C, 68.34; H, 5.42; N, 17.71. Found: C, 68.29; H, 5.30; N, 17.65.

N-Dimethyl-N2-(quinazolin-4-yl)-4-fluorobenzamidine (4f) To a solution of 8 (2.20 g, 15.2 mmol) in dry DC1Cl6 (60 ml) were added N2-Dimethyl-4-fluorobenzamidine (2.79 g, 16.7 mmol), POCl 3 (2.15 ml, 23.1 mmol), and triethylamine (6.40 ml, 45.9 mmol) sequentially, and the reaction mixture was refluxed for 24 h. After the workup procedure described above, the residue was purified by silica gel column chromatography (ethyl acetate/n-hexane, 1:1) to give 4f (350 mg, 10%) as a colorless oil. 1H-NMR (CDCl3): 0.14 (3H, s, Me), 2.00 (3H, s, NMe), 2.33 (3H, s, NMe), 6.91—7.12 (2H, m, H-4, 5), 7.56—7.73 (2H, m, H-5, 6), 8.01 (1H, d, J = 9.9 Hz, changed to singlet after addition of D2O, NH). IR (KBr) cm⁻¹: 3210, 3130 (br) (NH or OH). FAB-MS m/z: 295 (M⁺+H). Anal. Calcld for C17H15FN4 · 0.25AcOEt: C, 68.34; H, 5.42; N, 17.71. Found: C, 68.29; H, 5.30; N, 17.65.

General Procedure for the Reaction of 4 with NH2OH-HCl to give 10 To a solution of amidine (4) in dry methanol was added NH2OH-HCl, and the reaction mixture was stirred at room temperature. Water was added, and then it was made basic with NaHCO3 aq. The precipitate was filtered, washed with water then recrystallized from methanol to give 10.

N-[2-[1,2,4]Oxadiazol-5-yl]phenyl]formamide Oxime (10a) 4a (145 mg, 0.52 mmol) was allowed to react with NH2OH-HCl (61.0 g, 0.878 mmol) in dry methanol (10 ml) for 5 h. 10a (53.4 mg, 36%) was obtained as colorless needles, mp 165—167°C. 1H-NMR (DMSO-d6) 7.30—7.53 (2H, m, H-6), 7.53—7.56 (2H, m, H-5), 7.80 (1H, d, J = 7.7 Hz, H-8), 3.72 (1H, s, H-4), 3.76 (1H, s, H-2). FAB-MS m/z: 201 (M⁺+H). Anal. Calcld for C8H7N2O: C, 64.20; H, 5.17; N, 21.19. Found: C, 64.20; H, 5.17; N, 21.19.

Preparation of Platelet Blood was collected from a male Albino rabbit (3—3.5 kg weight) with 0.1 volume of 3.8% sodium citrate as the anticoagulant. After mixing, platelet rich plasma (PRP) was obtained by removing erythrocytes and leukocytes by centrifugation (4 °C, 1000 rpm, 15 min). Platelet poor plasma (PPP) was prepared by further centrifugation (4 °C, 3000 rpm, 15 min). The PRP was diluted by PPP to be 3.0 x 105 cells/ml, and then used for the aggregation.

Measurement of Platelet Aggregation The plasma described above (221 µl) was preincubated at 37°C for 2 min. Synthetic compounds 7a—g and 10a—g (2 µl) (DMSO solution) was added, followed by an addition of aggregating agent 25 µl (arachidonic acid 25 µg/ml) 1 min later. Platelet aggregation was measured by continuous recording of light transmission through plasma for 10 min using an aggregometer (Sysmex, AA-100) at 37°C. Cilostazol was used as a positive control. All compounds were used at a 30 µM final concentration. The inhibition rate was calculated according to the method already described.44

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References and Notes