# Studies on Antiplatelet Agents. I. Synthesis and Platelet Inhibitory Activity of 5-Alkyl-2-aryl-4-pyridylimidazoles[1)]

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**5-Alkyl-2-aryl-4-pyridylimidazoles were synthesized and tested in rat *ex vivo* platelet aggregation studies. Among these compounds, 2-(2-fluorophenyl)-5-methyl-4-(3-pyridyl)imidazole (25) was most potent, and showed 98% inhibition at a dose of 10 mg/kg (*p.o.*). 25 had inhibitory activity on cyclooxygenase, thromboxane $A_2$ ($TXA_2$) synthetase, and phosphodiesterase, and also showed inhibited KCl-induced contraction of rat aorta. All compounds have little acute toxicity and appear to be free of adverse effects on the stomach.**

**Keywords** platelet aggregation inhibitor; vasorelaxant activity; enzyme activity; 5-alkyl-2-aryl-4-pyridylimidazole; acute toxicity; ulcerogenicity

## Introduction

Recently, much information regarding the role of platelets in various cardiovascular diseases has accumulated,[2)] and many compounds have been synthesized in a search for inhibitors of platelet aggregation[3)] such as aspirin,[4)] E-5510,[5)] iloprost,[6)] and CV-4151[7)]; the effectiveness of these compounds has been demonstrated through laboratory and clinical studies.

Aspirin has been studied most extensively. However, it is wellknown that the platelet aggregation inhibitory activity of aspirin is based on an irreversible inhibition of cyclooxygenase so that aspirin inhibits not only a synthesis of thromboxane $A_2$ ($TXA_2$) in platelets but that of prostaglandin $I_2$ in vascular endothelal cells. As a result, aspirin induces stomach ulcers[8)] (This is often called "aspirin dilemma").

We considered that compounds, possessing platelet aggregation inhibitory activity due to the inhibition of not only cyclooxygenase but other enzymes such as $TXA_2$ synthetase and phosphodiesterase which were involved in platelet aggregation, would eliminate the ulcerogenic activity, and might be more valuable than aspirin as an anti-thrombotic drug.

We recently synthesized 5-alkyl-2-aryl-4-pyridylimidazoles[9)] and tested them in rat *ex vivo* platelet aggregation studies. Among these compounds, 2-(2-fluorophenyl)-5-methyl-4-(3-pyridyl)imidazole (**25**) was most potent, and inhibited cyclooxygenase, $TXA_2$ synthetase, and phosphodiesterase.

Novel imidazoles such as **25** showed less ulcerogenic activity in animal models than aspirin, and exhibited more potent antiplatelet activity *ex vivo* and *in vitro*. Moreover, **25** showed inhibitory activity on KCl-induced contraction of rat aorta, while aspirin has no effect. This may be beneficial in thrombosis.

Here, we describe the synthesis, structure–activity relationships, and pharmacology of novel imidazoles.

**Chemistry** Imidazole derivatives (**10**, **12**—**16**, **18**—**32** and **36**—**48**) were obtained from 3-hydroxy- (**9a**) or 1-hydroxy-imidazole derivatives (**9b**) by treatment with triethyl phosphite as shown in Chart 2. **9a** and **9b** were prepared by condensation of 1-hydroxyimino-2-oxo-1-pyridylethane derivatives (**3**—**8**) with the corresponding aldehydes (**2**) in a mixture of ammonium acetate and acetic acid (method A). 2-(2,4-Dimethoxyphenyl)-5-methyl-4-(3-pyridyl)imidazole (**11**) was prepared from 1-hydroxyimino-1-(3-pyridyl)-2-propanone[10)] (**3**) *via* *N*-hydroxyimidazole (**9c**), obtained from **3** in aqueous ammonia solution, by treatment with phosphorous trichloride (method B). These *N*-hydroxyimidazoles (**9a**—**c**) were used immediately for the following deoxygenation reaction without further purification. In the synthesis of the 2-benzylimidazole derivative (**42**), phenylacetaldehyde dimethyl acetal was used instead of aldehyde (**2**).

The syntheses of **3**—**8** are summarized in Chart 3. **3** was prepared from 3-pyridylacetic acid hydrochloride (**1**) *via* 1-(3-pyridyl)-2-propanone.[11)] The syntheses of **4**—**7** were done similarly. 2-Hydroxyimino-1-(3-pyridyl)-1-ethanone (**8**) was synthesized from 3-acetylpyridine with isoamyl nitrite in the presence of sodium ethoxide in ethanol.

2-(2-Methoxy-4-methylsulfonylphenyl)-5-methyl-4-(3-pyridyl)imidazole (**17**) was obtained from **16** by oxidation reaction with $KMnO_4$ as shown in Chart 4.

2-(2-Aminophenyl)-5-methyl-4-(3-pyridyl)imidazole (**33**) was obtained from 5-methyl-2-(2-nitrophenyl)-4-(3-pyridyl)imidazole which was prepared in the same way as **25**. 2-(2-Acylaminophenyl)-5-methyl-4-(3-pyridyl)imidazoles (**34**, **35**) were prepared from **33** (Chart 4).

2-(2-Fluorophenyl)-5-methyl-4-(1-methyl-1,2,5,6-tetrahydropyrid-3-yl)imidazole (**49**) was prepared from **25**, *via* 3-[2-(2-fluorophenyl)-5-methylimidazol-4-yl]-1-methylpyridinium iodide as shown in Chart 5.

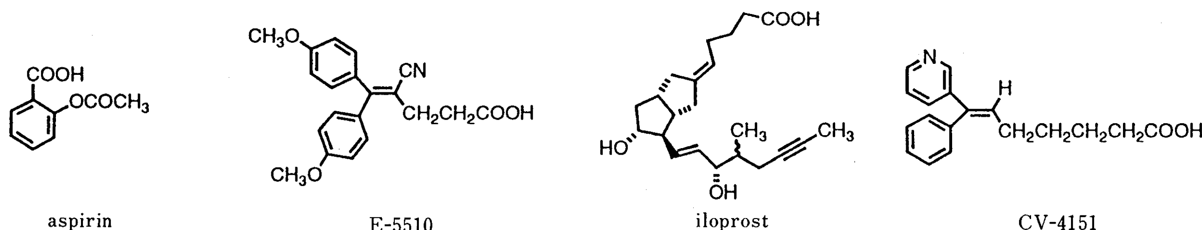


Chart 1

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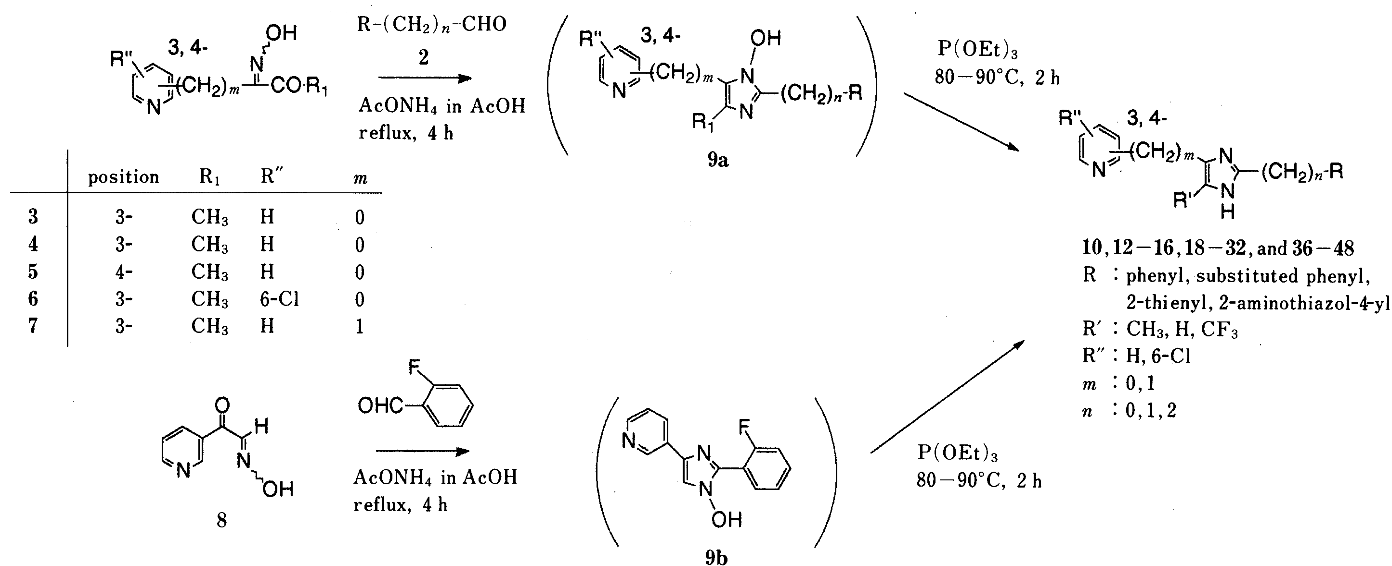
[method A]




| | position | $R_1$ | R″ | *m* |
|---|---|---|---|---|
| **3** | 3- | $CH_3$ | H | 0 |
| **4** | 3- | $CH_3$ | H | 0 |
| **5** | 4- | $CH_3$ | H | 0 |
| **6** | 3- | $CH_3$ | 6-Cl | 0 |
| **7** | 3- | $CH_3$ | H | 1 |

[method B]

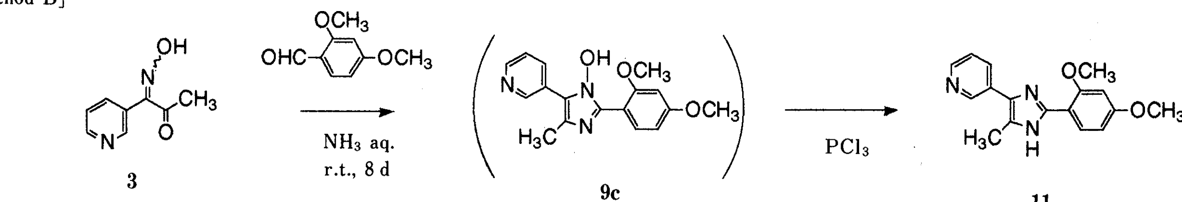
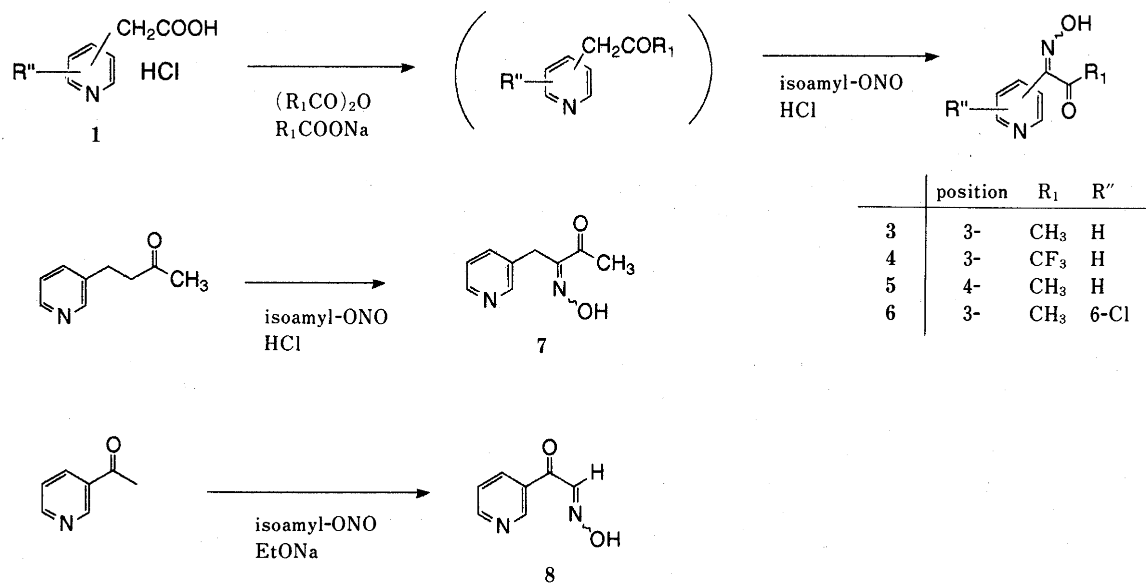



Chart 2




| | position | $R_1$ | R″ |
|---|---|---|---|
| **3** | 3- | $CH_3$ | H |
| **4** | 3- | $CF_3$ | H |
| **5** | 4- | $CH_3$ | H |
| **6** | 3- | $CH_3$ | 6-Cl |

Chart 3

**Pharmacological Results and Discussion**

All of the imidazole compounds in this study were evaluated for inhibitory activity on platelet aggregation induced by collagen in rat *ex vivo*.

To investigate the substituent effects on the benzene ring, the activities of 5-methyl-2-substituted phenyl-4-(3-pyridyl)imidazoles (**10**—**25**) were synthesized and tested, and those results are summarized in Tables I and II. The results showed that the activities of these compounds were affected by substitution on the benzene ring. The introduction of methylsulfonyl (**17**) and hydroxy groups (**23**) onto the benzene ring caused greatly decreased the *ex vivo* activity, compared with the non-substituted compound (**10**). The amino (**19**) and acetylamino derivatives (**20**) were

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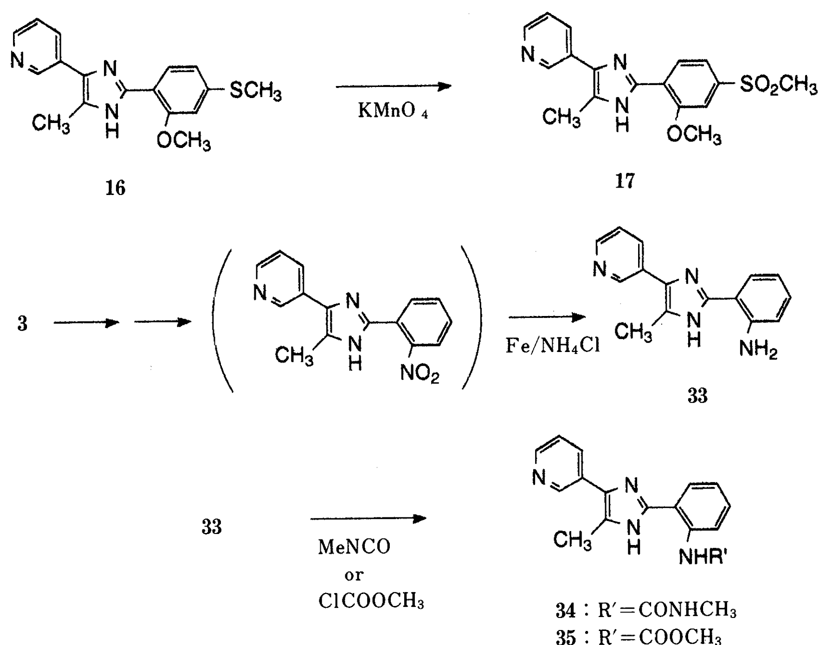


Chart 4

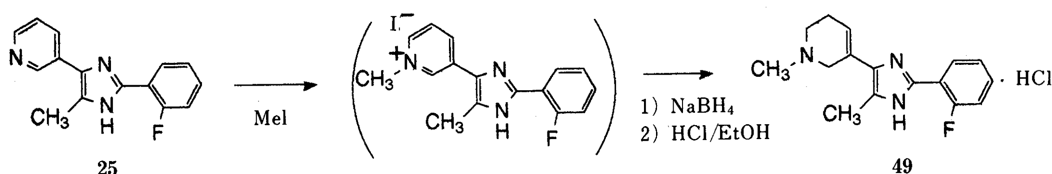


Chart 5

two-fold less active than **10**. On the other hand, the introduction of chloro (**13**—**15**, **21**, **22**) and fluoro (**24**, **25**) substituents almost doubled the *ex vivo* activity compared with **10**. The introduction of methoxyl (**11**, **12**), methylthio (**16**), and methyl groups (**18**, **22**) had little effect on activity. We therefore concluded that mono- and trisubstituted benzene derivatives tended to be more potent than di- and nonsubstituted ones in the relationship between substitution type on the benzene ring and activity. **21** and **25**, which were tri- and monosubstituted benzene derivatives, respectively, were thus selected as lead compounds.

To increase the inhibitory activity of the lead compounds, several derivatives of **21** and **25** were synthesized and their activities were evaluated at a lower dose (10 mg/kg) than used in the above studies (32 mg/kg).

The results of compounds having substituents at the 2-position of the benzene ring (derivatives of **25**) are summarized in Tables I and III. However, these whole derivatives such as alkoxyl (**26**—**30**), methyl (**31**), methylthio (**32**), acylamino (**33**—**36**), and chloro derivatives (**37**) actually decreased in activity.

2,4,5-Trisubstituted benzene compounds (**38**, **39**), derived from compound **21**, were synthesized and their *ex vivo* activities are summarized in Table III. Among these compouns, **39** had more potent activity than **21**, while **38** had less activity. However, the activity of **39** is less than that of **25**. These results showed that **25** was the most potent compound.

To study the structure–activity relationship on **25**, further modification of **25** were performed (Table IV). Substitutions at 5-position of the imidazole ring with trifluoromethyl (**40**) and hydrogen (unsubstituted) (**41**), and introductions of methylene chain between the imidazole and the phenyl groups (**42**, **43**) decreased the activity compared with **25**. Heteroaryl derivatives at 2-position of imidazole (2-thienyl; **44**, 2-amino-thiazolyl; **45**) and modifications of pyridyl moiety at 4-position of imidazole, *i.e.*, 4-pyridyl (**46**), 4-chloro-3-pyridyl (**47**), (3-pyridyl)-methyl (**48**), and 1-methyl-1,2,5,6-tetrahydropyrid-3-yl (**49**), also had less activity than **25**. As a result, no compound which exceeded **25** in potency was obtained in this study.

The most potent compound **25** and the trisubstituted derivative **21** were subsequently subjected to further detailed pharmacological tests which are summarized in Table V. These new imidazole derivatives strongly inhibited *in vitro* collagen-induced platelet aggregation in rabbit platelet-rich plasma (PRP). The $IC_{50}$ value of **25** was $1.7 \times 10^{-6}$ g/ml, which was three times more potent than aspirin. However, neither **21** nor **25** exhibited any activity on ADP induced platelet aggregation, similarly to aspirin. To investigate the mechanism of anti-platelet aggregation activities of these imidazoles, inhibitory activities on various enzymes ($TXA_2$ synthetase, cyclooxygenase, and phosphodiesterase) which were known to play important roles in platelet aggregation, were studied. As shown in Table V, these imidazole derivatives had inhibitory activity on all enzymes, unlike aspirin which showed it only on cyclooxygenase.

Moreover, **21** and **25** displayed inhibitory activity on

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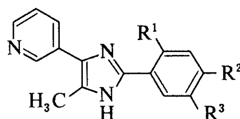
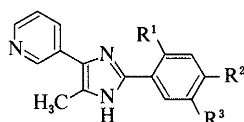
TABLE I. Yield, Melting Point, and Analytical Data of 5-Methyl-2-phenyl-4-(3-pyridinyl)imidazole Derivaties




| | $R^1$ | $R^2$ | $R^3$ | Yield[a] (%) | mp (°C) | Formula | Analysis (%) Calcd | | | Found | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | C | H | N | C | H | N |
| **10** | H | H | H | 32.6 | 206—208 | $C_{15}H_{13}N_3 \cdot 1H_2O$ | 75.99 | 5.61 | 17.72 | 76.00 | 5.65 | 17.51 |
| **11** | $OCH_3$ | $OCH_3$ | H | 58.4 | 164—166 | $C_{17}H_{17}N_3O_2$ | 69.13 | 5.80 | 14.22 | 68.93 | 5.82 | 14.12 |
| **12** | H | $OCH_3$ | H | 45.6 | 199—201 | $C_{16}H_{15}N_3O \cdot 2H_2O$ | 72.43 | 5.69 | 15.83 | 72.28 | 5.77 | 15.62 |
| **13** | $OCH_3$ | Cl | H | 41.0 | 145—147 | $C_{16}H_{14}ClN_3O$ | 64.11 | 4.71 | 14.02 | 64.18 | 4.59 | 13.95 |
| **14** | H | H | Cl | 60.6 | 242—243 | $C_{15}H_{12}ClN_3$ | 66.79 | 4.48 | 15.57 | 66.56 | 4.54 | 15.44 |
| **15** | $OCH_3$ | H | Cl | 86.0 | 193—195 | $C_{16}H_{14}ClN_3O$ | 64.11 | 4.71 | 14.01 | 63.72 | 4.74 | 13.99 |
| **16** | $OCH_3$ | $SCH_3$ | H | 69.9 | 153—156 | $C_{15}H_{12}N_3OS$ | 65.57 | 5.50 | 13.49 | 65.45 | 5.53 | 13.18 |
| **17** | $OCH_3$ | $SO_2CH_3$ | H | 10.9 | 115—118 | $C_{17}H_{17}N_3O_3S \cdot 4H_2O$ | 55.39 | 5.41 | 11.39 | 55.79 | 5.01 | 11.01 |
| **18** | $OCH_3$ | $CH_3$ | H | 61.9 | 125—128 | $C_{17}H_{17}N_3O \cdot 4H_2O$ | 71.25 | 6.26 | 14.66 | 71.37 | 6.15 | 14.61 |
| **19** | $OCH_3$ | $NH_2$ | H | 27.8 | 95—100 | $C_{16}H_{16}N_4O \cdot 3H_2O$ | 63.26 | 6.17 | 18.44 | 63.27 | 5.98 | 18.35 |
| **20** | $OCH_3$ | NHAc | H | 55.6 | 215—218 | $C_{18}H_{18}N_4O_2 \cdot 5H_2O$ | 61.87 | 6.05 | 16.03 | 62.01 | 5.76 | 16.07 |
| **21** | $OCH_3$ | NHAc | Cl | 77.5 | 239—240 | $C_{18}H_{17}ClN_4O_2$ | 60.59 | 4.80 | 15.70 | 60.31 | 4.72 | 15.60 |
| **22** | $OCH_3$ | $CH_3$ | Cl | 70.0 | 185—187 | $C_{17}H_{16}ClN_3O \cdot 5H_2O$ | 63.25 | 5.30 | 13.01 | 63.36 | 4.93 | 13.09 |
| **23** | H | $OCH_3$ | OH | 22.8 | 267—269 | $C_{16}H_{15}N_3O_2 \cdot 4H_2O$ | 66.60 | 5.51 | 14.56 | 66.61 | 5.25 | 14.58 |
| **24** | H | F | H | 60.0 | 123—124 | $C_{15}H_{12}FN_3$ | 71.13 | 4.77 | 16.59 | 70.79 | 4.73 | 16.39 |
| **25** | F | H | H | 51.2 | 194—195 | $C_{17}H_{17}FN_3$ | 71.13 | 4.77 | 16.59 | 71.05 | 4.75 | 16.49 |
| **26** | $OCH_3$ | H | H | 65.6 | 198—200 | $C_{16}H_{15}N_3O$ | 72.43 | 5.69 | 15.83 | 72.19 | 5.67 | 15.64 |
| **27** | OEt | H | H | 60.0 | 166—168 | $C_{17}H_{17}N_3O \cdot 1/4H_2O$ | 71.93 | 6.21 | 14.80 | 72.06 | 6.20 | 14.70 |
| **28** | $OCH_2COOEt$ | H | H | 55.7 | 142—147 | $C_{19}H_{19}N_3O_3 \cdot 1/4H_2O$ | 66.75 | 5.74 | 12.29 | 66.84 | 5.70 | 12.09 |
| **29** | $OCH_2CCH$ | H | H | 54.6 | 167—169 | $C_{18}H_{15}N_3O$ | 74.72 | 5.22 | 14.52 | 74.68 | 5.33 | 14.51 |
| **30** | $OCH_2$(4-Cl)Ph | H | H | 67.8 | 106—108 | $C_{22}H_{18}ClN_3O$ | 70.30 | 4.82 | 11.17 | 70.34 | 4.84 | 11.21 |
| **31** | $CH_3$ | H | H | 31.6 | 129—130 | $C_{16}H_{15}N_3$ | 77.08 | 6.06 | 16.85 | 77.19 | 6.02 | 16.73 |
| **32** | $SCH_3$ | H | H | 37.8 | 200—201 | $C_{16}H_{15}N_3S \cdot 1/4H_2O$ | 67.22 | 5.46 | 14.69 | 67.44 | 5.36 | 14.30 |
| **33** | $NH_2$ | H | H | 52.3 | 221—222 | $C_{15}H_{14}N_4 \cdot 1/10H_2O$ | 71.46 | 5.67 | 22.22 | 71.09 | 5.65 | 21.92 |
| **34** | $NHCONHCH_3$ | H | H | 43.4 | 230—231 | $C_{17}H_{17}N_5O \cdot 1/10H_2O$ | 66.04 | 5.60 | 22.65 | 66.46 | 5.64 | 22.29 |
| **35** | $NHCOOCH_3$ | H | H | 31.9 | 125—126 | $C_{17}H_{16}N_4O_2$ | 66.22 | 5.23 | 18.17 | 66.43 | 5.28 | 17.90 |
| **36** | $NHSO_2CH_3$ | H | H | 29.7 | 245—246 | $C_{16}H_{16}N_4O_2S$ | 58.82 | 4.92 | 17.06 | 58.53 | 4.88 | 16.90 |
| **37** | Cl | H | H | 36.5 | 108—109 | $C_{17}H_{17}ClN_3$ | 64.11 | 4.71 | 14.02 | 64.18 | 4.59 | 13.95 |
| **38** | $OCH_3$ | $OCH_3$ | NHAc | 64.0 | 250—252 | $C_{19}H_{20}N_4O_3$ | 64.75 | 5.72 | 15.89 | 64.67 | 5.60 | 15.93 |
| **39** | $OCH_3$ | NHAc | $NO_2$ | 86.3 | 220—221 | $C_{18}H_{17}N_5O_4 \cdot 1/5H_2O$ | 55.58 | 5.02 | 18.00 | 55.77 | 4.93 | 18.02 |

*a*) Yield from **3**.

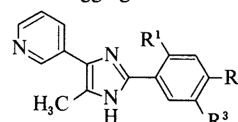
TABLE II. 5-Methyl-2-phenyl-4-(3-pyridinyl)imidazole Derivatives and Their Inhibitory Activities on Platelet Aggregation at 32 (mg/kg)




| | $R^1$ | $R^2$ | $R^3$ | Inhibitory activity[a] (%) |
|---|---|---|---|---|
| **10** | H | H | H | 44.7 |
| **11** | $OCH_3$ | $OCH_3$ | H | 55.5 |
| **12** | H | $OCH_3$ | H | 59.9 |
| **13** | $OCH_3$ | Cl | H | 75.4 |
| **14** | H | H | Cl | 72.6 |
| **15** | $OCH_3$ | H | Cl | 78.5 |
| **16** | $OCH_3$ | $SCH_3$ | H | 55.3 |
| **17** | $OCH_3$ | $SO_2CH_3$ | H | 4.7 |
| **18** | $OCH_3$ | $CH_3$ | H | 49.3 |
| **19** | $OCH_3$ | $NH_2$ | H | 22.6 |
| **20** | $OCH_3$ | NHAc | H | 16.6 |
| **21** | $OCH_3$ | NHAc | Cl | 87.8 |
| **22** | $OCH_3$ | $CH_3$ | Cl | 79.5 |
| **23** | H | $OCH_3$ | OH | 0.4 |
| **24** | H | F | H | 108.0 |
| **25** | F | H | H | 108.6 |

*a*) Inhibitory activities on rat platelet aggregation induced collagen *ex vivo* were measured after 1 h of oral administration of compounds (32 mg/kg).

TABLE III. Further Modification of Compounds **21**, **25** and Their Inhibitory Activities on Platelet Aggregation at 10 (mg/kg)




| | $R^1$ | $R^2$ | $R^3$ | Inhibitory activity[a] (%) |
|---|---|---|---|---|
| **26** | $OCH_3$ | H | H | 48.8 |
| **27** | OEt | H | H | 45.3 |
| **28** | $OCH_2COOEt$ | H | H | 11.6 |
| **29** | $OCH_2CCH$ | H | H | 38.5 |
| **30** | $OCH_2$(4-Cl)Ph | H | H | 14.1 |
| **31** | $CH_3$ | H | H | 6.2 |
| **32** | $SCH_3$ | H | H | 32.3 |
| **33** | $NH_2$ | H | H | 24.7 |
| **34** | $NHCONHCH_3$ | H | H | 46.3 |
| **35** | $NHCOOCH_3$ | H | H | 72.6 |
| **36** | $NHSO_2CH_3$ | H | H | 54.6 |
| **37** | Cl | H | H | 8.5 |
| **25** | F | H | H | 98.0 |
| **38** | $OCH_3$ | $OCH_3$ | NHAc | 24.0 |
| **39** | $OCH_3$ | NHAc | $NO_2$ | 86.3 |
| **21** | $OCH_3$ | NHAc | Cl | 65.0 |

*a*) Inhibitory activities on rat platelet aggregation induced by collagen *ex vivo* were measured after 1 h of oral administration of compounds (10 mg/kg).

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Table IV. Other Imidazole Derivatives and Their Inhibitory Activities on Platelet Aggregation

(General structure: imidazole ring with $R^1$, $R^2$, $R^3$ substituents; N, N–H)

| No. | $R^1$ | $R^2$ | $R^3$ | Inhibitory[a] activity (%) dose (mg/kg) 32.0 | 10.0 | Yield[b] (%) | mp (°C) | Formula | Analysis (%) Calcd (Found) C | H | N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **40** | 3-Py | 2-F-Phenyl | $CF_3$ | 66.1 | 44.0 | 7.0 | 172—173 | $C_{15}H_9FN_3$ | 58.63 (58.68 | 2.95 2.89 | 13.67 13.76) |
| **41** | 3-Py | 2-F-Phenyl | H | 35.8 | | 18.4 | 144—145 | $C_{14}H_{10}FN_3 \cdot 3/10H_2O$ | 68.73 (68.73 | 4.36 4.21 | 17.17 17.38) |
| **42** | 3-Py | Benzyl | $CH_3$ | 78.6 | 60.5 | 32.1 | 171—172 | $C_{16}H_{15}N_3$ ·fumaric acid | 65.74 (65.40 | 5.24 5.26 | 11.50 11.43) |
| **43** | 3-Py | $CH_2CH_2$–(phenyl)–$OCH_3$, $OCH_3$ | $CH_3$ | | 15.0 | 57.1 | 134—136 | $C_{19}H_{21}N_3O_2 \cdot 1/4H_2O$ | 69.59 (69.59 | 6.60 6.51 | 12.81 12.68) |
| **44** | 3-Py | 2-Thienyl | $CH_3$ | | 66.0 | 61.6 | 174—176 | $C_{13}H_{11}N_3S \cdot HCl \cdot 3/2H_2O$ | 44.75 (44.92 | 4.72 4.46 | 12.31 11.95) |
| **45** | 3-Py | (thiazolyl: S, N)–$NH_2$ | $CH_3$ | 54.4 | | 48.6 | >250 | $C_{12}H_{11}N_5S \cdot 1/2H_2O$ | 54.11 (54.37 | 4.54 4.36 | 26.29 26.08) |
| **46** | 4-Py | 2-F-Phenyl | $CH_3$ | 2.2 | | 81.6 | 192—193 | $C_{15}H_{12}FN_3$ | 71.13 (71.24 | 4.77 4.79 | 16.59 16.67) |
| **47** | Cl–(pyridyl, N=) | 2-F-Phenyl | $CH_3$ | 76.0 | 10.4 | 63.4 | 224—225 | $C_{15}H_{11}N_3ClF$ | 62.61 (62.53 | 3.85 3.78 | 14.60 14.64) |
| **48** | (pyridyl, N=)–$CH_2$ | (phenyl)–$OCH_3$, $OCH_3$ | $CH_3$ | 9.3 | | 18.0 | 58—59 | $C_{18}H_{19}N_3O_2 \cdot 1/10H_2O$ | 69.47 (69.33 | 6.21 6.14 | 13.50 13.61) |
| **49** | (tetrahydropyridyl, N–$CH_3$) | 2-F-Phenyl | $CH_3$ | | 36.9 | 40.7 | 132—136 | $C_{16}H_{18}FN_3 \cdot HC \cdot H_2O$ | 50.53 (50.35 | 6.36 6.38 | 11.04 10.86) |
| **25**[c] | 3-Py | 2-F-Phenyl | $CH_3$ | 108.6 | 98.0 | | | | | | |

*a*) Inhibitory activities on rat platelet aggregation induced by collagen *ex vivo* were measured after 1 h of oral administration of compounds (32 and 10 mg/kg). *b*) Analysis data are described in Table I. *c*) Yield from the corresponding hydroxyimino-pyridylethanone derivatives (**3**—**8**).

Table V. Pharmacological Properties of **21**, **25**, and Aspirin

| | **25** | **21** | Aspirin |
|---|---|---|---|
| *In Vitro* rabbits[a] | | | |
| Collagen | $1.7 \times 10^{-6}$ | $1.1 \times 10^{-5}$ | $5.6 \times 10^{-6}$ |
| ADP | $>10^{-4}$ | $>10^{-4}$ | $>10^{-4}$ |
| Inhibitory activities on enzymes[a] | | | |
| $TXA_2$ synthetase | $1.1 \times 10^{-7}$ | NT[b] | $>10^{-4}$ |
| Cyclooxygenase | $1.6 \times 10^{-5}$ | $4.1 \times 10^{-5}$ | $4.0 \times 10^{-5}$ |
| Phosphodiesterase | $3.5 \times 10^{-5}$ | $1.4 \times 10^{-5}$ | $>1.8 \times 10^{-5}$ |
| Inhibitory activity on a KCl induced contraction[a] | $2.5 \times 10^{-5}$ | $6.6 \times 10^{-5}$ | $>10^{-4}$ |
| Acute toxicity 56 mg/kg | 0/5 | 0/5 | NT[b] |

*a*) The evaluation method is described in Experimental section. Values: $IC_{50}$ (g/ml). *b*) NT, not tested.

KCl-induced contraction of rat aorta while aspirin had no effect; the activity in this case might be due to inhibition of phosphodiesterase, and may be beneficial in treatment of thrombotic disease. Furthermore, the acute toxicity of these imidazoles was very low.

Finally, we examined the side effects of **21** and **25** on the rat stomach, since aspirin was known to induce stomach ulcer and its use was therefore restricted. The results along with the *ex vivo* activities are shown in Table VI. Aspirin induced stomach ulcers at a low-dose ($UD_{50} = 16.5$ mg/kg), although it was effective in the rat *ex vivo* at 10 mg/kg. On the other hand, **21** and **25** had no effect on ulcer formation in rats even at doses of 100 and 32 mg/kg, respectively, while these compounds exhibited the inhibitory activities *ex vivo* at a dose of 10 mg/kg.

Further detailed pharmacological tests are being per-

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TABLE VI. The Ulcerogenic and Platelet Inhibitory Activities in Rat *ex Vivo* of **21**, **25**, and Aspirin

| | Dose | **25** | **21** | Aspirin |
|---|---|---|---|---|
| Induced ulcers in rats[a] | | | | |
| | 32 mg/kg | 0/5 | 0/5 | $UD_{50}$ |
| | 100 mg/kg | 0/5 | 2/5 | = 16.5 mg/kg |
| Activities *ex vivo* in rats[b] | | | | |
| | 3.2 mg/kg | 13.8% | NT[c] | 7.5% (1.0 mg/kg) |
| | 10 mg/kg | 98.0% | 65.0% | 86.0% |
| | 32 mg/kg | 108.6% | 87.7% | 100.0% |

*a*) The evaluation method is described in Experimental section. *b*) Inhibitory activities on rat platelet aggregation induced by collagen *ex vivo* were measured after 1 h of oral administration of compounds. *c*) NT, not tested.

TABLE VII. Yield, Melting Point, IR, and Analytical Data of Hydroxyimino-oxo-pyridinylimidazole Derivatives (**3**—**8**)[a]

| | Yield (%) | mp (°C) | IR (Nujol) $cm^{-1}$ | Formula | Analysis (%) Calcd (Found) C | H | N |
|---|---|---|---|---|---|---|---|
| **3** | 58.5 | 203—204 | 2400 (br), 1880 (br), 1680, 1600, 1580, | $C_9H_8N_2O_2$ | 58.53 | 4.91 | 17.06 |
| | | (203—204)[9] | 1510 | | (58.42 | 4.85 | 16.83) |
| **4** | 26.7 | 190—191 | 2400 (br), 1880 (br), 1720, 1605, 1585, | $C_8H_5F_3N_2O_2$ | 44.04 | 2.31 | 12.84 |
| | | | 1510 | | (43.85 | 2.47 | 12.99) |
| **7** | 36.1 | 126—130 | 2700 (br), 1850(br), 1680, 1640, 1600, | $C_9H_{10}N_2O_2$ | 60.66 | 5.65 | 15.72 |
| | | | 1580, 1510 | | (60.56 | 5.72 | 15.67) |
| **8** | 46.0 | 154—157 | 1660, 1590, 1570, 1510 | $C_7H_6N_2O_2$ | 56.00 | 4.02 | 18.65 |
| | | | | | (55.85) | 4.13 | 18.88) |

*a*) **5** and **6** were used for the next reaction without isolation.

formed in order to select a candidate as a new and effective anti-platelet agent.

**Experimental**

Melting point determinations were performed in a capillary melting point apparatus (Thomas Hoover). All melting points are uncorrected. The structures of all compounds were confirmed by their infrared (IR) (Hitachi 260-10) and 60 and 90 MHz proton nuclear magnetic resonance ($^1$H-NMR) (JEOL PMX-60SI and VARIAN EM-390) spectra. The mass spectra were measured with a Hitachi M-80 mass spectrometer. All compounds were analyzed for C, H, N, and the results were within 0.4% of the calculated theoretical values. No attempt was made to maximize the yields.

**1-Hydroxyimino-1-(3-pyridyl)-2-propanone (3)** A mixture of 3-pyridylacetic acid hydrochloride (**1**) (50.0 g, 0.288 mol) and sodium acetate (AcONa) (47.6 g, 0.576 mol) in acetic anhydride ($Ac_2O$) (216 ml, 1.440 mol) was stirred at 75—80 °C for 11 h. The reaction mixture was poured into a mixture of water and ethyl acetate (AcOEt), and the pH of the mixture was adjusted to 7 with $K_2CO_3$. After separation of the layer, the aqueous phase was further extracted with AcOEt, and the combined organic extracts were dried over $MgSO_4$. After the solvent was removed *in vacuo*, the resulting material (39.3 g) was dissolved in a mixture of tetrahydrofuran (THF) (36 ml), water (85 ml) and conc. HCl (36 ml). A mixture of isoamyl nitrite (46.0 ml, 0.346 mol) and THF (50 ml) was added to the reaction mixture, and the whole mixture was stirred at room temperature for 18 h. The pH of the reaction mixture was adjusted to 7 with an aqueous solution of $K_2CO_3$. The resulting precipitates were collected by filtration and washed with water and isopropyl alcohol (IPA). The precipitates were recrystallized from IPA to give **3** (27.64 g, 58.5%).

In the synthesis of **4**, $(CF_3CO)_2O$ and $CF_3COONa$ were used instead of $Ac_2O$ and AcONa. **5**, **6** and **7** were obtained by the same method as **3**, **5** and **6** were used for the following reaction without isolation. The chemical data of these hydroxyimino derivatives (**3**, **4**, **7** and **8**) are summarized in Table VII. Configuration of the hydroxyimino moiety [(*E*) or (*Z*) form] of **3**—**8** was not determined.

**2-Hydroxyimino-1-(3-pyridyl)-1-propanone (8)** To an ice-cooled mixture of NaOEt (5.62 g, 0.0826 mol), isoamyl nitrite (11.9 ml, 0.0894 mol) and EtOH (120 ml) was added dropwise a solution of 3-acetylpyridine (8.33 g, 0.0688 mol) in EtOH (40 ml), and the mixture was stirred at room temperature for 24 h. The pH of the reaction mixture was adjusted to 8 with 1 N HCl, and the mixture was concentrated under vacuum. The resulting precipitates were collected by filtration and washed with water and isopropyl ether (IPE). The precipitates were recrystallized from IPA to give **8** (5.17 g, 46.0%).

**Typical Procedure for Preparation of Imidazoles Method A: 2-(2-Fluorophenyl)-5-methyl-4-(3-pyridyl)imidazole (25)** A mixture of **3** (10 g, 0.0609 mol), 2-fluorobenzaldehyde (15.1 g, 0.122 mol) and ammonium acetate (47.0 g, 0.609 mol) in acetic acid (AcOH) (200 ml) was refluxed for 4 h. After the reaction mixture was allowed to cool to room temperature, it was poured into water (1000 ml), and the pH of the mixture was adjusted to 11 with an aqueous solution of $K_2CO_3$. The mixture was washed with AcOEt, and the aqueous solution was neutralized with 10% aqueous HCl and extracted with $CHCl_3$ (three times). The combined extracts were washed with water and brine, dried over $MgSO_4$, and then evaporated *in vacuo*. A mixture of the resulting residue (15.5 g) and triethyl phosphite (22.8 ml, 0.133 mol) in dimethylformamide (DMF) (120 ml) was stirred at 80—90 °C for 2 h. After cooling, the reaction mixture was poured into water (1000 ml) whereupon some of the dissolved products precipitated. After collection by filtration, the precipitates were recrystallized from EtOH to give **25** (7.19 g, 51.2%).

Other imidazoles **10**, **12**—**16**, **18**—**32**, and **36**—**47** were prepared similarly. For the preparation of 2-benzylimidazole (**42**), phenylaldehyde dimethyl acetal was used. In the syntheses of **40** and **41**, **4** and **8** were used instead of **3**, respectively. For the syntheses of the modified pyridyl derivatives **46**—**48**, the appropriate hydroxyimino compounds **5**, **6** and **7** were used, respectively. The chemical data of whole imidazole derivatives (**10**—**49**) are summarized in Tables I and IV.

**Method B: 2-(2,4-Dimethoxyphenyl)-5-methyl-4-(3-pyridyl)imidazole (11)** A mixture of **3** (1.50 g, 9.14 mmol), 2,4-dimethoxybenzaldehyde

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(1.52 g, 9.14 mmol) in dioxane (30 ml), EtOH (8 ml) and conc. aqueous $NH_3$ (40 ml) was stirred at room temperature for 8 d. After evaporation of the solvent *in vacuo*, the residue was purified by silica gel column chromatography [$CHCl_3$–MeOH (95:5)] to give 1-hydroxy-2-(2,4-dimthoxyphenyl)-5-methyl-4-(3-pyridyl)imidazole (**9c**), which was used in the next reaction without further purification. To an ice-cooled mixture of the above **9c** and DMF (40 ml), was added $PCl_3$ (3.0 ml, 18.3 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with water, neutralized with a saturated aqueous solution of $NaHCO_3$, and extracted with AcOEt (three times). The extracts were washed with water, brine, and dried over $MgSO_4$. After evaporation, the resulting residue was purified by silica gel column chromatography [$CHCl_3$–MeOH (95:5)]. The product was triturated with IPE to give **11** (0.94 g, 34.8%).

**2-(2-Methoxy-4-methylsulfonylphenyl)-5-methyl-4-(3-pyridyl)imidazole (17)** To a solution of **16** (0.93 g, 2.99 mmol) in AcOH (10 ml), was added a suspension of $KMnO_4$ (0.80 g, 5.08 mmol) in water (15 ml) at room temperature, and the mixture was stirred at the same temperature for 1 h. The pH of the mixture was adjusted to 8 with a saturated aqueous solution of $NaHCO_3$, and then $CHCl_3$ was added to the mixture. After separation of the layer, the aqueous phase was further extracted with $CHCl_3$. The combined organic extracts were washed with water, drid over $Na_2SO_4$, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography [$CHCl_3$–MeOH (95:5)] to give **17** (0.16 g, 15.6%).

**2-(2-Aminophenyl)-5-methyl-4-(3-pyridyl)imidazole (33)** 5-Methyl-2-(2-nitrophenyl)-4-(3-pyridyl)imidazole was obtained in a similar manner to that of **25**, and was used for the following reduction reaction without purification. To a mixture of Fe (63.54 g, 1.06 mol) and $NH_4Cl$ (3.21 g, 0.06 mol) in EtOH (520 ml) and water (160 ml), was added a mixture of the above nitro compound (28.07 g, 0.100 mol) and EtOH (250 ml) while being refluxed and vigorously stirred, and the mixture was then refluxed for 1 h. After filtered to remove dissolved matter and washing thoroughly with EtOH, the entire filtrate was evaporated *in vacuo*, and the resulting precipitates were recrystallized from EtOH and IPE to give **33** (17.92 g, 52.3%).

**5-Methyl-2-[2-(3-methylureido)phenyl]-4-(3-pyridyl)imidazole (34)** A mixture of 2-(2-aminophenyl)-5-methyl-4-(3-pyridyl)imidazole (**33**) (0.50 g, 2.00 mmol), MeNCO (0.15 ml, 2.60 mmol), THF (5 ml), and MeOH (2 ml) was stirred at room temperature for 5 h. The resulting precipitate was collected by filtration, recrystallized from EtOH and $CHCl_3$, and washed with EtOH and $Et_2O$ to give **34** (0.51 g, 83%).

**2-(2-Methoxycarbonylaminophenyl)-5-methyl-4-(3-pyridyl)imidazole (35)** To an ice-cooled mixture of 2-(2-aminophenyl)-5-methyl-4-(3-pyridyl) imidazole (**33**) (4.00 g, 16.0 mmol), triethylamine (2.45 ml, 17.6 mmol) and $CHCl_3$ (50 ml), $ClCOOCH_3$ (1.36 ml, 17.6 mmol) were added. The mixture was stirred at room temperature for 10 min, and was poured into a mixture of AcOEt and water. The resulting precipitates were recrystallized from EtOH (450 ml) to give **35** (3.01 g, 61.0%).

**2-(2-Fluorophenyl)-5-methyl-4-(1-methyl-1,2,5,6-tetrahydropyrid-3-yl)-imidazole Hydrochloride (49)** A mixture of **25** (4.0 g, 15.8 mmol), MeI (9.83 ml, 0.158 mol) and acetone (40 ml) was stirred at room temperature for 3 h. The resulting precipitates were collected by filtration, and were washed with acetone to give 3-[2-(2-fluorophenyl)-5-methylimidazol-4-yl]-1-methylpyridinium iodide (5.37 g, 86.0%), mp 266—267 °C. IR (Nujol): 1629, 1585, 1534, 1500 $cm^{-1}$. MS *m/z*: 268 ($M^+ + 1$). These salts were used for the following reaction without further purification.

A solution of the salts (4.73 g, 0.0120 mol) in MeOH (47 ml) was treated with $NaBH_4$ (1.35 g, 0.0360 mol) in small portions at 0 °C. After stirring at room temperature for 3 h, the solvent was removed under reduced pressure. The residue was diluted with water and AcOEt, and the separated organic layer was washed with water and brine, and dried over $MgSO_4$. After evaporation, the residue was dissolved in EtOH, and an EtOH solution of HCl was added. Following evaporation under reduced pressure, the resulting precipitates were recrystallized from EtOH and $Et_2O$ to give **49** (1.96 g, 47.4%).

**Pharmacological Tests *ex Vivo* Studies on Platelet Aggregation:** Male Sprague-Dawley rats weighing about 250 g were used after overnight fasting. One hour after oral administration of test compound (32 mg/kg or 10 mg/kg) or vehicle of test compound (control), blood was collected into a tube containing 0.1 vol. of 3.8% sodium citrate. To 0.45 ml of blood, 0.05 ml of collagen (final concentration 5.0 μg/ml) was added and the mixture incubated for 5 min at 37 °C with shaking. The reaction was terminated by addition of 1 ml of 10 mM phosphate buffered saline (pH = 7.4) containing 11.5 mM EDTA and 1% formalin. The reaction mixture was centrifuged at 70 × *g* for 5 min and platelet count of the upper phase was measured by a Platelet Analyzer 810 (Backer instruments). Platelet aggregation was calculated according to the following formula:

$$\text{platelet aggregation (\%)} = (A - B)/A \times 100$$

*A*: platelet count after addition of vehicle of collagen
*B*: platelet count after addition of collagen

$$\text{inhibition (\%)} = (C - D)/C \times 100$$

*C*: platelet aggregation (%) of control
*D*: platelet aggreagtion (%) of test compound

*In Vitro* Studies on Platelet Aggregation: Male Japanese White rabbits weighing about 2 kg were used. Blood was collected into plastic vessels containing 3.8% sodium citrate (1 volume with 9 volume blood). PRP was obtained by centrifugation of the remaining blood at 120 × *g* for 15 min. To 225 μl of PRP were added 25 μl of test compound dissolved in 25 mM Tris–acetate solution (pH = 7.4) containing 120 mM NaCl, and the mixture was stirred for 2 min at 37 °C. To the solution, 5 μl of collagen (2.5 μg/ml) was added to induce aggregation. Aggregation was measured using an aggregometer (NKK Hema-Tracer 1). Activities of inhibitors (test compounds) were expressed as $IC_{50}$ values, *i.e.*, doses required to inhibit the platelet aggregation response by 50%.

$TXA_2$ Synthetase Inhibitory Activity[12]: Aspirin-treated human platelet microsomes (APM, Ran Biochem, Israel) were used as a source of $TXA_2$ synthetase. APM was suspended with 50 mM Tris–HCl buffer (pH = 7.5), containing 0.1 M NaCl. To 90 μl of APM suspension, 10 μl of test drug solution was added and incubation followed for 3 min at 25 °C. To this reaction mixture, 2 μl of $PGH_2$ solution (10 μg/ml in acetone) was added and the entire mixture was incubated for 3 min at 25 °C. The reaction was stopped by the addition of 10 μl of $FeCl_2$ solution (25 nM in $H_2O$) and left for 15 min at room temperature. The reaction mixture was centrifuged at 10000 rpm for 5 min. $TXB_2$ in the supernatant was measured by radioimmunoassay (Amersham). $IC_{50}$ (inhibition concentration of $TXB_2$ production by 50%) values were graphically calculated.

Cyclooxygenase Inhibitory Activity[13]: Microsomal fraction from sheep seminal vehicle (Ran Biochem) was used as a source of cyclooxygenase. The reaction mixture consisted of 0.1 M Tris–HCl (pH = 7.6), 1 mM epinephrine, 2 mM glutathione, 240 μg of the microsomal enzyme, and the drug to be tested. The reaction was started by the addition of 10 μM [$^{14}$C]arachidonic acid (58 mCi/mmol), performed at 37 °C for 5 min and stopped by the addition of 50 μl of 1 N HCl. Prostaglandins were extracted with 1.5 ml of ethylacetate, and the separated organic layer was dried with nitrogen gas, dissolved into 40 μl of methanol and applied to a thin-layer plate (Merck, Kieselgel 60F). The solvent used for the chromatography was a mixture of ethylacetate and acetic acid (100:2). The prostaglandin $E_2$ ($PGE_2$) fraction was scraped out and the radioactivity was counted with a toluene scintillator.

Phosphodiesterase Inhibitory Activity[14]: Cyclic AMP phosphodiesterase was obtained from rabbit platelets. PRP was centrifuged at 1000 × *g* for 10 min and the pellet was suspended in 25 mM Tris–acetate buffer (pH = 7.4) containing 120 mM NaCl. The pellet was washed twice using the same buffer and finally resuspended in 40 mM Tris–HCl buffer (pH = 7.4). The suspension of cells was sonicated 3 times for 10 s (Tomy, UR-150P). The platelet lysate was centrifuged at 10000 × *g* for 20 min and then recentrifuged at 100000 × *g* for 60 min. The supernatant was stored at −70°C and used as phosphodiesterase.

The phosphodiesterase activity was measured in 500 μl reaction mixture consisting of 40 mM Tris–HCl buffer (pH = 7.4), 1 mM $MgCl_2$, 1.5 × $10^7$ M cyclic AMP (containing $10^{-8}$ M $^3$H-cyclic AMP) and the crude cytosolic enzyme (approximately 10 μg protein). After 10 min at 30 °C, the reaction was terminated by immersing the reaction tube in a boiling water bath for 2.5 min. Snake venom (50 μl of 1 mg/kg atrox crotalus) was then added for 10 min at 30 °C to convert the 5′-AMP to the uncharged nucleotide, adenosine. The addition of 1 ml of an ion-exchange resin slurry (AG 1 × 2) was done to bind all of the unconverted cyclic AMP. After centrifugation, an aliquot (0.25 ml) of the supernatant was removed for quantitative analysis in a liquid scintillation counter.

Vasorelaxant 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, durg solution (dissolved in dimethyl sulfoxide (DMSO)) was added cumulatively and, finally, $10^{-4}$ M of papaverine was added to obtain maximum relaxation. Activities of the test compound

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were expressed as $ED_{50}$ values, *i.e.*, dose required to relax the isolated rat aorta by 50%.

Gastro-Ulcerogenic Activity: Drugs were given *p.o.* to male Sprague-Dawley rats 5 h before autopsy. Gastric lesions were quantified using a scoring system.

**References**

1) H. Takasugi, K. Ito, S. Nishino, A. Tanaka, and T. Takaya, Eur. Pat. 257897 (1988) [*Chem. Abstr.*, **110**, 8207*h* (1988)].
2) *a)* B. Coller, *New Engl. J. Med.*, **322**, 339 (1990); *b)* M. D. Trip, V. M. Cats, F. J. C. vanCapelle, and J. Vreeken, *ibid.*, **322**, 1549 (1990); *c)* W. G. Henderson, S. Goldman, J. G. Copeland, T. E. Moritz, and L. A. Harker, *Ann. Int. Med.*, **111**, 743 (1989); *d)* B. Stein, V. Fuster, D. M. Israel, M. Cohen, L. Badimon, J. Badimon, and J. H. Chesebro, *J. Am. Chem. Soc.*, **14**, 813 (1989).
3) *a)* N. Seko, K. Yoshino, K. Yokota, and G. Tsukamoto, *Chem. Pharm. Bull.*, **39**, 651 (1991); *b)* T. Nishi, K. Yamamoto, T. Shimizu, T. Kanbe, Y. Kimura, and K. Nakagawa, *ibid.*, **31**, 798 (1983).
4) *a)* R. De Caterina, D. Giannessi, A. Boem, W. Bernini, D. Battaglia, C. Michelassi, F. Dell'Amico, A. L'Abbate, P. Patrignani, and C. Patrono, *Thromb Haemat*, **54**, 528 (1985); *b)* W. S. Fields, N. A. Lemak, R. F. Frankowski, and R. J. Hardy, *Stroke*, **8**, 301 (1977); *c)* The Canadian Cooperative Study Group, *N. Engl. J. Med.*, **299**, 53 (1978).
5) T. Fujimori, K. Harada, T. Saeki, M. Koguchi, K. Akasaka, Y. Yamagishi, and I. Yamatsu, *Arzneim.-Forsch./Drug Res.*, **37**, Nr. 10, 1143 (1987).
6) E. Tremoli, S. Colli, and R. Paoletti, *Thromb. Res.*, Suppl., **11**, 33 (1990).
7) I. S. Watts, B. P. White, K. A. Wharton, and P. Lumley, *Br. J. Pharmacol.*, **98**, Dec. Proc. Suppl., 842P (1989).
8) C. Patrono, *Trends in Pharm. Sci.*, **10**, 453 (1989).
9) Both tautmers (see below) of the imidazole ring may be able to exist at room temperature, however, we have not confirmed this, but they are numbered as shown in Chart 6.

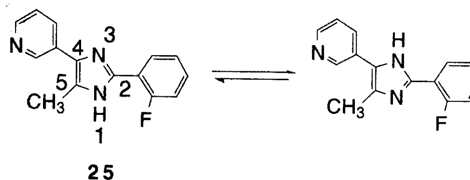

Chart 6

10) R. G. Hunt and S. T. Reid, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 2462.
11) *a)* D. L. Krottinger, R. M. Schumacher, E. H. Sund, and T. J. Weaver, *J. Chemical and Engineering Data*, **19**, 392 (1974); *b)* S. Pavlov and V. Arsenijevic, *Glas. Hem. Drus., Beograd*, **32**, 469 (1967).
12) B. Hammarston and P. Faladaeu, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 3691 (1977).
13) K. Matsuda, K. Ohnishi, E. Misaka, and M. Yamazaki, *Biochem. Pharmacol.*, **32**, 1347 (1983).
14) G. Brooker, L. J. Thomas, Jr., and M. M. Appleman, *Biochemistry*, **7**, 4177 (1968).

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