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Syntheses of 5-Substituted Oxazole-4-carboxylic Acid Derivatives with Inhibitory Activity on Blood Platelet Aggregation

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Methyl 5-substituted oxazole-4-carboxylates were synthesized by the reaction of methyl α-isocyanoacetate with acylating reagents in the presence of bases. The oxazole methyl esters were converted into the carboxylic acids and the carboxamides. Then, *N*-alkyl-oxazole-4-carboxamides were prepared through the oxazole-4-carboxylic acid chloride. Furthermore, 2-substituted oxazoles were synthesized by cyclization of *N*-acyl-α-benzoylglycine methyl esters, which were obtained by acylation of α-benzoylglycine methyl ester followed by ammonolysis of the resulting oxazole methyl esters. These oxazole compounds were evaluated for inhibitory activity on blood platelet aggregation *in vitro* and *ex vivo*. Some of these compounds showed the inhibitory activity comparable to that of aspirin. Of these, 5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxamide was the most active compound in the *ex vivo* test.

Keywords——isocyano compound; oxazole synthesis; anti-platelet agent; structure–activity relationship

In recent years, much information regarding the role of platelets in various cardiovascular diseases has accumulated, and the effectiveness of platelet-active drugs in these diseases has been demonstrated through laboratory studies and clinical trials of the drugs.[1)]

On the other hand, in a series of studies on syntheses of amino acids and related compounds, we previously established a convenient synthetic method for methyl 5-substituted oxazole-4-carboxylates using an anionic amino acid synthon, methyl α-isocyanoacetate.[2)] Fortunately, in our screening of various pharmacological activities of the oxazole compounds obtained by this method, it was observed that some of them possessed inhibitory activity on platelet aggregation. In the present study, we have investigated in detail the syntheses and the activities of various kinds of oxazole carboxylic acid derivatives.

Chemistry

Three types of oxazole compounds used in this study were synthesized as shown in Charts 1, 2 and 3.

Methyl 5-substituted oxazole-4-carboxylates (III, **1**—**20**) were prepared by the reaction of methyl α-isocyanoacetate (IIa, R^2=OMe) with acyl halides (I) in the presence of Et_3N or *tert*-BuOK according to the previous reports.[2-4)] 5-Substituted oxazole-4-carboxylic acids (IVa, **21**—**26**) and carboxamides (IVb, **27**—**32**) were obtained by saponification and ammonolysis, respectively, of the corresponding methyl esters (III) (Chart 1).

N-Alkyl 5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxamides (VI, **33**—**35**) were prepared by the reaction of various amines with the oxazole carboxylic acid chloride (V) which was obtained from the carboxylic acid (IVa, R^2=OH, **26**) and thionyl chloride (Chart 2). Moreover, the morpholinoamide (**36**) was prepared by the reaction of *N*-(α-isocyano-

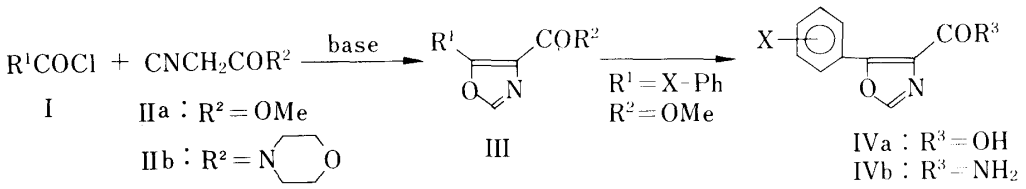


Chart 1

acetyl)morpholine (IIb, $R^2 = N\overset{\frown}{O}$)[4] with 3,4,5-trimethoxybenzoyl chloride in the presence of a metallic base in a similar way to that shown in Chart 1.

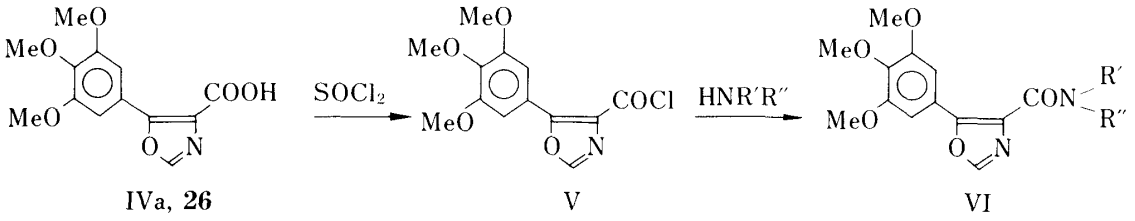


Chart 2

2-Substituted 5-(3,4,5-trimethoxyphenyl)oxazole compounds (X, **37**—**41**) were synthesized by dehydration–cyclization of *N*-acyl-α-(3,4,5-trimethoxybenzoyl)glycine methyl esters (VIII), which were prepared by acylation of α-(3,4,5-trimethoxybenzoyl)glycine methyl ester (VII),[2] using phosphorus oxychloride as a dehydrating agent. Subsequent ammonolysis of the methyl esters (IX) afforded the 2-substituted oxazole-4-carboxamides (X) (Chart 3).

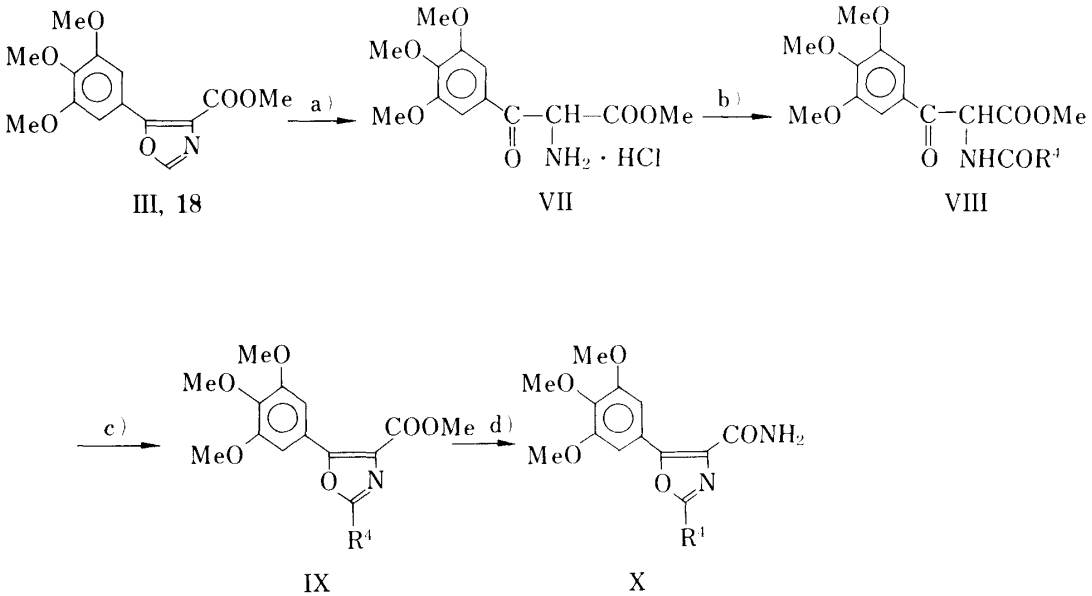
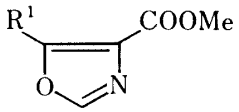


Chart 3

Inhibitory Activity on Blood Platelet Aggregation

All of the oxazole compounds in this study were evaluated for *in vitro* inhibitory activity on platelet aggregation induced by collagen in rat platelet-rich plasma by the method described previously.[5] The *in vitro* active compounds were subsequently subjected to an *ex*

TABLE I. Physicochemical Properties and Inhibitory Activities on Platelet Aggregation of **1**—**20**



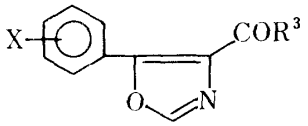
No.	R^1	mp (°C)	Analysis (%) Calcd (Found)			Activity[a] *in vitro* (*ex vivo*)
			C	H	N	
1	Ph	81—82		[b]		—
2	2-F-Ph	61—63	59.73 (59.93	3.65 3.70	6.33 6.36)	+
3	3-F-Ph	60—61	59.73 (59.88	3.65 3.66	6.33 6.36)	—
4	4-F-Ph	90—92	59.73 (59.49	3.65 3.85	6.33 6.33)	+ (±)
5	2-Cl-Ph	100—102	55.59 (55.61	3.39 3.50	5.89 5.77)	—
6	3-Cl-Ph	109—111	55.59 (55.87	3.39 3.64	5.89 5.93)	—
7	4-Cl-Ph	111—112	55.59 (55.57	3.39 3.46	5.89 5.74)	+ (±)
8	2,4-Cl_2-Ph	134—136	48.55 (48.37	2.59 2.63	5.14 5.10)	+ (±)
9	2,6-Cl_2-Ph	90—91	48.55 (48.49	2.59 2.60	5.14 5.12)	—
10	3,4-Cl_2-Ph	143—144		[b]		+ (±)
11	4-Me-Ph	64—65	66.35 (66.39	5.11 5.06	6.45 6.38)	+ (−)
12	2,4-Me_2-Ph	63—64	67.52 (67.48	5.66 5.62	6.06 6.00)	++ (±)
13	4-Et-Ph	53—54	67.52 (67.54	5.66 5.68	6.06 5.97)	++ (−)
14	4-Pr^{iso}-Ph	42—43	68.55 (68.68	6.16 6.16	5.71 5.67)	++ (±)
15	4-Bu^{tert}-Ph	83—84	69.48 (69.49	6.61 6.57	5.40 5.55)	++ (−)
16	4-MeO-Ph	74—75	61.80 (61.54	4.75 4.73	6.01 5.97)	+ (−)
17	3,4-$(MeO)_2$-Ph	112—113	59.31 (59.21	4.98 4.88	5.32 5.40)	+ (−)
18	3,4,5-$(MeO)_3$-Ph	139—140		[b]		+ (±)
19	2-Thienyl	82—83	51.67 (51.47	3.37 3.28	6.70 6.68)	+
20	2-Furyl	97—98	55.96 (55.80	3.65 3.54	7.25 7.15)	—

a) Symbols (−, ±, +, and ++) are defined in the experimental section.
b) Lit. 2).

vivo test using rats.

The inhibitory activities of a series of methyl 5-substituted oxazole-4-carboxylates (**1**—**20**) at a concentration of 100 μg/ml are summarized in Table I. As shown in the table, although methyl 5-phenyloxazole-4-carboxylate (**1**), as a typical compound in this series, scarcely exhibited the inhibitory activity *in vitro*, the 5-(4-halophenyl)oxazole compounds (**4**

TABLE II. Physicochemical Properties and Inhibitory Activities on Platelet Aggregation of **21**—**32**



No.	X	R^3	mp (°C)	Analysis (%) Calcd (Found)			Activity[a] *in vitro* (*ex vivo*)
				C	H	N	
21	4-Me	OH	192—194	65.02 (64.86	4.46 4.83	6.89 6.85)	−
22	2,4-Me_2	OH	180—182	66.35 (66.51	5.11 5.06	6.45 6.48)	−
23	4-Et	OH	150—152	66.35 (66.28	5.11 5.10	6.45 6.50)	+ (±)
24	4-MeO	OH	154—156	60.27 (60.45	4.14 4.08	6.39 6.47)	−
25	3,4-$(MeO)_2$	OH	188—190[b]	57.83 (57.55	4.45 4.39	5.62 5.53)	−
26	3,4,5-$(MeO)_3$	OH	176—177	55.91 (55.85	4.69 4.91	5.02 5.05)	−
27	4-Me	NH_2	226—228	64.09 (64.23	5.92 6.10	13.72 13.81)	+ (−)
28	2,4-Me_2	NH_2	188—190	66.65 (66.79	5.60 5.52	12.96 12.97)	++ (+)
29	4-Et	NH_2	194—196	66.65 (66.65	5.60 5.56	12.96 13.06)	++ (+)
30	4-MeO	NH_2	208—209	60.55 (60.48	4.62 4.55	12.84 12.99)	+ (±)
31	3,4-$(MeO)_2$	NH_2	182—183	58.06 (58.00	4.87 4.97	11.29 11.08)	+ (±)
32	3,4,5-$(MeO)_3$	NH_2	170—171	56.11 (56.10	5.07 5.05	10.07 10.11)	+ (++)

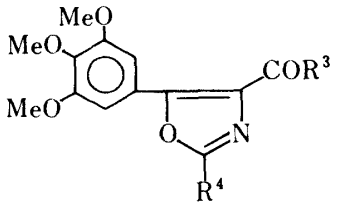
a) Symbols are defined in the experimental section.
b) dec.

and **7**) showed the activity. The 2,4- and 3,4-dichlorophenyl derivatives (**8** and **10**) also had the activity. On the other hand, even among the halophenyl derivatives, the 3-fluoro-, 2-chloro-, 3-chloro-, and 2,6-dichlorophenyloxazoles (**3**,**5**,**6**, and **9**) did not show the activity. These results suggested that substitution at the 4-position on the phenyl ring contributes to the inhibitory action.

Furthermore, it was found that the compound (**11**) having a 4-methylphenyl group in place of the 4-chlorophenyl group at the 5-position on the oxazole skeleton showed inhibitory activity, and the compound (**12**) possessing a 2,4-dimethylphenyl moiety was more active than **11**. The activity of **12** was comparable to that of aspirin (acetylsalicylic acid), used as a standard drug.

To examine the effect of substituent bulkiness on the phenyl ring of **11**, the methyl group was replaced by ethyl, isopropyl and *tert*-butyl groups. These three compounds (**13**, **14** and **15**) were found to be as active as **12**, indicating that such a replacement results in an increase in the inhibitory activity. The activities of the oxazole compounds having a methoxy group on the phenyl ring were also determined. The 4-methoxy-, 3,4-dimethoxy-, and 3,4,5-trimethoxyphenyl compounds (**16**, **17**, and **18**) were all active, suggesting that the introduction of

TABLE III. Physicochemical Properties and Inhibitory Activities on Platelet Aggregation of **33**—**41**



No.	R^3	R^4	mp (°C)	Analysis (%) Calcd (Found)			Activity[a] *in vitro* (*ex vivo*)
				C	H	N	
33	NHMe	H	105—106	57.52 (57.56	5.52 5.48	9.58 9.67)	+
34	$NHPr^{iso}$	H	95—97	59.99 (59.97	6.29 6.28	8.75 8.77)	+ (−)
35	$N<^{Me}_{Me}$	H	132—134	58.81 (58.81	5.92 5.92	9.15 9.23)	−
36		H	142—144	58.61 (58.60	5.79 5.75	8.04 7.99)	−
37	NH_2	Me	191—193	57.53 (57.46	5.52 5.72	9.59 9.55)	+ (±)
38	NH_2	Pr^{iso}	125—127	59.99 (59.63	6.29 6.18	8.75 8.49)	+ (±)
39	NH_2	Bu^{tert}	142—144	61.07 (60.67	6.63 6.61	8.38 8.15)	+ (±)
40	NH_2		162—163	63.32 (63.26	6.71 6.65	7.77 7.54)	−
41	NH_2	Ph	175—177	64.40 (64.47	5.12 5.22	7.91 7.69)	−

a) Symbols are defined in the experimental section.

bulky groups into the phenyl ring increases the potency. In addition, it was found that the introduction of a thienyl group (**19**) in place of the phenyl group at the 5-position on the oxazole ring exhibited the inhibitory activity, but the furyl compound (**20**) did not show the activity. Unfortunately, none of the *in vitro* active compounds in this series showed *ex vivo* activity at a dose of 100 mg/kg (*p.o.*).

In view of the effectiveness of the ester moiety of the oxazole compounds, the acid derivatives (**21**—**26**) and the carboxamides (**27**—**32**) were synthesized, and their inhibitory activities on platelet aggregation were examined. The results indicated that the conversion of the ester derivatives into the acids decreased the activity. On the other hand, surprisingly, the introduction of an amide moiety markedly increased both the *in vitro* and *ex vivo* potencies. In particular, **32** was the most potent of these amide derivatives in the *ex vivo* test, and its inhibitory activity was comparable to that of aspirin (Table II). Thus, **32** was selected for further study as a candidate of anti-platelet drug.

To find other candidates possessing higher activity than **32**, modification of the 4-carboxamide group to form the *N*-alkylamides (**33**—**36**) was carried out, but both activities were decreased, as shown in Table III. Furthermore, we synthesized various 2-substituted 5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxamides (**37**—**41**). The inhibitory activities of these compounds are listed in Table III. Although the compounds **37**—**39** were active *in vitro*, the *ex vivo* activities were much weaker than that of **32**. In addition, **40** and **41**, having bulky

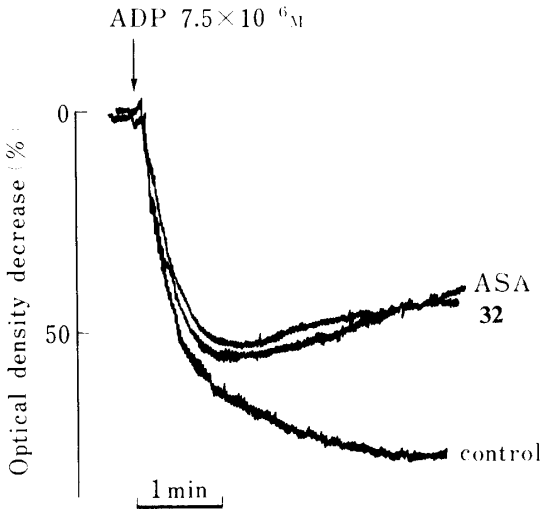


Fig. 1. Effects of Compound **32** and Acetylsalicylic Acid (ASA) on ADP-Induced Platelet Aggregation in Human PRP

substituents, were inactive even in the *in vitro* test.

For the most active compound (**32**) in this study, an *in vitro* test was carried out using human platelets instead of rat platelets. At a concentration of 100 μg/ml, the secondary phase of adenosine 5′-diphosphate (ADP)-induced platelet aggregation was almost completely inhibited, while the primary aggregation was unaffected, as shown in Fig. 1. This inhibitory pattern is similar to that of aspirin, and the degree of the inhibition was also similar to that of aspirin. However, the mechanism of its inhibitory action remains to be investigated.

Experimental

Apparatus——Melting points were measured with a Yamato melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded with a Shimadzu IR-27G spectrophotometer and nuclear magnetic resonance (NMR) spectra with a Hitachi Perkin-Elmer R-20A high resolution NMR spectrometer using tetramethylsilane as an internal standard. Column chromatography was carried out on silica gel (Kieselgel 60, 0.063—0.200 mm, E. Merck). Platelet counts were determined with a Microcellcounter (CC-1002 type, Toa Medical Electronics Co., Ltd.). Platelet aggregation was measured in a Sienco aggregometer (model DP247-E. Sienco Inc.).

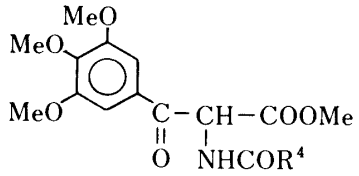
Materials——Methyl α-isocyanoacetate and *N*-(α-isocyanoacetyl)morpholine were prepared according to the reported method.[6] Methyl 5-substituted oxazole-4-carboxylates were also synthesized by the procedure previously reported[2] and obtained in 70—85% yields.

Preparation of 5-(3,4,5-Trimethoxyphenyl)oxazole-4-carboxylic acid (26)——Methyl 5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxylate (**18**) (8.8 g) was dissolved in a mixture of 85% KOH (2.1 g), H_2O (50 ml) and MeOH (200 ml). The solution was allowed to stand for 18 h at r.t. and then evaporated to dryness *in vacuo*. The residue was dissolved in water. The aqueous solution was washed with Et_2O and then acidified with conc. HCl. The resultant oil was extracted with AcOEt, and the organic layer was washed with brine and dried over $MgSO_4$. The solution was concentrated *in vacuo* and the crude product was recrystallized from AcOEt to yield 6.6 g of **26** (79%). Other oxazole-4-carboxylic acids (IVa, **21**—**25**) were obtained in a similar manner in 75—85% yields.

Preparation of 5-(3,4,5-Trimethoxyphenyl)oxazole-4-carboxamide (32). The oxazole methyl ester (**18**) (5.8 g) was dissolved in 20% NH_3–MeOH (100 ml) and the mixture was allowed to stand for 3 d at r.t. The mixture was evaporated to dryness *in vacuo* and the residue was recrystallized from MeOH to afford 4.1 g of **32** (75%). The oxazole-4-carboxamides (IVb, **27**—**31**) were prepared in the same way in 75—85% yields.

Preparation of *N*-Methyl-5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxamide (33)——A mixture of the oxazole-4-carboxylic acid (**26**) (4.0 g), $SOCl_2$ (4 ml) and toluene (40 ml) was refluxed for 2 h and then concentrated *in vacuo* to give a crystalline residue, 5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxylic acid chloride; mp 128—133 °C; IR ν_{max}^{Nujol} cm^{-1}: 3120, 1750, 1590. The acid chloride dissolved in tetrahydrofuran (THF) (30 ml) was added to a mixture of methylamine hydrochloride (1.5 g), Et_3N (2.3 g) and THF (30 ml) at 0—10 °C. The mixture was stirred for 1 h, then evaporated to dryness *in vacuo*. The residue was extracted with AcOEt, and the solution was washed successively with 10% HCl, satd. $NaHCO_3$, and brine, and dried over $MgSO_4$. The solvent was removed *in vacuo* and the product was separated by column chromatography using $CHCl_3$ as an eluent. Recrystallization of the crude crystals from iso-Pr_2O gave 3.6 g of **33** (86%). Compounds **34** and **35** were prepared by a similar procedure in 83 and 95% yields, respectively.

TABLE IV. Physicochemical Properties of Methyl *N*-Acyl-α-(3,4,5-trimethoxybenzoly)glycinates



R^4	Yield (%)	mp (°C)	Analysis (%) Calcd (Found)		
			C	H	N
Me	92	131—133	55.38 (55.40	5.89 5.91	4.31 4.32)
Pr^{iso}	88	115—117	57.78 (57.82	6.56 6.46	3.96 3.94)
Bu^{tert}	87	83—85	58.84 (58.84	6.86 6.87	3.81 3.80)
H	87	121—124	61.06 (61.36	6.92 6.98	3.56 3.56)
Ph	90	119—120	62.01 (61.96	5.46 5.45	3.62 3.56)

Preparation of 4-Morpholinocarbonyl-5-(3,4,5-trimethoxyphenyl)oxazole (36)——A solution of 3,4,5-trimethoxybenzoyl chloride (4.5 g) in THF (50 ml) was added to a mixture of *N*-(α-isocyanoacetyl)morpholine (3.0 g), *tert*-BuOK (2.2 g) and THF (50 ml) below 0 °C. The mixture was stirred for 1 h at r.t. and then evaporated to dryness *in vacuo*. The residue was extracted with AcOEt, and the extract was washed with water and dried over $MgSO_4$. The solvent was removed *in vacuo* and the resulting crystals were collected by filtration. Recrystallization from AcOEt–iso-Pr_2O afforded 4.0 g of **36** (59%).

Preparation of 2-Methyl-5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxamide (37)——The α-(3,4,5-trimethoxybenzoyl)glycine methyl ester hydrochloride (VII) (12 g), which was prepared by the reported method,[2] was added to a mixture of AcOEt (100 ml), $NaHCO_3$ (10 g) and water (100 ml) precooled to 0—5 °C. Acetic anhydride (4.8 g) was added dropwise to the mixture and the whole was stirred for 3 h at r.t. Then the AcOEt layer was separated, washed with 10% HCl and brine, and dried over $MgSO_4$. The solvent was removed *in vacuo* and the residue was crystallized with iso-Pr_2O. The crystals were recrystallized from AcOEt to afford *N*-acetyl-α-(3,4,5-trimethoxybenzoyl)glycine methyl ester (11.2 g, 93%). Other *N*-acylglycine derivatives (VIII) were also prepared under the same conditions as above. The yields and characterization of the products are shown in Table IV.

The *N*-acetylglycine ester (10 g) was dissolved in *N*,*N*-dimethylformamide (DMF) (50 ml), and $POCl_3$ (9.4 g) was added at r.t. After being stirred for 2 h, the solution was poured into water. The mixture was extracted with AcOEt and the solution was washed with satd. $NaHCO_3$ soution. After being dried over $MgSO_4$, the solution was evaporated to dryness *in vacuo* and the resulting crystals of methyl 2-methyl-5-(3,4,5-trimethoxyphenyl)oxazole-4-carboxylate (IX, R^3 = Me) were collected by filtration. The crystals were dissolved in 20% NH_3–MeOH (100 ml) and the usual work-up gave 7.5 g of **37** (83.5%). Other 2-substituted oxazole-4-carboxylates (X, **38**—**41**) were obtained by a similar method in 80—85% yields.

Pharmacological Tests——*In Vitro*: Platelet aggregation was determined by the method described elsewhere[5] using rat and human platelet-rich plasma (PRP). The platelet aggregation-inhibiting activity (inhibition %) of a test compound was calculated by the following formula:

$$\text{inhibition } \%\ (I) = 1 - \left[\frac{\text{maximal aggregation (\%) in the presence of a test compound (100 } \mu\text{g/ml)}}{\text{maximal aggregation (\%) in the absence of a test compound (control)}} \right] \times 100$$

The potency of the platelet aggregation–inhibiting activity of the test compound was expressed as follows:

−: $I < 10$

+: $10 \leq I < I_{ASA}$ (the inhibition % of aspirin)

++: $I_{ASA} \leq I$

Ex Vivo: The test compound (100 mg/kg) dissolved or suspended in 0.25% carboxymethylcellulose (CMC) aqueous solution (10 ml/kg) was orally administered to male Sprague-Dawley rats (body weight 150—200 g, three rats in each group) which had been fasted for about 20 h. One hour after oral administration of the compound, 4.5 ml of blood was collected from the abdominal aorta under ether anesthesia into a plastic syringe containing 0.5 ml of aqueous 3.8% (w/v) trisodium citrate solution.

The platelet aggregation-inhibiting activity (inhibition %) of the test compound was calculated by the following formula:

$$\text{inhibition } \% = 1 - \left[\frac{\begin{array}{l}\text{mean value of maximal aggreagation} \\ (\%) \text{ in the medicated group}\end{array}}{\begin{array}{l}\text{mean value of maximal aggregation} \\ (\%) \text{ in the non-medicated group} \\ (i.e., \text{ control group})\end{array}} \right] \times 100$$

The potency of platelet aggregation-inhibiting activity of the test compound was expressed as (−) if all three rats showed less than 10% inhibition of platelet aggregation; (±) if one or two rats showed not less than 10% inhibition; (+) if all three rats showed not less than 10% inhibition but less than the inhibition (%) of ASA-treated rats; (++) all three rats showed not less than the inhibition (%) of ASA-treated rats.

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References

1) W. Friedewald, R. I. Levy, and E. Braunwald, *Circulation*, **62** (Supple V), V-1 (1980).
2) M. Suzuki, T. Iwasaki, K. Matsumoto, and K. Okumura, *Syn. Commun.*, **2**, 237 (1972).
3) M. Suzuki, T. Iwasaki, M. Miyoshi, K. Okumura, and K. Matsumoto, *J. Org. Chem.*, **38**, 3571 (1973).
4) U. Schöllkopf and R. Schröder, *Angew. Chem.*, **83**, 358 (1971); R. Schröder, U. Schöllkopf, E. Blume, and D. Hoppe, *Justus Liebigs Ann. Chem.*, **1975**, 533.
5) A. Shinjo, Y. Sasaki, M. Inamatsu, and T. Morita, *Thromb. Res.*, **13**, 941 (1978).
6) K. Matsumoto, M. Suzuki, N. Yoneda, and M. Miyoshi, *Synthesis*, **1977**, 249.