Synthesis and Antiplatelet Activities of *N*-Arylmethyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-one Derivatives

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A series of new 1- and 2-arylmethyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-one derivatives were synthesized and examined for their antiplatelet activities. Some of these compounds showed significant inhibitory activities. Among them, 1-phenylmethyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (4a), 2-(2′-methoxyphenyl)methyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(2*H*)-one (3e) and 2-(3′-methoxyphenyl)methyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-(2*H*)-one (3f) were the most effective. These inhibitors acted in a concentration-dependent manner. The antiplatelet effect of compound 3f is due to the inhibition of thromboxane A_2 formation and the blockade of thromboxane A_2/prostaglandin endoperoxide receptor in washed rabbit platelets.

Keywords pyrano[2,3-*c*]pyrazol-6-one; alkylation; *N*-arylmethyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-one; 2D NMR; antiplatelet activity; structure–acivity relationship; thromboxane A_2 formation; thromboxane A_2/prostaglandin endoperoxide receptor

Introduction

In the course of our project toward the investigation of the synthesis and biological activities of pyrano[2,3-*c*]pyrazol-6-ones, we have reported the pharmacological activities of some derivatives of 3,4-dimethylpyrano[2,3-*c*]pyrazol-6-ones.[1-5]

Recently, 2-phenylmethylpyrano[2,3-*c*]pyrazol-6(2*H*)-one (**3a**) and 1-phenylmethylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (**4a**) were synthesized and found to reveal significant antiplatelet activity (Tables V, VI). Therefore, a series of *N*-arylmethyl analogues of compounds **3a** and **4a** were synthesized and evaluated for their antiplatelet activities.

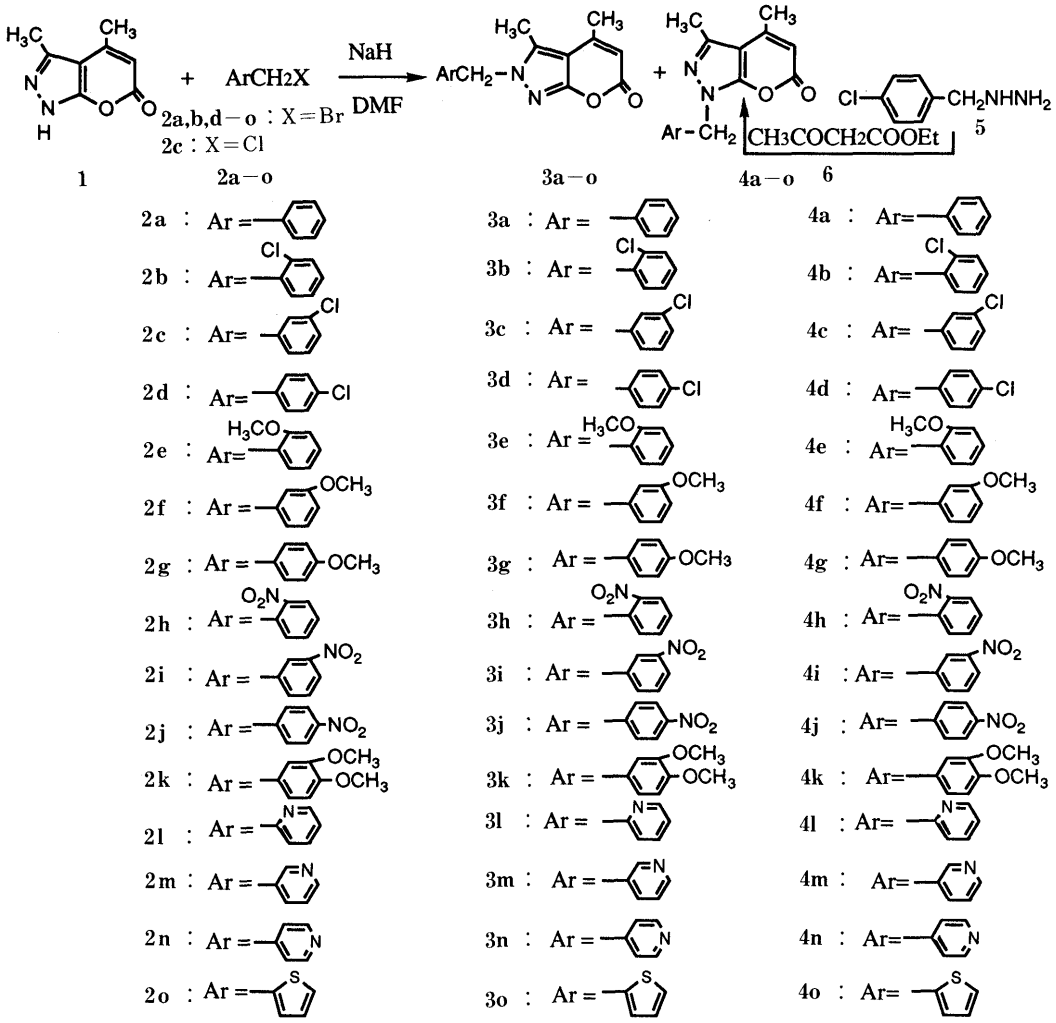


Chart 1

This report describes the synthesis and the antiplatelet activities of these analogues.

Results and Discussion

Chemistry As shown in Chart 1, when compound **1** was treated with NaH in dry dimethylformamide (DMF), followed by reaction with *p*-chlorobenzyl bromide (**2d**) at 45 ± 5 °C, two products (**3d**, **4d**) were obtained. The relative yield of these two products was around 5 : 1. Based on high resolution mass spectroscopy, the molecular formula of both products was determined to be $C_{15}H_{13}ClN_2O_2$, which indicated that the two compounds could be isomers of *N*-(*p*-chlorophenyl)methylpyrano[2,3-*c*]pyrazol-6-ones (**3d**, **4d**).

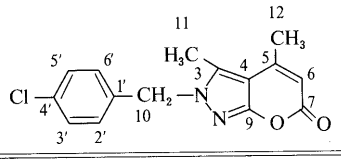
In the previous paper of this series,[1] we reported that N^1-alkyl and N^2-alkyl derivatives of compound **1** exhibit characteristic difference in their physical properties such as *Rf* value thin layer chromatography (TLC), ultraviolet (UV) λ_{max}, chemical shifts of the 3-CH_3 signal in proton nuclear magnetic resonance (^{1}H-NMR) spectra. From the difference of *Rf* value, UV λ_{max}, and chemical shifts of 3-CH_3, the two structures of **3d** and **4d** were tentatively assigned. The assignments were further confirmed by two dimensional (2D) NMR techniques including ^{1}H–^{13}C correlation spectroscopy (COSY) and long range ^{1}H–^{13}C COSY (COLOC) experiments.

The chemical shifts and ^{1}H–^{13}C correlations in the 2D NMR spectra of compounds **3d** and **4d** are shown in Tables I and II respectively. The results of examinantion of the COLOC spectra were shown by arrows in formulas I and II (Fig. 1).

As shown in Fig. 1,3-C (δ 135.5) of compound **3d** (formula I) experienced two bond coupling ($^2J_{CH}$) with 11-H_3 (δ 2.41) and three bond coupling ($^3J_{CH}$) with 10-H_2 (δ 5.19). However, in the case of compound **4d** (formula II), 3-C (δ 143.4) experienced only two bond coupling with 11-H_3 (δ 2.38), whereas 9-C (δ 150.5) has a $^3J_{CH}$ to 10-H_2 (δ 5.15).

Based on the above analysis, the major product (**3d**) was determined to be 2-(*p*-chlorophenyl)methylpyrano[2,3-*c*]pyrazol-6(2*H*)-one, and the minor product (**4d**) was 1-(*p*-chlorophenyl)methylpyrano[2,3-*c*]pyrazol 6-(1*H*)-one. Furthermore, compound **4d** could also be obtained by reacting *p*-chlorophenylmethylhydrazine (**5**) with ethyl acetoacetate (**6**) (Chart 1).

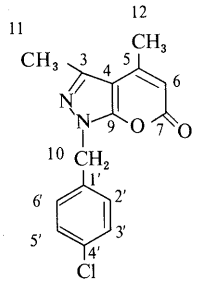
TABLE I. Chemical Schift and ^{1}H–^{13}C Correlation of NMR Spectra of Compound **3d**



No. of carbon	δ H[a]	δ C[a]
3		135.5
4		102.5
5		150.2
6	5.72 (1H, q, J=1.2 Hz)	109.3
7		161.2
9		158.4
10	5.19 (2H, s)	52.5
11	2.41 (3H, s)	11.4
12	2.30 (3H, d, J=1.2 Hz)	29.3
1′		134.2
2′, 6′	7.06 (2H, d, J=8.4 Hz)	128.4
3′, 5′	7.26 (2H, d, J=8.4 Hz)	129.1
4′		134.0

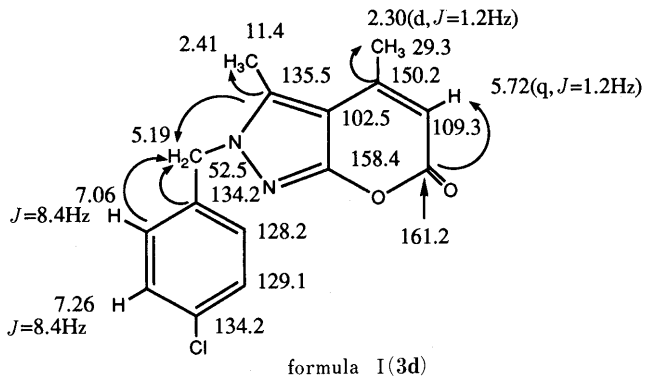
a) ^{1}H and ^{13}C-NMR spectra were measured in $CDCl_3$.

TABLE II. Chemical Schift and ^{1}H–^{13}C Correlation of NMR Spectra of Compound **4d**



No. of carbon	δ H[a]	δ C[a]
3		143.4
4		100.9
5		153.2
6	5.68 (1H, q, J=1.2 Hz)	104.7
7		159.7
9		150.5
10	5.15 (2H, s)	50.5
11	2.38 (3H, s)	14.4
12	2.33 (3H, d, J=1.1 Hz)	29.3
1′		133.8
2′, 6′	7.28 (1H, d, J=8.4 Hz)	129.0
3′, 5′	7.23 (1H, d, J=8.4 Hz)	129.4
4′		134.2

a) ^{1}H- and ^{13}C-NMR spectra were measured in $CDCl_3$.



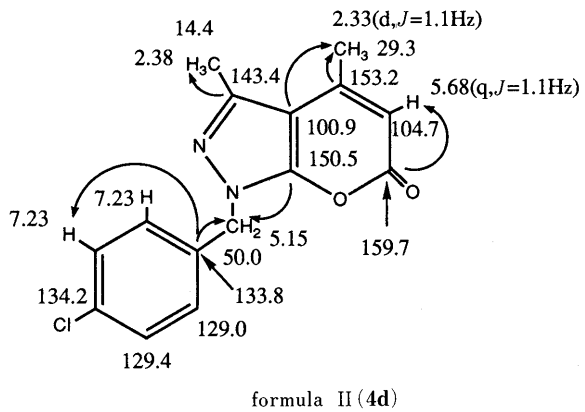
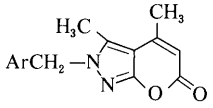


Fig. 1. COLOC Results of Compound **3d** and **4d**

TABLE III. Physical Constants and Spectral Data for N^2-Substituted Pyrano[2,3-*c*]pyrazol-6-ones



3a—o

Compd. No.	Recrystn. solvent[b]	Yield (%)	TLC,[a] *Rf*	mp (°C)	Formula[c]	Analysis (%) Calcd (Found) C	H	N	IR (KBr) C=O cm^{-1}	UV λ_{max} (solvent), nm (ε, $\times 10^4$)
3a	A	60	0.68	161—162	$C_{15}H_{14}N_2O_2$	70.85 (70.86)	5.55 5.54	11.2 11.04)	1710	($CHCl_3$) 302 (4.40)
3b	A	75	0.64	181—183	$C_{15}H_{13}ClN_2O_2$	62.42 (62.35)	4.54 4.56	9.70 9.62)	1715	($CHCl_3$) 304 (2.02)
3c	B	77	0.44	131—134	$C_{15}H_{13}ClN_2O_2$	62.42 (64.36)	4.54 4.53	9.70 9.74)	1705	($CHCl_3$) 300 (2.82)
3d	A	74	0.43	163—166	$C_{15}H_{13}ClN_2O_2$	62.41 (62.21)	4.54 4.55	9.70 9.71)	1707	($CHCl_3$) 304 (1.70)
3e	A	81	0.56	161—163	$C_{16}H_{16}N_2O_2$	67.59 (67.58)	5.67 5.62	9.85 9.87)	1712	($CHCl_3$) 304 (1.70)
3f	B	83	0.53	107—110	$C_{16}H_{16}N_2O_2$	67.59 (67.58)	5.67 5.66	9.85 9.76)	1722	($CHCl_3$) 304 (1.70)
3g	A	83	0.44	148—150	$C_{16}H_{16}N_2O_2$	67.59 (67.47)	5.67 5.63	9.85 9.82)	1705	($CHCl_3$) 304 (1.70)
3h	B	38	0.68	225—227	$C_{15}H_{13}N_3O_4$	60.20 (60.18)	4.38 4.35	14.04 14.01)	1720	($CHCl_3$) 304 (1.7)
3i	B	40	0.4	207—210	$C_{15}H_{13}N_3O_4$	60.20 (60.14)	4.38 4.36	14.04 14.04)	1718	($CHCl_3$) 304 (1.70)
3j	B	42	0.42	205—207	$C_{15}H_{13}N_3O_4$	60.20 (60.09)	4.38 4.37	14.04 14.01)	1718	($CHCl_3$) 304 (1.70)
3k	C	90	0.38	162—163	$C_{17}H_{18}N_2O_4$	64.96 (64.95)	5.77 5.77	8.91 8.88)	1710	($CHCl_3$) 306 (2.12)
3l	B	70	0.09	137—140	$C_{14}H_{13}N_3O_2$	65.87 (65.86)	5.13 5.13	16.46 16.45)	1732	($CHCl_3$) 304 (1.83)
3m	C	74	0.40	164—166	$C_{14}H_{13}N_3O_2$	65.87 (65.38)	5.13 5.12	16.46 16.45)	1710	($CHCl_3$) 302 (1.76)
3n	A	60	0.38	180—181	$C_{14}H_{13}N_3O_2$	65.87 (65.75)	5.13 5.12	16.46 16.53)	1707	($CHCl_3$) 300 (1.96)
3o	C	86	0.49	124—127	$C_{13}H_{12}N_2O_2S$	59.98 (59.96)	4.65 4.65	10.76 10.78)	1725	($CHCl_3$) 304 (1.59)

a) Adsorbent, Silica gel 60 F_{254}; solvent, $CHCl_3$/EtOH (25 : 2). *b*) A, $CHCl_3$ + EtOH; B, EtOAc; C, EtOH. *c*) Analyzed for C, H, N; analytical results were within ± of the theoretical value.

Compound **1** was then allowed to react with a variety of arylmethylhalides (**2c—o**) to afford the corresponding *N*-arylmethyl-3,4-dimethylpyrano[2,3*c*]pyrazol-6-ones (**3c—o** and **4c—o**). The N^1-arylmethyl and N^2-arylmethyl derivatives exhibited characteristic differences in their physical properties (Tables III, IV).

Biological Activity The synthesized compounds (**3a—o** and **4a—o**) were tested for their antiplatelet activities in washed rabbit platelets. As shown in Tables V and VI, compounds **3a**, **3e—g**, **3k**, **3o**, **4a**, **4f**, **4g**, **4k**, **4m** and **4o** were found to have significant inhibitory activity. Among these compounds, **4a**, **3e** and **3f** were the most potent inhibitors of platelet aggregation induced by arachidonic acid (AA) and collagen. However, in platelet activating factor (PAF)-induced platelet aggregation, compound **3a** was the most potent inhibitor. These inhibitions were in a concentration-dependent manner as shown in Fig. 2 using compound **3f** as an example in arachidonate- and collagen-induced platelet aggregation. The pharmacological profile of compound **3f** was similar to that of aspirin.

Compound **3f** was investigated for its inhibitory mechanism of action in washed rabbit platelets. We found that the antiplatelet effect of **3f** is due to the inhibition of thromboxane (TX)A_2 formation and blockade of the TXA_2/prostaglandin (PG) endoperoxide receptor. Details will be reported elsewhere.[6]

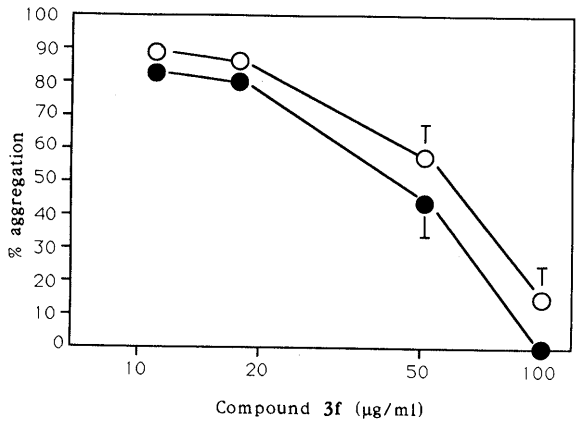
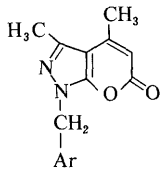


Fig. 2. Concentration–Response Curve of **3f** on the Aggregation of Washed Rabbit Platelets Induced by Arachidonic Acid and Collagen

Platelets were preincubated with various concentrations of **3f** or DMSO (0.5%) at 37 °C for 3 min, then arachidonic acid (100 μM) or collagen (10 μg/ml) was added to trigger the aggregation. Percentages of maximal aggregation are presented as means ± S.E.M. (n=8). ○—○, collagen; ●—●, arachidonic acid.

TABLE IV. Physical Constants and Spectral Data for N^1-Substituted Pyrano[2,3-*c*]pyrazol-6-ones



4a—o

Compd. No.	Recrystn. solvent[b]	Yield (%)	TLC,[a] *Rf*	mp (°C)	Formula[c]	Analysis (%) Calcd (Found) C	H	N	IR (KBr) C=O cm^{-1}	UV λ_{max} (solvent), nm (ε, $\times 10^4$)
4a	A	20	0.84	128—129	$C_{15}H_{14}N_2O_2$	70.85 (70.84)	5.55 5.55	11.02 11.01)	1720	($CHCl_3$) 316 (3.80)
4b	A	15	0.80	128—130	$C_{15}H_{13}ClN_2O_2$	62.4 (62.29)	4.54 4.53	9.70 9.73)	1730	($CHCl_3$) 316 (1.47)
4c	B	15	0.87	151—154	$C_{15}H_{13}ClN_2O_2$	62.4 (62.38)	4.54 4.53	9.70 9.69)	1731	($CHCl_3$) 316 (1.47)
4d	B	15	0.69	175—178	$C_{15}H_{13}ClN_2O_2$	62.4 (62.35)	4.54 4.53	9.70 9.72)	1725	($CHCl_3$) 316 (1.27)
4e	A	13	0.78	161—163	$C_{16}H_{16}N_2O_3$	67.59 (67.48)	5.67 5.66	9.85 9.85)	1745	($CHCl_3$) 304 (1.7)
4f	B	14	0.75	107—110	$C_{16}H_{16}N_2O_3$	67.59 (67.61)	5.67 5.68	9.85 9.37)	1725	($CHCl_3$) 304 (1.7)
4g	A	15	0.68	138—139	$C_{16}H_{16}N_2O_3$	67.59 (67.48)	5.67 5.66	9.85 9.81)	1725	($CHCl_3$) 316 (1.51)
4h	B	9	0.78	210—211	$C_{15}H_{13}N_3O_4$	60.20 (60.21)	4.38 4.37	14.04 14.03)	1735	($CHCl_3$) 316 (1.00)
4i	B	10	0.55	182—184	$C_{15}H_{13}N_3O_4$	60.20 (62.19)	4.38 4.38	14.04 14.09)	1720	($CHCl_3$) 316 (1.00)
4j	B	15	0.53	224—226	$C_{15}H_{13}N_3O_4$	60.20 (62.19)	4.38 4.38	14.04 14.03)	1720	($CHCl_3$) 316 (1.00)
4k	B	9	0.27	148—151	$C_{17}H_{18}N_2O_4$	64.96 (64.81)	5.77 5.76	8.91 8.88)	1735	($CHCl_3$) 306 (2.12)
4l	B	15	0.14	130—134	$C_{14}H_{13}N_3O_2$	65.87 (65.74)	5.13 5.12	16.46 16.45)	1735	($CHCl_3$) 316 (1.05)
4m	C	10	0.53	137—139	$C_{14}H_{13}N_3O_2$	65.87 (65.75)	5.13 5.12	16.46 16.45)	1722	($CHCl_3$) 316 (1.00)
4n	B	9	0.42	132—136	$C_{14}H_{13}N_3O_2$	65.87 (65.67)	5.13 5.12	16.46 16.45)	1732	($CHCl_3$) 314 (1.20)
4o	B	9	0.78	108—110	$C_{13}H_{12}N_2O_2S$	59.98 (59.97)	4.65 4.49	10.76 10.79)	1725	($CHCl_3$) 304 (1.59)

a) Adsorbent, Silica gel 60 F_{254}; solvent, $CHCl_3$/EtOH (25 : 2). *b*) A, $CHCl_3$ + EtOH; B, EtOAc; C, EtOH. *c*) Analyzed for C, H, N; analytical results were within ± of the theoretical value.

A combination of TXA_2 synthetase inhibitor with TXA_2/PG endoperoxide receptor blockade has been proposed as an improved antithrombotic approach.[7,8] Indeed, such dual inhibition achieved by combining two different pharmacological principles exerts antiplatelet effects superior to those of the single action compounds *in vivo*.[9]

The *N*-arylmethylpyrano[2,3-*c*]pyrazol-6-one derivatives, structurally different from the known inhibitor of TXA_2 formation with the antagonism of TXA_2/PG endoperoxide receptor, could provide a novel structural prototype for antiplatelet agents.

In the series of N^2-arylmethyl derivatives (**3a—o**), the lead compound (**3a**) showed significant antiplatelet activity. The substituent effects on the benzene ring of compound **1** was then examined. It was found that methoxy substitution at the benzene ring (**3e—g** and **3d**) resulted in potent inhibitory activity, whereas, chloro-(**3b—d**) and nitro-(**3h—j**) substituted derivatives always caused spontaneous aggregation of platelets. On the other hand, the replacement of the phenyl group of compound **3a** by a thienyl group, which lead to **3o**, might maintain high activity, whereas pyridinyl substituted derivatives (**3l—n**) were less active than the lead compound (**3a**). The N^1-arylmethyl derivatives (**4a—o**) showed a tendency similar to the N^2-arylmethyl derivatives (**3a—o**).

In conclusion, a series of new 1- and 2-arylmethyl-3,4-dimethylpyrano[2,3-*c*]pyrazol-6-one derivatives (**3a—o** and **4a—o**) were synthesized and examined for their antiplatelet activity.

In this screening test, we found that some compounds showed significant antiplatelet effects. Among them, compound **3f** is an inhibitor of TXA_2 formation with antagonism of TXA_2/PG endoperoxide receptor[6]; compound **3k** and **3o** not only showed significant antiplatelet activity, but also possessed a vasorelaxing action in rat thoracic aorta caused by Ca^{2+}-channel blocker (data not shown). Compound **4a** was the strongest among the compounds tested, and its potency was similar to that of aspirin. Thus compounds **3k**, **3o** and **4a** were selected for further pharmacological studies and the results will be reported elsewhere.

TABLE V. Effects of Compounds **3a—o** on the Platelet Aggregation Induced by Arachidonic Acid, Collagen and PAF

Agent No.	Concentration (μg/ml)	AA	Collagen	PAF
Control		79.8±1.5	96.3±2.9	82.6±7.8
3a	100	3.9±2.7[c]	10.7±2.3[c]	7.2±4.7[c]
3b	100	Caused spontaneous aggregation		
3c	100	Caused spontaneous aggregation		
3d	100	Caused spontaneous aggregation		
3e	20	89.9±3.7		
	50	0±0[c]		
	100	0±0[c]	8.2±2.9[c]	73.7±4.8
3f	100	0±0[c]	15.2±4.5[c]	83.6±3.6
3g	50	74.5±10.7	85.1±3.2	
	100	14.5±6.2[c]	73.6±3.9[c]	
3h	100	Caused spontaneous aggregation		
3i	100	Caused spontaneous aggregation		
3j	100	Caused spontaneous aggregation		
3k	100	6.3±4.4[c]	31.2±5.8[c]	57.6±2.7[b]
3l	100	63.1±7.0[b]	80.5±1.6	84.2±1.3
3m	100	35.5±10.4[b]	69.1±1.7	76.4±3.3
3n	100	51.9±3.7[b]	92.2±4.8	72.5±4.1
3o	100	8.2±1.8[c]	75.8±3.0	85.6±1.3
Control	100	92.5±1.0(5)	91.2±1.1(3)	
Aspirin	10	0.0±0.0[c]	65.5±3.9(3)[b]	
	5	43.1±16.7(5)[c]		

Platelets were incubated with each compound or 0.5% DMSO (control) at 37 °C for 3 min, then adenosine diphosphate (ADP) (20 μM), AA, (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added to trigger the aggregation. Percentages of aggregation are presented as means+S.E.M. ($n=3$—4), *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ as compared with the respective control value.

TABLE VI. Effects of Compounds **4a—o** on the Platelet Aggregation Induced by Arachidonic Acid, Collagen and PAF

Agent No.	Concentration (μg/ml)	AA	Collagen	PAF
Control		84.7±0.8	89.7±1.2	90.1±4
4a	2	89.6±1.6		
	5	47.1±20.9[b]		
	10	40.4±20.3[b]		
	20	6.7±2.9[c]	83.5±3.4[a]	
	50	0±0[c]	54.5±10.6[b]	82.3±2.0[b]
	100	0±0[c]	5.2±2.7[c]	68.9±7.7[b]
4b	100	Caused spontaneous aggregation		
4c	100	Caused spontaneous aggregation		
4d	100	Caused spontaneous aggregation		
4e	100	81.8±1.4	85.2±3.5	89.7±1.2
4f	100	15.1±8.8[c]	33.6±5.9[c]	38.9±12.8[c]
4g	100	6.5±3.7[c]	14.5±6.4[c]	73.6±3.9
4h	100	Caused spontaneous aggregation		
4i	100	Caused spontaneous aggregation		
4j	100	Caused spontaneous aggregation		
4k	100	4.3±3.2[b]	40.4±7.5[b]	44.4±10.3[c]
4l	100	80.1±3.2[a]	89.1±1.3	
4m	10	83.1±1.9		
	20	76.9±1.3[b]	83.5±3.0[b]	
	50	14.5±11.9[c]	67.3±3.4[c]	
	100	18.7±7.7[c]	45.5±5.1[c]	
4n	100	72.2±7.2[a]	83.2±2.9[b]	
4o	100	14.5±3.2[c]	3.2±2.2[c]	32.0±5.2[c]
Control		92.5±1.0(5)	91.2±1.1(3)	
Aspirin	10	0.0±0.0[c]	65.5±3.9[b]	
	5	43.1±16.7(5)[c]		

The experimental condition and data expression are the same as described in the footnote of Table V.

Experimental

Chemistry All melting points are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-440 spectrometer in KBr. NMR spectra were taken at 90 MHz on a JEOL FX-90Q spectrometer and a Varian VXR-300 FT-NMR spectrometer with tetramethylsilane (TMS) as an internal reference in $CDCl_3$, or dimethyl sulfoxide (DMSO)-d_6. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad. Mass spectra (MS) were measured with an HP 5995 GC-MS instrument and a JMS-D-300 spectrometer. The UV spectra were recorded on a Hewlett Packard Diode Array UV-VIS spectrometer (HP-8452A). Elemental analyses were performed by Chung Shan Institute of Science Technology, R.O.C., and National Cheng-Kung University, Tainan, Taiwan, R.O.C.

***N*-Arylmethylation of 3,4-Dimethylpyrano[2,3-*c*]pyrazol-6-one (1)** 3,4-Dimethylpyrano[2,3-*c*]pyrazol-6-one (**1**) (8.0 g, 0.05 mol) was dissolved in dry DMF (80 ml), and NaH (80% in oil, 1.5 g, 0.1 mol) was added portionwise with stirring for 30 min at 30 °C. Arylmethyl halides (**2a—o**) (0.05 mol) was then added portionwise at 30—40 °C. Stirring was continued for an additional 30 min, and then the reaction mixture was poured into ice water and extracted with $CHCl_3$. The organic layer was washed with water, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography (benzene–silica gel) to give **3a—o** (Table III) and **4a—o** (Table IV).

Reaction of *p*-Chlorophenylmethylhydrazines (5) with Ethyl Acetoacetate (6) Ethyl acetoacetate (26.0 g, 0.2 mol) was heated at 140 °C and the *p*-chlorophenylmethylhydrazine (**5**) (28.0 g, 0.1 mol) was added dropwise (or portionwise) with stirring. Water and EtOH that formed in the reaction were distilled off, and the mixture was heated under reflux. Upon completion of the reaction, the mixture was allowed to stand at room temperature. The solid thus separated was collected and purified by column chromatography (benzene–silica gel) to afford **4d** (Table IV).

Pharmacology Reagent: Collagen (type I, bovine achilles tendon) obtained from Sigma Chem. Co. was homogenized in 25 mM acetic acid and stored (1 mg/ml) at 70 °C. PAF was purchased from Calbiochem-Behring Co. and dissolved in chloroform. AA, ethylenediaminetetraacetic acid (EDTA), and bovine serum albumin were purchased from Sigma Chem. Co.

Platelet Aggregation: Blood was collected from the rabbit marginal vein, anticoagulated with EDTA (6 mM) and centrifuged for 10 min at 90×*g* and room temperature. Platelet suspension was prepared from this EDTA-anticoagulated platelet-rich plasma according to the washing procedures described previously.[10] Platelet numbers were counted by a Coulter counter (Model ZM) and adjusted to 4.5×10^8 platelets/ml. The platelet pellets were finally suspended in Tyrode's solution of the following composition (mM): NaCl (136.8), KCl (2.8), $NaHCO_3$ (11.9), $MgCl_2$ (2.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0) and glucose (11.2), containing bovine serum albumin (0.35%). The Platelet suspension was stirred at 1200 rpm and the aggregation was measured at 37 °C by the turbidimetric method as described by O'Brien[11] using a Chromo-Log Lumi aggregometer. In order to eliminate the effect of the solvent in the aggregation, the final concentration of DMSO was fixed at 0.5%. Percentage of aggregation was calculated using the absorbance of platelet suspension as 0% aggregation and the absorbance of Tyrode's solution as 100% aggregation.

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References

1) S. C. Kuo, L. J. Huang, and H. Nakamura, *J. Med. Chem.*, **27**, 539 (1984).
2) J. P. Wang, M. F. Hsu, T. H. Tsai, L. J. Huang, and C. M. Teng, *Thromb. Res.*, **36**, 113 (1984).
3) J. P. Wang, M. F. Hsu, and L. J. Huang, *Thromb. Res.*, **39**, 501 (1985).
4) S. C. Kuo, C. M. Teng, and J. P. Wang, *Drugs of the Future*, **10**, 761 (1985).
5) S. C. Kuo, L. J. Huang, J. M. Lai, and H. Nakamura, *J. Taiwan Pharm. Assoc.*, **39**, 234 (1987).
6) C. H. Lin, S. C. Kuo, L. J. Huang, T. F. Huang, and C. M. Teng, *Thromb. Res.*, **66**, 61 (1992).
7) G. A. Fitzgerald, I. A. G. Reilly, and A. K. Pedersen, *Circulation*, **72**, 1194 (1985).
8) P. Gresele, E van Houtte, J. Arnout, H. Deckmyn, and J. Vermylen, *Lancet*, **1**, 991 (1984).
9) P. Gresele, J. Arnout, H. Deckmyn, and J. Vermylen, *Adv. Prostaglandin Thromboxane Leukotriene Res.*, **17**, 248 (1987).
10) S. C. Kuo, C. M. Teng, J. G. Lee, F. N. Ko, S. C. Chen, and T. S. Wu, *Planta Medica*, **56**, 164 (1990).
11) J. R. O'Brien, *J. Clin Pathol.*, **15**, 452 (1962).