[Chem. Pharm. Bull. 33(7)2809—2820(1985)]

Synthesis and Inhibitory Effect on Platelet Aggregation of 2-Phenyl-1(2*H*)-phthalazinone Derivatives

AKIKO SUGIMOTO, KEIKO SAKAMOTO, YOHKO FUJINO, YOSHIMI TAKASHIMA, and MASAYUKI ISHIKAWA*

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2–3–10, Surugadai, Kanda, Chiyoda-ku, Tokyo 101, Japan

(Received September 3, 1984)

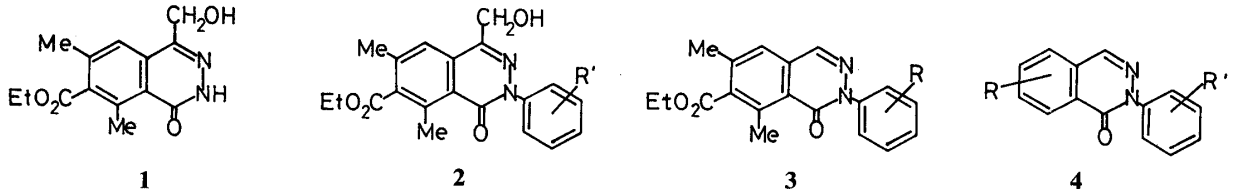
2-Phenyl-1(2*H*)-phthalazinone derivatives (**4**) were synthesized by the reactions of corresponding *o*-phthalaldehydic acids (**5**) with phenylhydrazine derivatives. The preparation of **5** was carried out by decarboxylation of keto-carboxylic acids (**6**) or hydroxylation of phthalides (**8**) *via* their bromo derivatives (**9**).

The derivatives (**4**) were tested for inhibitory activity on platelet aggregation. None of them showed any appreciable effect on platelet aggregation induced by adenosine diphosphate. Several compounds (**4l**, **4m**, **16a**, and **16c**), however, effectively inhibited platelet aggregation induced by arachidonic acid.

Keywords——2-phenyl-1(2*H*)-phthalazinone; antiaggregating activity; structure–activity relationship; 7-ethoxycarbonyl-2-phenyl-1(2*H*)-phthlazinone; 7-bromo-2-phenyl-1(2*H*)-phthalazinone; 7-chloro-2-phenyl-1(2*H*)-phthalazinone

In a series of studies on antiatherosclerotic agents, we found that 7-ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-1(2*H*)-phthalazinone (EG-626) (**1**) showed potent inhibitory effects on platelet aggregation induced by adenosine diphosphate (ADP) or arachidonic acid (AA).[1] 7-Ethoxycarbonyl-4-hydroxymethyl-6,8-dimethyl-2-phenyl-1(2*H*)-phthalazinone derivatives (**2**), having a phenyl group at the 2 position in **1**, inhibited platelet aggregation induced by AA without affecting aggregation induced by ADP.[2] Furthermore, the inhibitory potencies of 7-ethoxycarbonyl-6,8-dimethyl-2-phenyl-1(2*H*)-phthalazinone derivatives (**3**), in which the hydroxymethyl group at the 4 position in **2** is replaced by a hydrogen atom, were increased markedly, though the mode of action was similar to that of **2**.[3] We have synthesized 2-phenyl-1(2*H*)-phthalazinone derivatives (**4**) having various substituents on the benzene ring in order to investigate the structure–activity relationships of phthalazinone derivatives.

In this paper, we describe the synthetic procedures for **4** and the antiaggregating activity of the products.



1 **2** **3** **4**

Fig. 1

Synthesis

For the preparation of **4**, it was convenient to use the reaction of the corresponding *o*-phthalaldehydic acids (3-hydroxyphthalides) (**5**) with phenylhydrazine derivatives. The compounds (**5**) were synthesized by two basic routes, with a number of variations as shown in

Chart 1: (A) decarboxylation of keto carboxylic acids (**6**) under acidic conditions[4]; (B) hydroxylation of phthalides (**8**) obtained by the reduction of phthalic anhydrides (**7**)[5-7] or phthalic acid monoesters (**10**), *via* bromination and subsequent hydrolysis.

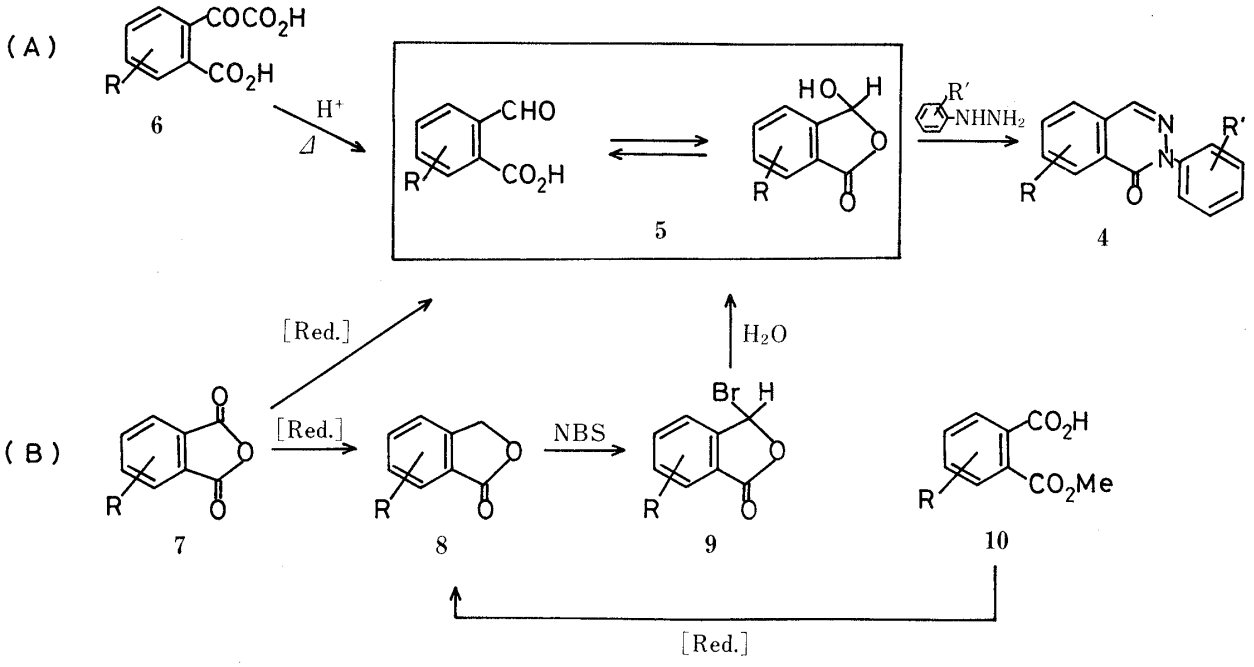


Chart 1

The syntheses of four nitro-2-phenyl-1(2*H*)-phthalazinones (**4a**—**d**) are outlined in Chart 2. In the case of nitro derivatives, method (B) was employed because of difficulties in preparing the corresponding keto carboxylic acids (**6**; $R=NO_2$). It was reported that reduction of 3-nitrophthalic anhydride (**7a**) with $NaBH_4$ preferentially gave 4-nitrophthalide (**8d**).[7] However, subsequent bromination with *N*-bromosuccinimide (NBS) was unsuccessful, presumably due to steric hindrance. Thus, the synthesis of 3-hydroxy-4-nitrophthalide (**5d**) was carried out according to the procedure described in the literature,[8] that is, the reduction of 3-nitrophthalimide (**11**) with 2 mol of $NaBH_4$ gave 3-hydroxy-4-nitrophthalimidine (**12**), and hydrolysis of **12** afforded **5d** in good yield. For the preparation of 3-hydroxy-7-nitrophthalide (**5c**), we examined the following route. It is known that the 2-methyl ester of 3-nitrophthalic acid (**10c**) can be prepared by the methanolysis of **7a** as a major product.[9] Using the technique described by Ishizumi *et al.*[10] for phthalic acid monoester, **10c** was converted to the corresponding mixed anhydride with ethyl chloroformate, followed by reduction with $NaBH_4$ to afford 7-nitrophthalide (**8c**) in moderate yield. Treatment of 3-bromo-7-nitrophthalide (**9c**), which had been prepared by bromination of **8c**, with aqueous 5% HCl solution gave 3-hydroxy-7-nitrophthalide (**5c**). On the other hand, methanolysis of 4-nitrophthalic anhydride (**7b**) gave a mixture of two monomethyl esters (**10a** and **10b**). The mixture was converted to the mixed anhydride, and reduction with $NaBH_4$ as described above afforded 5-nitrophthalide (**8a**) and 6-nitrophthalide (**8b**). At this step, it was found that the ratio of the products **8a**/**8b** was 7/3 by column chromatography. Direct reduction of **7b** with $NaBH_4$ gave a mixture of **8a**, **8b** and the dihydroxymethyl derivative (**13**). To our knowledge, nitration of phthalide (**14**)[11] is the best procedure to obtain 6-nitrophthalide (**8b**). The preparation of **8b** was achieved by this method in about 75% yield. Compounds **8a** and **8b** were converted to 3-hydroxyphthalides (**5a**, **5b**) in a manner similar to that described above. Without isolation and purification, the products (**5a**—**c**) were heated with phenylhydrazine in MeOH to give the corresponding nitro-2-phenyl-1(2*H*)-phthalazinone derivatives (**4a**—**c**).

The conversion of **5b** to **4b** by treatment with phenylhydrazine has already been reported by Borsche *et al.*[11] A similar result was achieved in the case of the reaction of **5d** or **12** with phenylhydrazine, but the latter compound gave the product (**4d**) in lower yield.

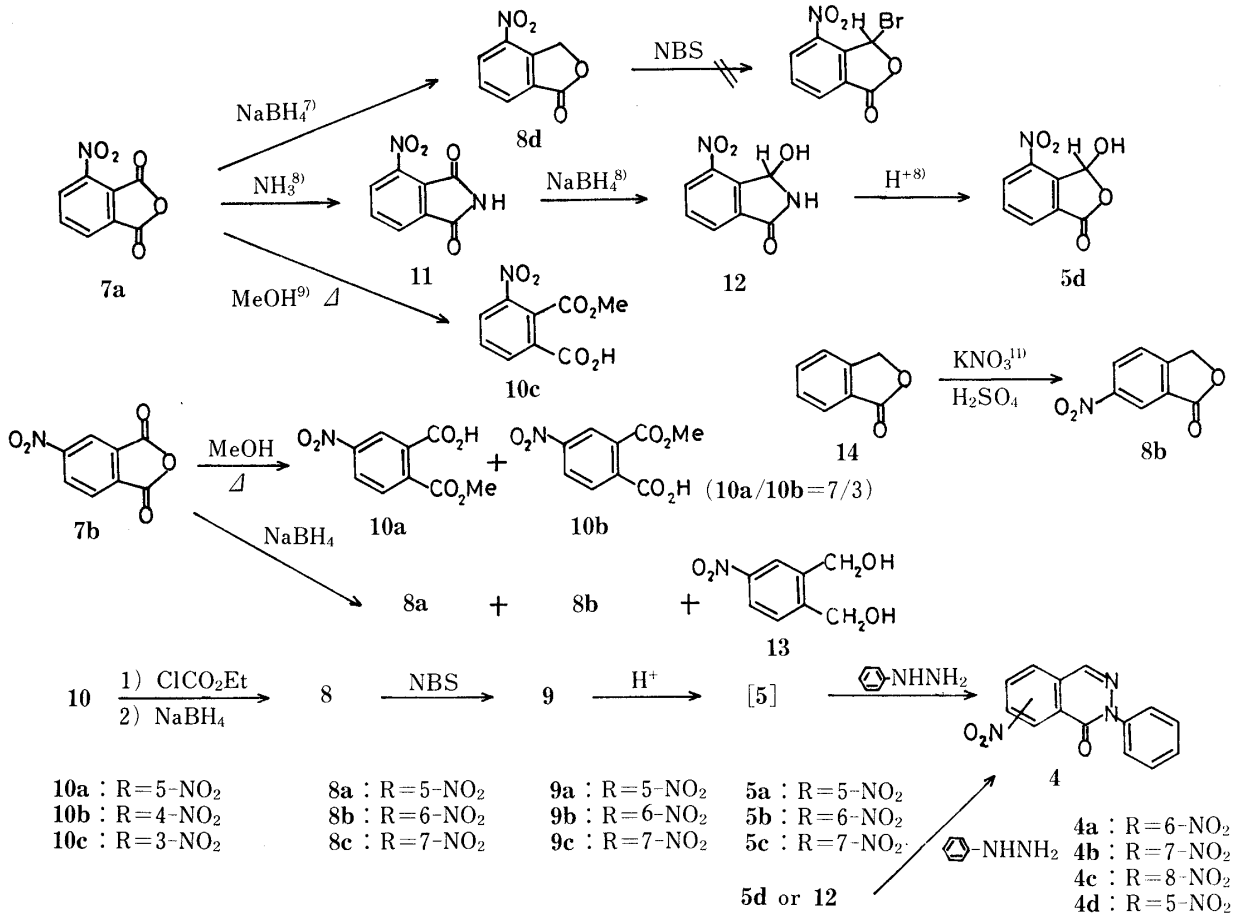
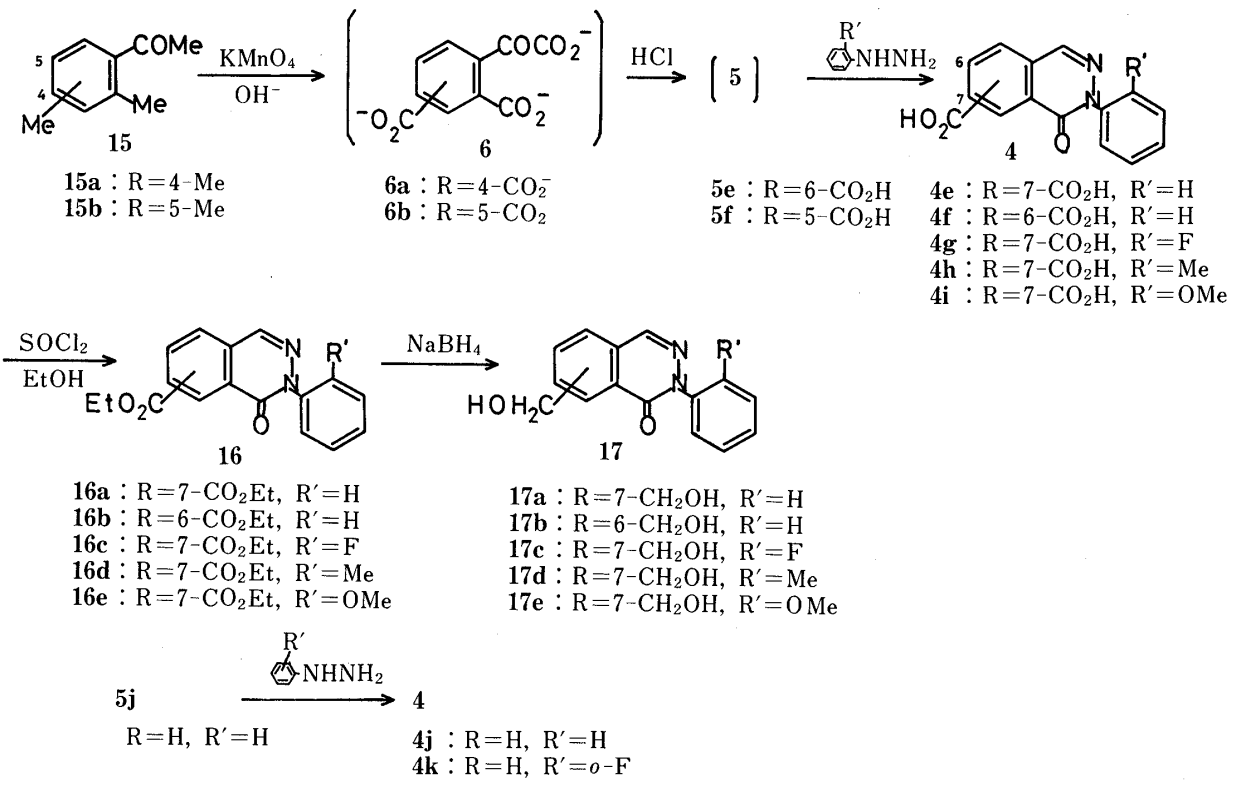


Chart 2

6-, and 7-Ethoxycarbonyl-2-phenyl-1(2*H*)-phthalazinone (**16b**, **16a**), and the reduced products (**17b**, **17a**) at the ethoxycarbonyl group were prepared by method (A) as shown in Chart 3. We have previously reported that *ortho* substituents on the 2-phenyl group affect the inhibitory activity on AA-induced platelet aggregation in the case of **2** or **3**. Therefore, some derivatives (**16c**—**e**, **17c**—**e**, and **4k**) bearing a 2-*ortho* substituted phenyl group were also prepared for comparison with the 2-phenyl derivatives (**16a**, **17a**, and **4j**).

2-Phenyl-1(2*H*)-phthalazinone derivatives having various substituents at the 7 position were obtained as described below (Chart 4). Acetophenone derivatives (**19**) were prepared by Friedel-Crafts reaction of the corresponding toluene derivatives (**18**), accompanied by positional isomers (**20**). As judged from the nuclear magnetic resonance (NMR) spectra, the ratios of products (**19** and **20**) formed from **18** were as follows: **18a**, 3 : 2; **18b**, 1 : 1. However, in the case of **18c**, the only product was **19c**. Without isolation and purification, the mixture (**19** and **20**) was oxidized with $KMnO_4$ to give keto carboxylic acids. According to procedure (A), the keto carboxylic acids were converted to aldehydic acids, then cyclized with phenylhydrazine to give 7-halogeno- or 7-methoxy-2-phenyl-1(2*H*)-phthalazinones (**4l**, **m** or **4n**). At this time, other condensed products which can be assumed to be **21** formed from **20** were dissolved in alkaline solution and separated from the reaction mixture of **4l**, **m** and **21a**, **b**.



15
15a : R=4-Me
15b : R=5-Me

6
6a : R=4-CO_2^-
6b : R=5-CO_2

5e : R=6-CO_2H
5f : R=5-CO_2H

4
4e : R=7-CO_2H, R′=H
4f : R=6-CO_2H, R′=H
4g : R=7-CO_2H, R′=F
4h : R=7-CO_2H, R′=Me
4i : R=7-CO_2H, R′=OMe

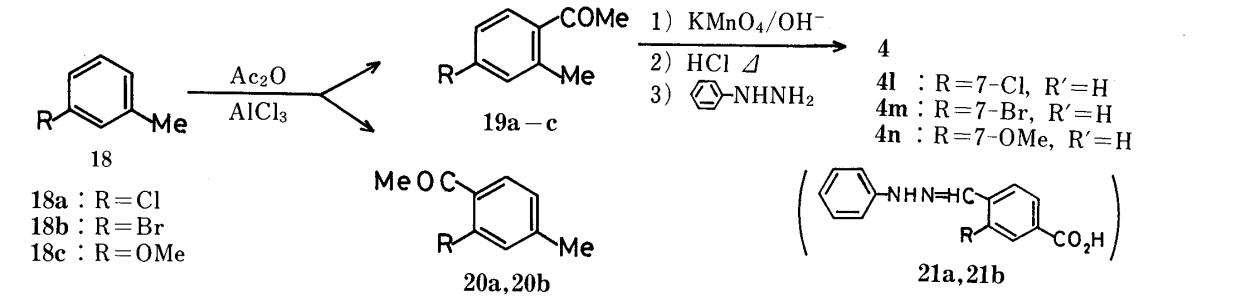
16
16a : R=7-CO_2Et, R′=H
16b : R=6-CO_2Et, R′=H
16c : R=7-CO_2Et, R′=F
16d : R=7-CO_2Et, R′=Me
16e : R=7-CO_2Et, R′=OMe

17
17a : R=7-CH_2OH, R′=H
17b : R=6-CH_2OH, R′=H
17c : R=7-CH_2OH, R′=F
17d : R=7-CH_2OH, R′=Me
17e : R=7-CH_2OH, R′=OMe

5j
R=H, R′=H

4
4j : R=H, R′=H
4k : R=H, R′=*o*-F

Chart 3



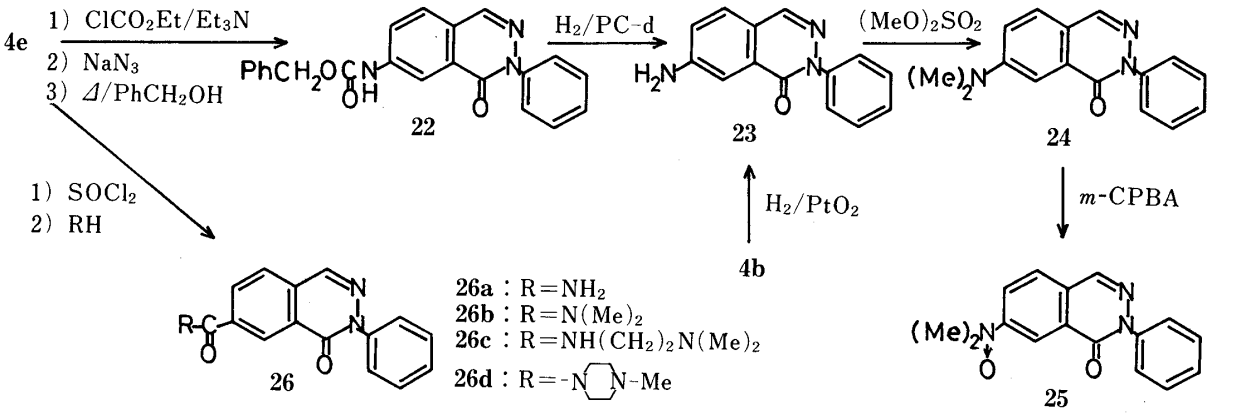
18
18a : R=Cl
18b : R=Br
18c : R=OMe

19a–c

20a,20b

21a,21b

4
4l : R=7-Cl, R′=H
4m : R=7-Br, R′=H
4n : R=7-OMe, R′=H



22

23

24

4b

26
26a : R=NH_2
26b : R=$N(Me)_2$
26c : R=$NH(CH_2)_2N(Me)_2$
26d : R=-N⟨⟩N-Me

25

Chart 4

7-Amino-, and 7-carbamoyl-2-phenylphthalazinone derivatives were synthesized as shown in Chart 4. By Curtius rearrangement,[12] the 7-carboxy-2-phenylphthalazinone (**4e**) was converted to the benzylcarbamate (**22**), which was cleaved by catalytic hydrogenolysis to give the 7-amino derivative (**23**). Compound **23** was also obtained by catalytic hydrogenation

of the 7-nitro derivative (**4b**) with PtO_2 in good yield. Preparation of **23** by method (A) from 3-methylaniline was unsuccessful because decarboxylation failed to occur smoothly. Dimethylation of **23** was carried out according to Quast *et al.*[13] The product (**24**) was oxidized with *m*-chloroperbenzoic acid in the usual manner to give the *N*-oxide (**25**). The 7-amide derivatives (**26**) were prepared by the general method for the synthesis of amide from carboxylic acid *via* the acid chloride.

The reactions of the 7-chloromethyl derivative (**27**), prepared from (**17a**), with amines or amino alcohol proceeded in good yield to give 7-aminomethyl derivatives (**28a, b**) or the corresponding ether (**28c**) as shown in Chart 5. Nucleophilic substitution of the 7-bromo-2-phenylphthalazinone (**4m**) with CuCN or CuSEt[14] was carried out smoothly to give 7-cyano-2-phenylphthalazinone (**29**) or 7-ethylmercapto-2-phenylphthalazinone (**30**). The 7-carboxylic acid derivative (**4e**) was also prepared from 7-cyanophthalazinone (**29**) by hydrolysis under acidic conditions. Oxidation of **30** with *m*-chloroperbenzoic acid gave the corresponding sulfoxide (**31**) and sulfone (**32**) derivatives in good yields.

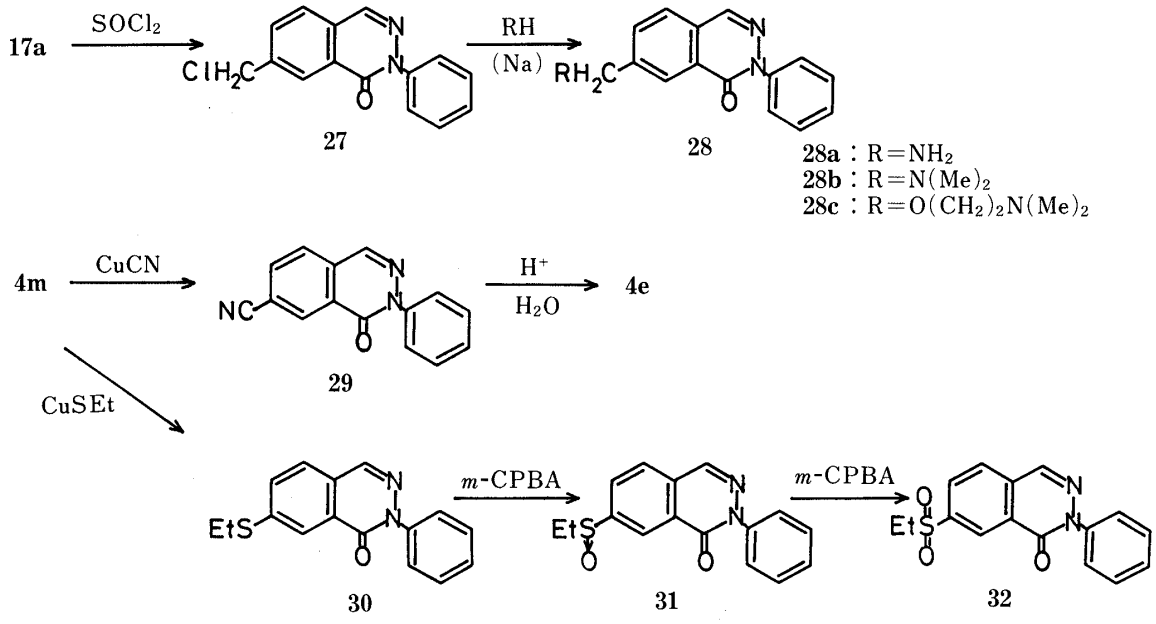


Chart 5

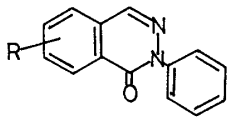
Biological Activity and Discussion

The inhibitory activity on platelet aggregation was assessed by the optical density method of Born and Cross[15] using rabbit platelet-rich plasma (PRP). Aggregation was induced by using either adenosine diphosphate (ADP, 10 μM) or arachidonic acid (AA, 137 μM). The results are summarized in Tables I and II, and the inhibitory activity is expressed as IC_{100}. Since none of the compounds, prepared as described above, showed any appreciable inhibitory effect at a concentration of 100 μM on platelet aggregation induced by ADP, the results for ADP-induced aggregation are not shown.

As shown in Table I, the derivatives (**4b**, **16a**, and **17a**) possessing a substituent at position 7 showed the most potent inhibitory activity. In addition, among 6-substituted derivatives, **16b**, a compound possessing an ethoxycarbonyl group, exceptionally exerted a fairly potent inhibitory activity. However, the remaining derivatives listed in Table I did not show any activity at a concentration of 100 μM.

The profile of inhibitory activity of 7-substituted 2-phenyl-1(2*H*)-phthalazinone derivatives is summarized in Table II. In the previous papers,[2,3] we reported that the potency in inhibiting platelet aggregation decreases in order of *ortho*, *para*, and *meta*, when the activities

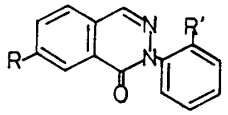
TABLE I. Inhibition of AA-Induced Platelet Aggregation (IC_{100} μM)

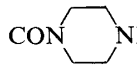


R	C-5	C-6	C-7	C-8
CO_2Et	—	10	5	—
CH_2OH	—	≧100	25	—
NO_2	≧100	≧100	25	≧100

—; not determined.

TABLE II. Physical Constants and Antiaggregating Activities of 7-Substituted-1(2*H*)-Phthalazinone



Compd. No.	R	R′	mp °C (Solvent)	Formula	Inhib. of AA-induced platelet aggregation IC_{100} (μM)
4b[11]	NO_2	H	172.5—173 (EtOH)	$C_{14}H_9N_3O_3$	25
4j[16]	H	H	104—105 (EtOH)	$C_{14}H_{10}N_2O$	25
4k	H	F	120.5—121.5 (EtOH)	$C_{14}H_9FN_2O$	25
4l	Cl	H	160—161 (EtOH)	$C_{14}H_9ClN_2O$	5
4m	Br	H	157—158 (EtOH)	$C_{14}H_9BrN_2O$	5
4n	OMe	H	168—171 (EtOH)	$C_{15}H_{12}N_2O_2$	10
16a	CO_2Et	H	166—167 (EtOH)	$C_{17}H_{14}N_2O_3$	5
16c	CO_2Et	F	217—218 (EtOH)	$C_{17}H_{13}FN_2O_3$	5
16d	CO_2Et	Me	139—141 (EtOH)	$C_{18}H_{16}N_2O_3$	10
16e	CO_2Et	OMe	114—116 (EtOH)	$C_{18}H_{16}N_2O_4$	50
17a	CH_2OH	H	141—142 (EtOH)	$C_{15}H_{12}N_2O_2$	25
17c	CH_2OH	F	143—145 ($EtOH-Et_2O$)	$C_{15}H_{11}FN_2O_2$	10
17d	CH_2OH	Me	112—115 ($EtOH-Et_2O$)	$C_{16}H_{14}N_2O_2$	50
17e	CH_2OH	OMe	183—185 (MeOH)	$C_{16}H_{14}N_2O_3$	≧100
23	NH_2	H	216—219 (MeOH)	$C_{14}H_{11}N_3O$	≧100
24	$N(Me)_2$	H	140—141 ($MeOH-Et_2O$)	$C_{16}H_{15}N_3O$	50
25	$O\leftarrow N(Me)_2$	H	159—160 ($EtOH-Et_2O$)	$C_{16}H_{15}N_3O_2$	≧100
26b	$CON(Me)_2$	H	150—152 (EtOH)	$C_{17}H_{15}N_3O_2$	25
26d		H	149—152 (MeOH–hexane)	$C_{20}H_{20}N_4O_2$	≧100
28a	CH_2NH_2	H	70—72 (Benzene)	$C_{15}H_{13}N_3O$	≧100
28b	$CH_2N(Me)_2$	H	113—115 ($EtOH-Et_2O$)	$C_{17}H_{17}N_3O$	50
28c	$CH_2O(CH_2)_2N(Me)_2$	H	48—49 (EtOH–hexane)	$C_{19}H_{21}N_3O_2$	≧100
29	CN	H	232—233 (MeOH)	$C_{15}H_9N_3O$	10
30	SEt	H	144.5—145.5 (Et_2O)	$C_{16}H_{14}N_2OS$	10
31	$O\leftarrow SEt$	H	124—125 ($EtOH-Et_2O$)	$C_{16}H_{14}N_2O_2S$	50
32	SO_2Et	H	192—194 (EtOH–hexane)	$C_{16}H_{14}N_2O_3S$	50

of positional isomers of the phenyl group at the 2 position in **2** or **3** are compared. As for the kind of substituent at the *para* position on the 2-phenyl group, electron-withdrawing groups markedly reduced the potency, whereas electron-donating groups tended to retain the activity of the parent compound among **2** or **3**.[2,3] In the case of an *ortho* substituent, the compounds

having fluorine, chlorine or a methyl group constituted the most active group in the series of **2** and **3**.[2,3] Among the present compounds (**4**), we tested only *ortho*-substituted 2-phenyl derivatives. The potency of *ortho*-fluoro derivatives (**4k**, **16c**, **17c**) was equal to or stronger than that of the parent compounds (**4j**, **16a**, **17a**) in every case, but the introduction of an *ortho*-methyl or *ortho*-methoxy group (**16d**, **e**, **17d**, **e**) decreased the potency about 2 to 10 times in comparison with the non-substituted compounds. However, it remains to be clarified whether the difference in activity is due to the ionic effect of the substituent or to a restriction of free rotation or other factors. Among the 2-phenyl-1(2*H*)-phthalazinone derivatives having various kinds of substituents at the 7 position, several compounds show fairly potent inhibition. 2-Phenyl-1(2*H*)-phthalazinone (**4j**), the basic structure of **4**, completely inhibited the platelet aggregation induced by AA at a concentration of 25 μM. The activities of the 7-hydroxymethyl (**17a**), 7-nitro (**4b**), and 7-dimethylamide (**26b**) derivatives were comparable to that of **4j**. The 7-methoxy (**4n**), 7-mercaptoethyl (**30**), and 7-cyano (**29**) derivatives showed increased inhibitory activity in comparison with **4j**. On the other hand, we have predicted high potency of 7-ethoxycarbonyl derivatives having the structure **3**.[3] In fact, **16a** and **16c** showed the most potent inhibitory activities, together with 7-halogeno derivatives (**4l**, **m**), in this series. Meanwhile, it should be emphasized that substituents containing an oxygen atom attached to nitrogen or sulfur, such as $(Me)_2N\rightarrow O$ in **25**, $EtS\rightarrow O$ in **31** and $EtSO_2$ in **32** did not show retained or enhanced biological activity relative to the parent compound (**24**, **30**), in contrast to the case of the ester moiety EtOCO in **16a**. We have reported[2,3] that the antiaggregating activity of **2** and **3** results from inhibition of cyclooxygenase and we suggested that their mechanisms of action may be similar to those of nonsteroidal antiinflammatory agents such as aspirin and indomethacin. Though the pharmacological profiles and the structure–activity relationship of **4** were not elucidated in detail, the mode of action of **4** on platelet aggregation induced by AA or ADP was similar to that of **2** or **3**. Thus, we speculate that the antiaggregating activity arises from a mechanism similar to that of **2** or **3**.

In conclusion, we have found several derivatives of 2-phenyl-1(2*H*)-phthalazinone, such as **4l**, **4m**, **16a**, and **16c**, having fairly potent inhibitory action on platelet aggregation. The results of detailed pharmacological studies on these compounds will be reported in a subsequent paper.

Experimental

All melting points were determined in a capillary tube (Yamato Mp-1 apparatus) and are uncorrected. NMR and infrared (IR) spectra were recorded by using JEOL C-60HL and Hitachi 285 spectrometers, respectively. Mass spectra (MS) were recorded on Hitachi RMU-7M and JEOL JMS-D300 mass spectrometers.

7-Nitrophthalide (8c)——3-Nitrophthalic acid 2-methyl ester (**10c**) (12.3 g, 54.6 mmol) [obtained from 3-nitrophthalic anhydride (**7a**)][9] and triethylamine (5.52 g, 54.6 mmol) were dissolved in 100 ml of tetrahydrofuran (THF). A solution of ethyl chloroformate (5.93 g, 54.6 mmol) in THF (30 ml) was added dropwise to the mixture under stirring at 0 °C. The mixture was stirred for an additional 30 min at 0 °C, then the precipitate was filtered off and washed with THF. The filtrate was added dropwise to a solution of $NaBH_4$ (5.18 g, 137 mmol) in 50% H_2O–THF (v/v %) (100 ml), keeping the internal temperature below 10 °C. When the addition was completed, the mixture was stirred at room temperature for 1 h. The mixture was acidified with 1 N HCl and concentrated under reduced pressure, and the residue was extracted with $CHCl_3$. The extract was dried over $MgSO_4$, and evaporated. The residue was recrystallized from EtOH to give **8c** (6.25 g, 63.9%). mp 166—167.5 °C (lit.[6] 164—166 °C). MS *m/e*: 179 (M^+), 150. NMR (DMSO-d_6) δ: 5.52 (2H, s), 8.04 (3H, s). IR ν_{max}^{KBr} cm^{-1}: 1770, 1530, 1350.

5-Nitrophthalide (8a)——4-Nitrophthalic anhydride (**7b**) (10 g, 51.8 mmol) was refluxed in MeOH (100 ml) for 24 h. After evaporation, the residue was recrystallized from benzene–hexane to give a mixture of the corresponding monomethyl esters **10a** and **10b**. Yield, 10.7 g (92%). The mixrure was treated as described above for the preparation of **8c**. After extraction with $CHCl_3$ followed by evaporation, the residue was purified by column chromatography over silica gel with benzene. The fraction eluted first was recrystallized from EtOH to give **8a** (2.4 g, 31.9%). mp 149.5—150 °C. MS *m/e*: 179 (M^+), 150, 133. NMR ($CDCl_3$) δ: 5.47 (2H, s), 8.09 (1H, d, J=9 Hz), 8.40 (1H, d, J=2 Hz), 8.45 (1H, dd, J=9, 2 Hz). IR ν_{max}^{KBr} cm^{-1}: 1770, 1530, 1350. The second fraction gave **8b** (1 g, 13.2%). The

melting point and spectrochemical dara were in good agreement with those of the compound prepared by the following method.

6-Nitrophthalide (8b)——6-Nitrophthalide (**8b**) was prepared by the nitration of phthalide (**14**) in the manner described by Borsche *et al.*[11] mp 146.5—147 °C (acetic acid) (yield, 71%). MS *m/e*: 179 (M^+), 150, 133. NMR (DMSO-d_6) δ: 5.57 (2H, s), 7.97 (1H, d, J=9 Hz), 8.52 (1H, s), 8.58 (1H, d, J=9 Hz). IR ν_{max}^{KBr} cm^{-1}: 1770, 1520, 1350.

Direct Reduction of 4-Nitrophthalic Anhydride (7b) with $NaBH_4$——Sodium borohydride (189 mg, 5 mmol) was suspended in 20 ml of THF. A solution of 4-nitrophthalic anhydride (**7b**) (500 mg, 2.59 mmol) in THF (20 ml) was added dropwise to the suspension at 0 °C under stirring. Stirring was continued for 1 h at 0 °C, then at room temperature for 4 h. The mixture was acidified with 10% HCl solution and extracted with a large amount of ether. The organic layer was washed with 5% $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel with benzene. The first eluted product, **8a** (125 mg; 27%), and the next, **8b** (96 mg; 20.7%), were each obtained as crystals. The product eluted last (**13**) (88 mg) was an oil. **13**; Yield, 18.6%. MS *m/e*: 183 (M^+), 165 (baseion peak, M^+-18), 149, 120, 91. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1520, 1350.

General Procedure for the Preparation of 4a—c from 8a—c——A mixture of nitrophthalide (**8**) (2 g, 11.2 mmol), *N*-bromosuccinimide (4.4 g, 24.7 mmol), a catalytic amount of benzoyl peroxide and CCl_4 (80 ml) was refluxed for 24 h. The solvent was removed and the residue was chromatographed over silica gel with $CHCl_3$ to give **9**. Yields: **9a**, 1.81 g, 63%; **9b**, 1.21 g, 42%; **9c**, 1.76 g, 61%. After refluxing of **9** with 5% HCl solution, the mixture was cooled to room temperature, then phenylhydrazine·HCl (1.2 eq) was added with stirring. Stirring was continued at room temperature for 30 min to 2 h. The resulting precipitate was filtered off (in the case of **5c**, the oily product was extracted with $CHCl_3$). The precipitate was dissolved in MeOH, and refluxed for 30 min. After concentration of the solvent, the residue was extracted with $CHCl_3$, washed with 5% $NaHCO_3$ solution, and dried over $MgSO_4$. The solvent was removed and the residue was recrystallized from EtOH to give **4**. **4a**; Yield, 43% (from **9a**). mp 202—203 °C. MS *m/e*: 267 (M^+), 236, 220. NMR ($CDCl_3$) δ: 7.40—7.80 (5H, m), 8.40 (1H, s), 8.61 (3H, m). IR ν_{max}^{KBr} cm^{-1}: 1680, 1515, 1335. *Anal.* Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N, 15.73. Found: C, 62.96; H, 3.41; N, 15.77. **4b**; Yield, 73% (from **9b**). mp 172.5—173 °C. (lit.[11] 171 °C). MS *m/e*: 267 (M^+), 237, 220. NMR ($CDCl_3$) δ: 7.26—7.80 (5H, m), 7.93 (1H, d, J=9 Hz), 8.39 (1H, s), 8.63 (1H, dd, J=9, 2 Hz), 9.31 (1H, d, J=2 Hz). IR ν_{max}^{KBr} cm^{-1}: 1660, 1515, 1340. *Anal.* Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N, 15.73. Found: C, 63.12; H, 3.50; N, 15.66. **4c**, Yield, 45% (from **9c**). mp 182—182.5 °C. MS *m/e*: 267 (M^+), 237, 192. NMR ($CDCl_3$) δ: 7.20—8.00 (8H, m), 8.33 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 1670, 1535, 1380, 1340. *Anal.* Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N, 15.73. Found: C, 63.20; H, 3.40; N, 15.89.

5-Nitro-2-phenyl-1(2*H*)-phthalazinone (4d)——3-Hydroxy-4-nitrophthalimidine (**12**) or 3-hydroxy-4-nitrophthalide (**5d**) (2.6 mmol), prepared according to Watanabe *et al.*,[8] was suspended in H_2O (10 ml). Phenylhydrazine·HCl (3 ml) was added to the suspension and the mixture was refluxed for 20 h, then extracted with $CHCl_3$. The extract was washed with H_2O, and dried over $MgSO_4$. The solvent was removed, and the residue was chromatographed over silica gel with $CHCl_3$ to give **4d**. Yield from **12**, 20.3%; from **5d**, 41.4%. mp 154—155 °C (EtOH). MS *m/e*: 267 (M^+), 250, 236, 220. NMR ($CDCl_3$) δ: 7.26—7.90 (5H, m), 7.89 (1H, t, J=8 Hz), 8.56 (1H, dd, J=8, 2 Hz), 8.83 (1H, dd, J=8, 2 Hz), 9.08 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 1675, 1510, 1340. *Anal.* Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N, 15.73. Found: C, 63.09; H, 3.41; N, 15.70.

7- or 6-Carboxy-2-phenyl-1(2*H*)-phthalazinone Derivatives (4e—i)——$KMnO_4$ (83.8 g, 530 mmol) was added to a solution of an acetophenone derivative (**15a** or **15b**) (12 g, 81 mmol) in 360 ml of H_2O containing 7 g of K_2CO_3 for 1 h with vigorous stirring; the temperature of the reaction mixture was maintained at 50 to 70 °C. After stirring of the mixture for an additional 2 h at 70 °C, 10 ml of EtOH was added to decompose the excess $KMnO_4$. The precipitated MnO_2 was filtered off and washed with H_2O. The filtrate and washings were combined and diluted with H_2O until the total volume of solution was 1150 ml. This solution was used as a stock solution (*ca.* 0.07 mmol/ml) of **6a** or **6b**. Twenty mmol of **6a** or **6b** was neutralized with conc. HCl solution, and 42 g (40 mmol) of sodium bisulfite was added, then the mixture was evaporated to dryness in an oil bath at 120 °C. The residue was stirred with 30 ml of conc. HCl sol. and evaporated to dryness. The treatment with aq. HCl sol. and evaporation were repeated 3 or 4 times. A solution consisting of 100 ml of H_2O and 22 mmol of a derivative of phenylhydrazine·HCl (dissolved in 50 ml of EtOH in the case of free base) was added to the residue, and the mixture was stirred at room temperature for 15 h. The precipitate was filtered off, dissolved in aq. 10% KOH sol. and extracted with CH_2Cl_2 to remove impurities. The H_2O layer was acidified with conc. HCl sol. to give **4e—i**. The crystalline precipitate was filtered off, washed with H_2O and dried in a desiccator. It was difficult to purify further, because the carboxylic acids were insoluble in organic solvents. Thus, the purification of the products was carried out after esterification. The yields of crude products **4** from **15** were as follows: **4e**, 75%, **4f**, 76.5%; **4g**, 72%; **4h**, 80.3%; **4i**, 70%.

7- and 6-Ethoxycarbonyl-2-phenyl-1(2*H*)-phthalazinone Derivatives (16)——The appropriate carboxy-2-phenyl-1(2*H*)-phthalazinone derivative (**4e—i**) (23 mmol) was suspended in 500 ml of EtOH. With stirring, 5 ml of $SOCl_2$ was added dropwise to the mixture at room temperature, and the whole was refluxed for 5 h, then cooled to room temperature. The precipitate was filtered off (occasionally, removal of some of the EtOH was necessary), and recrystallized from EtOH to give **16**. **16a**; Yield, 82%. mp 166—167 °C. MS *m/e*: 294 (M^+), 265, 249. NMR ($CDCl_3$) δ: 1.46 (3H, t, J=7 Hz), 4.45 (2H, q, J=7 Hz), 7.30—7.80 (5H, m), 7.79 (1H, d, J=9 Hz), 8.34 (1H, s), 8.51 (1H, dd,

J=9, 2 Hz), 9.15 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 1718, 1660, 1290, 1250. *Anal.* Calcd for $C_{17}H_{14}N_2O_3$: C, 69.37; H, 4.80; N, 9.52. Found: C, 69.47; H, 4.77; N, 9.49. **16b**; Yield, 70%. mp 136—137 °C. MS *m/e*: 294 (M^+), 265, 249. NMR ($CDCl_3$) δ: 1.46 (3H, t, J=7 Hz), 4.47 (2H, q, J=7 Hz), 7.30—7.95 (5H, m), 8.30—8.80 (2H, m), 8.47 (1H, s), 9.15 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 1720, 1670, 1300, 1250. *Anal.* Calcd for $C_{17}H_{14}N_2O_3$: C, 69.37; H, 4.80; N, 9.52. Found: C, 69.58; H, 4.85; N, 9.55. **16c**; Yield, 72%. mp 217—218 °C. MS *m/e*: 312 (M^+), 293, 275. NMR ($CDCl_3$) δ: 1.46 (3H, t, J=7 Hz), 4.47 (2H, q, J=7 Hz), 7.20—7.90 (4H, m), 7.83 (1H, d, J=9 Hz), 8.36 (1H, s), 8.55 (1H, dd, J=9, 2 Hz), 9.18 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{17}H_{13}FN_2O_3$: C, 65.38; H, 4.20; N, 8.97. Found : C, 65.55; H, 4.18; N, 9.00. **16d**; Yield, 74.8%. mp 139—141 °C. MS *m/e*: 308 (M^+), 291, 263. NMR ($CDCl_3$) δ: 1.46 (3H, t, J=7 Hz), 2.23 (3H, s), 4.56 (2H, q, J=7 Hz), 7.42 (4H, s), 7.89 (1H, d, J=9 Hz), 8.40 (1H, s), 8.59 (1H, dd, J=9, 2 Hz), 9.28 (1H, d, J=2 Hz). IR ν_{max}^{KBr} cm^{-1}: 1730, 1665, 1302, 1255. *Anal.* Calcd for $C_{18}H_{16}N_2O_3$: C, 70.11; H, 5.23; N, 9.09. Found: C, 70.15; H, 5.42; N, 9.08. **16e**; Yield, 69%. mp 114—116 °C. MS *m/e*: 324 (M^+), 293, 265. NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz), 3.61 (3H, s), 4.25 (2H, q, J=7 Hz), 7.30—7.60 (4H, m), 7.90 (1H, d, J=9 Hz), 8.42 (1H, s), 8.28 (1H, dd, J=9, 2 Hz), 9.26 (1H, d, J=2 Hz). IR ν_{max}^{KBr} cm^{-1}: 1730, 1670, 1295, 1250. *Anal.* Calcd for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64. Found: C, 66.46; H, 4.94; N, 8.61.

7- and 6-Hydroxymethyl-2-phenyl-1(2*H*)-phthalazinone Derivatives (17)——An ethoxycarbonyl-2-phenyl-1(2*H*)-phthalazinone derivative (**16**) (7.4 mmol) was dissolved in 100 ml of EtOH, then 10 mmol of $NaBH_4$ was added at room temperature and the mixture was refluxed for 2 h. After addition of dil. HCl sol., the solvent was removed under reduced pressure. The residue was extracted with $CHCl_3$. The organic layer was washed with saturated NaCl sol., and dried over $MgSO_4$. The $CHCl_3$ was evaporated off and the residue was recrystallized with EtOH to afford pure **17**. **17a**; Yield, 76%. mp 141—142 °C. MS *m/e*: 252 (M^+). NMR ($CDCl_3$) δ: 3.28 (1H, br s, disappeared on the addition of D_2O), 4.78 (2H, s), 7.30—7.90 (7H, m), 8.21 (1H, s), 8.37 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 3420, 1637. *Anal.* Calcd for $C_{15}H_{12}N_2O_2$: C, 71.41; H, 4.80; N, 11.11. Found: C, 71.56; H, 4.81; N, 11.23. **17b**; Yield, 71%. mp 151—153 °C. MS *m/e*: 252 (M^+). NMR ($CDCl_3$) δ: 3.10 (1H, br s, disappeared on the addition of D_2O), 4.85 (2H, s), 7.20—8.00 (7H, m), 8.27 (1H, s), 8.43 (1H, br s). *Anal.* Calcd for $C_{15}H_{12}N_2O_2$: C, 71.41; H, 4.80; N, 11.11. Found: C, 71.59; H, 4.62; N, 11.19. **17c**; Yield, 70%. mp 143—145 °C (EtOH–Et_2O), MS *m/e*: 270 (M^+), 251. NMR ($CDCl_3$) δ: 3.16 (1H, br s disappeared on the addition of D_2O), 4.82 (2H, s), 7.20—7.90 (6H, m), 8.23 (1H, s), 8.42 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 3400, 1635. *Anal.* Calcd for $C_{15}H_{11}FN_2O_2$: C, 66.66; H, 4.10; N, 10.37. Found : C, 66.48; N, 4.13; N, 10.26. **17d**; Yield, 69%. mp 112—115 °C (EtOH–H_2O). MS *m/e*: 266 (M^+), 249. NMR ($CDCl_3$) δ: 2.13 (3H, s), 2.58 (1H, br s, disappeared on the addition of D_2O), 4.89 (2H, s), 7.33 (4H, s), 7.75—7.85 (2H, m), 8.27 (1H, s), 8.48 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 3390, 1625. *Anal.* Calcd for $C_{16}H_{14}N_2O_2$: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.22; H, 4.31; N, 10.42. **17e**; Yield, 61%. mp 183—185 °C (MeOH). MS *m/e*: 282 (M^+), 251. NMR (DMSO-d_6) δ: 3.83 (3H, s), 4.72 (2H, d, J=6 Hz), 5.50 (1H, t, J=6 Hz, disappeared on the addition of D_2O), 7.00—7.60 (4H, m), 7.92 (2H, s), 8.25 (1H, br s), 8.45 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 3360, 1620. *Anal.* Calcd for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 67.89; H, 5.19; N, 9.88.

2-Phenyl- or 2-(*o*-Fluorophenyl)-1(2*H*)-phthalazinone (4j or 4k)——Phenylhydrazine·HCl or *o*-fluorophenylhydrazine·HCl (2.5 mmol) was added under stirring to *o*-phthalaldehydic acid (**5j**) (2 mmol) at room temperature. The mixture was stirred for an additional 5 to 10 h, then extracted with $CHCl_3$. The organic layer was washed with 10% KOH aq. solution and H_2O, then dried over $MgSO_4$ and concentrated. The residue was recrystallized from EtOH to give **4j** or **4k**. **4j**; Yield, 318 mg (71.5%). mp 104—105 °C (lit.[16] 105—106 °C. MS *m/e*: 222 (M^+). NMR ($CDCl_3$) δ: 7.20—7.90 (8H, m), 8.25 (1H, s), 8.40—8.60 (1H, m). IR ν_{max}^{KBr} cm^{-1}: 1670, 1590. *Anal.* Calcd for $C_{14}H_{10}N_2O$: C, 75.65; H, 4.54; N, 12.61. Found: C, 75.72; H, 4.55; N, 12.74. **4k**; Yield, 362 mg (75.3%). mp 120.5—121.5 °C. MS *m/e*: 240 (M^+), 221 ($M^+ - F$, base peak). NMR ($CDCl_3$) δ: 7.20—7.90 (7H, m), 8.25 (1H, s), 8.40—8.60 (1H, m). IR ν_{max}^{KBr} cm^{-1}: 1670, 1590, 1490. *Anal.* Calcd for $C_{14}H_9FN_2O$: C, 70.00; H, 3.78; N, 11.66. Found: C, 70.14; H, 3.91; N, 11.43.

7-Halogeno-, and 7-Methoxy-2-phenyl-1(2*H*)-phthalazinone (4l—n)——Acetophenone derivatives (**19**) were prepared from the corresponding 3-substituted toluenes (**18**) by the use of Friedel–Crafts acylation with Ac_2O in CS_2. By-products (**20**) were also formed in the cases of **18a** and **18b**. Without purification, the mixture of **19** and **20** was oxidized with $KMnO_4$ followed by decarboxylation as described above. On subsequent treatment with phenylhydrazine, the corresponding condensation products, (**4l—n**) and (**2l**) were obtained. The mixture was treated with 10% NaOH aq. solution and extracted with $CHCl_3$ to give **4l—n**, and **4l—n** were recrystallized from EtOH. **4l**; Yield, 40.2% (from the mixture of **19a** and **20a**). mp 160—161 °C. MS *m/e*: 256 and 258 (M^+), 192, 166. NMR ($CDCl_3$) δ: 7.30—7.80 (5H, m), 7.67 (1H, d, J=9 Hz), 7.80 (1H, d, J=9 Hz), 8.25 (1H, s), 8.48 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 3450, 3060, 2320, 1665. *Anal.* Calcd for $C_{14}H_9ClN_2O$: C, 65.51; H, 3.53; N, 10.91. Found: C, 65.66; H, 3.62; N, 10.94. **4m**; Yield, 41.3% (from the mixture of **19b** and **20b**). mp 157—158 °C. MS *m/e*: 302 and 300 (M^+). NMR ($CDCl_3$) δ: 7.33—7.80 (5H, m), 7.61 (1H, d, J=9 Hz), 7.97 (1H, dd, J=9, 2 Hz), 8.27 (1H, s), 8.68 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{14}H_9BrN_2O$: C, 55.84; H, 3.01; N, 9.30. Found: C, 55.95; H, 2.97; N, 9.36. **4n**; Yield, 28% (from **19c**). mp 168—171 °C. MS *m/e*: 252 (M^+). NMR ($CDCl_3$) δ: 3.95 (3H, s), 7.25—7.70 (5H, m), 7.55 (1H, d, J=9 Hz), 7.61 (1H, d, J=2 Hz), 7.78 (1H, dd, J=9, 2 Hz), 8.21 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 1650, 1610, 1585. *Anal.* Calcd for $C_{15}H_{12}N_2O_2$: C, 71.41; H, 4.80; N, 11.11. Found: C, 71.43; H, 4.87; N, 11.08. The basic layer was acidified with conc. HCl to pH 1. The precipitate (**21a**, **b**) was washed with H_2O. **21a**; MS *m/e*: 276 and 274 (M^+). **21b**; MS *m/e*: 320 and 318 (M^+).

7-Amino-2-phenyl-1(2*H*)-phthalazinone (23)——Curtius Reaction of **4e**: The procedure described by Weinstock[12] gave 900 mg of the corresponding acid azide from 7-carboxy-2-phenyl-1(2*H*)-phthalazinone (**4e**) (1 g, 3.76 mmol). Yield, 82.3%. mp 133—135 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 2150, 1700, 1280. The azide derivative (460 mg, 1.6 mmol) was dissolved in 20 ml of benzene and the solution was heated gently in the presence of 324 mg of benzyl alcohol. After the evolution of N_2 gas had ceased, refluxing was continued for an additional 6 h at 90 °C. The solvent was evaporated off and the residue was recrystallized from MeOH to afford **22** (411 mg, 72%). mp 193—194 °C. MS *m/e*: 371 (M^+), 263. NMR ($CDCl_3$) δ: 5.12 (2H, s), 7.30—7.70 (11H, m), 7.76 (1H, d, J=9 Hz), 8.17 (1H, s), 8.25 (1H, s), 8.32 (1H, d, J=9 Hz). IR ν_{max}^{KBr} cm^{-1}: 1730, 1630. A solution of 7-benzoxycarbonylamino-2-phenyl-1(2*H*)-phthalazinone (**22**) (371 mg, 1 mmol) in dioxane (50 ml) was hydrogenated over 10% palladium carbon (100 mg) at atmospheric pressure at room temperature. The catalyst was filtered off and the filtrate was evaporated. The residue was recrystallized from MeOH to give **23**. Yield, 204 mg (86%). mp 216—219 °C. MS *m/e*: 237 (M^+), 236, 149. NMR (DMSO-d_6) δ: 6.30 (2H, br s, disappeared on the addition of D_2O), 7.00—7.80 (8H, m), 8.26 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 3350, 3230, 1650, 1600. *Anal.* Calcd for $C_{14}H_{11}N_3O$: C, 70.87; H, 4.67; N, 17.71. Found: C, 70.81; H, 4.80; N, 17.66.

Reduction of the Nitro Compound (**4b**): 7-Nitro-2-phenyl-1(2*H*)-phthalazinone (**4b**) (534 mg, 2 mmol) was dissolved in 50 ml of hot dioxane and hydrogenated over PtO_2 (100 mg) at atmospheric pressure at room temperature. Crystallization from EtOH of the residue obtained by removal of the catalyst and solvent gave **23**. Yield, 431.8 mg (91%).

7-Dimethylamino-2-phenyl-1(2*H*)-phthalazinone (24)——A stirred mixture of the 7-amino compound (**23**) (1.69 g, 7.1 mmol), NaH (washed with hexane; 600 mg, 25 mmol) and THF (50 ml) was gently refluxed and treated dropwise with 3.6 g (21.3 mmol) of $(MeO)_2SO_2$. When the addition was complete, refluxing was continued for a further 4 h. The solution was allowed to cool to room temperature, then 5 ml of MeOH and 20 ml of aq. 10% NaOH sol. were added. The mixture was extracted with $CHCl_3$, dried over $MgSO_4$ and concentrated. The residue was chromatographed on a column of silica gel and eluted with $CHCl_3$ to give **24** (851 mg, 45%). mp 140—141 °C (MeOH–Et_2O). MS *m/e*: 265 (M^+). NMR ($CDCl_3$) σ: 3.15 (6H, s), 7.00—7.90 (7H, m), 8.15 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 1660, 1615, 1380. *Anal.* Calcd for $C_{16}H_{15}N_3O$: C, 72.43; H, 5.70; N, 15.84. Found: C, 72.55; H, 5.69; N, 15.98.

2-Phenyl-1(2*H*)-phthalazinone 7-Dimethylamino *N*-Oxide (25)——*m*-Chloroperbenzoic acid (414.2 mg, 2.4 mmol) was added to a solution of the 7-dimethylamino compound (**24**) (265 mg, 1 mmol) in 30 ml of $CHCl_3$. The mixture was stirred at room temperature for 21 h. After the addition of aq. 10% KOH sol., the whole was extracted with $CHCl_3$, washed, dried and concentrated. The residue was recrystallized from EtOH–Et_2O to give **25**. Yield, 152 mg (54%). mp 159—160 °C. MS *m/e*: 281 (M^+), 265, 237. NMR (DMSO-d_6) δ: 3.60 (6H, s), 7.61 (5H, br s), 8.10 (1H, s), 8.40 (1H, d, J=15 Hz), 8.69 (1H, br s), 8.98 (1H, dd, J=15, 3 Hz). *Anal.* Calcd for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.38; N, 14.94. Found: C, 68.05; H, 5.50; N, 14.78.

7-Carbamoyl-2-phenyl-1(2*H*)-phthalazinone Derivatives (26)——A mixture of the 7-carboxy derivative (**4e**) (3 mmol) and $SOCl_2$ (20 ml) was refluxed for 3 h, then $SOCl_2$ was evaporated off, and the residue was dissolved in 20 ml of CH_2Cl_2. An excess of the corresponding amine derivative was added to the solution and the mixture was stirred at room temperature for several hours, then treated with H_2O, extracted, washed, dried and concentrated to give **26**. **26a**; Yield, 60%. mp 243—245 °C (EtOH–Et_2O). MS *m/e*: 265 (M^+), 249, 221. NMR (DMSO-d_6) δ: 7.20—7.80 (5H, m), 8.00 (1H, d, J=9 Hz), 8.36 (1H, dd, J=9, 2 Hz), 8.55 (1H, s), 8.77 (1H, d, J=2 Hz). IR ν_{max}^{KBr} cm^{-1}: 3490, 1700, 1650, 1600. *Anal.* Calcd for $C_{15}H_{11}N_3O_2$: C, 67.91; H, 4.18; N, 15.84. Found: C, 67.78; H, 4.23; N, 15.99. **26b**; Yield, 52%. mp 150—152 °C (EtOH). MS *m/e*: 293 (M^+), 249, 221. NMR (DMSO-d_6) δ: 3.04 (6H, br s), 7.33—7.80 (5H, m), 8.05 (2H, br s), 8.32 (1H, s), 8.67 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 3350, 3090, 1670. *Anal.* Calcd for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.45; H, 4.98; N, 14.23. **26c**; Yield, 42.2%. mp 220—224 °C (EtOH–AcOEt–Et_2O). MS *m/e*: 336 (M^+), 292, 249, 221. NMR (DMSO-d_6) δ: 2.85 (6H, s), 3.50 (2H, m), 3.68 (2H, br t, J=6 Hz), 7.40—7.80 (5H, m), 8.10 (1H, d, J=9 Hz), 8.58 (1H, dd, J=9, 2 Hz), 8.67 (1H, s), 8.87 (1H, d, J=2 Hz), 9.41 (1H, br s, disappeared on the addition of D_2O). IR ν_{max}^{KBr} cm^{-1}: 3250, 3050, 1670. *Anal.* Calcd for $C_{19}H_{20}N_4O_2$: C, 67.84; H, 5.99; N, 16.66. Found: C, 67.71; H, 6.02; N, 16.80. **26d**; Yield, 90.6%. mp 149—152 °C (MeOH–hexane). MS *m/e*: 348 (M^+), 304, 291, 249. NMR ($CDCl_3$) δ: 2.30—2.70 (4H, m), 2.37 (3H, s), 3.50—4.00 (4H, m), 7.40—8.10 (7H, m). IR ν_{max}^{KBr} cm^{-1}: 3450, 3060, 1660, 1640. *Anal.* Calcd for $C_{20}H_{20}N_4O_2$: C, 68.95; H, 5.79; N, 16.08. Found: C, 68.72; H, 5.85; N, 16.03.

7-Chloromethyl-2-phenyl-1(2*H*)-phthalazinone (27)——A mixture of the 7-hydroxymethyl compound (**17a**) (1.26 g, 5 mmol) and $SOCl_2$ (10 ml) was refluxed for 1 h, then $SOCl_2$ was evaporated off and the residue was treated with aq. 10% K_2CO_3 sol. and $CHCl_3$. The $CHCl_3$ layer was washed, dried and evaporated, and the residue was recrystallized from EtOH to give **27** (1.1 g, 78%). mp 119—120 °C. MS *m/e*: 270 and 272 (M^+), 235, 207. NMR ($CDCl_3$) δ: 4.74 (2H, s), 7.20—8.05 (7H, m), 8.30 (1H, s), 8.53 (1H, br s).

7-Aminomethyl and 7-Aminoalkoxymethyl-1(2*H*)-phthalazinone Derivatives (28)——The appropriate amine derivative (2—4 mmol) was added to the 7-chloromethyl compound (**27**) (1 mmol) and the mixture was stirred at room temperature for 2—40 h. (**28a**, conc. NH_4OH; **28b**, $(Me)_2NH/CHCl_3$; **28c**, $(Me)_2N(CH_2)_2ONa$/THF). The product was extracted with $CHCl_3$ or AcOEt and worked up in the usual manner. **28a**; Yield, 82%. mp 70—72 °C (benzene). MS *m/e*: 251 (M^+), 223. NMR (DMSO-d_6) δ: 3.25 (2H, br s), 4.00 (2H, br s), 7.40—7.70 (5H, m), 7.98 (2H, s), 8.38 (1H, br s), 8.58 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 3050, 1650, 1600. *Anal.* Calcd for $C_{15}H_{13}N_3O$: C, 71.69; H, 5.21; N,

16.72. Found: C, 71.75; H, 5.22; N, 16.58. **28b**; Yield, 76%. mp 113—115 °C (EtOH–Et_2O) MS *m/e*: 279 (M^+), 236, 206. NMR ($CDCl_3$) δ: 2.33 (6H, s), 3.69 (2H, s), 7.30—8.20 (7H, m), 8.47 (1H, s), 8.60 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 3400, 3050, 2760, 1660, 1600. *Anal.* Calcd for $C_{17}H_{17}N_3O$: C, 73.09; H, 6.13; N, 15.04. Found: C, 73.23; H, 6.01; N, 15.23. **28c**; Yield, 42%. mp 48—49 °C. (Et_2O–hexane). MS *m/e*: 323 (M^+), 295. NMR ($CDCl_3$) δ: 2.30 (6H, s), 2.56 (2H, t, J=6 Hz), 3.62 (2H, t, J=6 Hz), 4.73 (2H, s), 7.30—8.00 (7H, m), 8.27 (1H, s), 8.45 (1H, s). IR ν_{max}^{KBr} cm^{-1}: 3050, 2930, 2760, 1665. *Anal.* Calcd for $C_{19}H_{21}N_3O_2$: C, 70.56; H, 6.55; N, 13.00. Found: C, 70.31; H, 6.42; N, 13.19.

7-Cyano-2-phenyl-1(2*H*)-phthalazinone (29)——A solution of 602 mg of the 7-bromo compound (**4m**) (2 mmol) and 206 mg of CuCN (2.3 mmol) in 10 ml of dimethylformamide was refluxed for 10 h. The solvent was removed under reduced pressure. In order to decompose the resulting complex, $FeCl_3 \cdot 6H_2O$ (800 mg), conc. HCl (0.5 ml) and H_2O (2 ml) were added to the residue. The mixture was heated at 70 °C for 20 min, extracted with $CHCl_3$, dried and concentrated, and the residue was recrystallized from MeOH to give **29**. Yield, 369 mg (75%). mp 232—233 °C. MS *m/e*: 247 (M^+). NMR (DMSO-d_6) δ: 7.40—7.70 (5H, m), 8.25 (1H, d, J=12 Hz), 8.28 (1H, s), 8.73 (1H, s), 8.80 (1H, d, J=12 Hz). IR ν_{max}^{KBr} cm^{-1}: 2230, 1680. *Anal.* Calcd for $C_{15}H_9N_3O$: C, 72.86; H, 3.67; N, 17.00. Found: C, 73.01; H, 3.74; N, 17.27.

Hydrolysis of 7-Cyano-2-phenyl-1(2*H*)-phthalazinone (29)——The 7-cyano compound (**29**) (100 mg, 0.4 mmol) was dissolved in 10 N NaOH solution (2 ml) and EtOH (2 ml), and the solution was refluxed for 3 h. After evaporation of the EtOH, the residue was acidified with conc. HCl solution. The precipitate was filtered off, washed with H_2O, and dried to give **4e**. Purification of the crude **4e** (97.6 mg, 90.6%) was carried out by esterification as described above.

7-Ethylmercapto-2-phenyl-1(2*H*)-phthalazinone (30)——A mixture of the 7-bromo compound (**4m**) (900 mg, 3 mmol), CuSEt[14] (1.13 g, 9 mmol) and dimethylformamide (50 ml) was refluxed for 15 h. The warm solution was poured onto cracked ice in the presence of excess hydrochloric acid. After being stirred for 30 min at room temperature, the mixture was extracted with $CHCl_3$, then the extract was washed with H_2O, dried over $MgSO_4$ and concentrated. The residue was subjected to chromatography on silica gel and eluted with $CHCl_3$ to give **30** (600 mg, 71%). mp 144.5—145.5 °C (Et_2O). MS *m/e*: 282 (M^+), 254, 253. NMR ($CDCl_3$) δ: 1.40 (3H, t, J=8 Hz), 3.15 (2H, q, J=8 Hz), 7.30—7.80 (7H, m), 8.23 (1H, s), 8.30 (1H, br s). IR ν_{max}^{KBr} cm^{-1}: 3040, 2970, 1645, 1344. *Anal.* Calcd for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found: C, 68.29; H, 4.95; N, 9.95.

Oxidation of the 7-Ethylmercapto Derivative to Sulfoxide (31) and Sulfone Derivatives (32)——The 7-ethylmercapto compound (**30**) (282 mg, 1 mmol) was dissolved in 50 ml of $CHCl_3$. After addition of *m*-chloroperbenzoic acid 190 mg (1.1 mmol), the mixture was stirred for 1 h at 0 °C, and aq. 10% K_2CO_3 solution was added. Conventional work-up of the reaction mixture and recrystallization from EtOH gave **31**. The addition of 1.1 more mol of *m*-chloroperbenzoic acid caused further oxidation to give the sulfone derivative (**32**). **31**; Yield, 82%. mp 124—125 °C. (EtOH–Et_2O). MS *m/e*: 298 (M^+), 282, 269. NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz), 2.77 (1H, q, J=7 Hz), 3.10 (1H, q, J=7 Hz), 7.30—7.82 (5H, m), 7.92 (1H, d, J=9 Hz), 8.24 (1H, dd, J=9, 2 Hz), 8.36 (1H, s), 8.60 (1H, d, J=2 Hz). IR ν_{max}^{KBr} cm^{-1}: 1660, 1650, 1339, 1075. *Anal.* Calcd for $C_{16}H_{14}N_2O_2S$: C, 64.41; H, 4.73; N, 9.39. Found: C, 64.43; H, 4.84; N, 9.44. **32**; Yield, 84.5%. mp 192—194 °C (EtOH). MS *m/e*: 314 (M^+), 285, 269. NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7 Hz), 3.22 (2H, q, J=7 Hz), 7.30—7.80 (5H, m), 7.93 (1H, d, J=8 Hz), 8.34 (1H, dd, J=8, 2 Hz), 8.40 (1H, s), 9.05 (1H, d, J=2 Hz). IR ν_{max}^{KBr} cm^{-1}: 1655, 1295, 1140. *Anal.* Calcd for $C_{16}H_{14}N_2O_3S$: C, 61.13; H, 4.49; N, 8.91. Found: C, 60.90; H, 4.46; N, 8.76.

Preparation of Platelet-Rich Plasma (PRP)——Blood samples were collected into tubes containing 1/10 volume of 3.8% aqueous sodium citrate through a cannula inserted into the carotid artery of rabbits. PRP was prepared by centrifugation of the blood samples at 150 g for 15 min at room temperature. The supernatant was used as PRP.

Platelet Aggregation Test——The turbidometric method of Born and Cross[15] modified to provide continuous stirring (1200 rpm) and maintenance of constant temperature (37 °C) was employed for assessing the ability of test compounds to inhibit platelet aggregation induced by aggregating agents. A 0.435 ml sample of PRP was placed in an aggregometer (SIENCO, dual sample aggregation meter, model DP-247E) and then various concentrations of a test compound or vehicle in a volume of 2.5 μl were added. After preincubation of the mixture of PRP and test compound or vehicle for 3 min, 10 μl of an aqueous solution of ADP (final concentration of 10 μM) or 10 μl of an aqueous solution of AA (final concentration of 137 μM) was added to induce platelet aggregation. Inhibition of platelet aggregation by a test compound was calculated by dividing the maximum deflection in the optical density curve by that without test compound. Test compounds were dissolved in dimethyl sulfoxide, which was present at a final concentration of 0.5% or less in all experiments; it had no effect at this concentration on any of the parameters studied. The number of platelets (ranging from 3.5 to 4.5 $\times 10^5$ platelets/μl of PRP) was determined with the aid of a Coulter counter (Coulter Electronics, Inc.). The results are given as mean IC_{100} values from three separate experiments.

Acknowledgment We thank Dr. H. Azuma of our Department for helpful discussions and a critical reading of the manuscript.

References

1) M. Ishikawa, Y. Eguchi, and A. Sugimoto, *Chem. Pharm. Bull.*, **28**, 2770 (1980).

2) A. Sugimoto, S. Ito, Y. Eguchi, H. Tanaka, Y. Takashima, and M. Ishikawa, *Eur. J. Med. Chem.*, **19**, 223 (1984).
3) A. Sugimoto, H. Tanaka, Y. Eguchi, S. Ito, Y. Takashima, and M. Ishikawa, *J. Med. Chem.*, **27**, 1300 (1984).
4) J. H. Gardner and C. A. Naylor, Jr., "Organic Synthesis," Coll. Vol. II, ed. by A. H. Blatt, John Wiley and Sons, Inc., New York, 1943, p. 523.
5) D. Taub, *Tetrahedron*, **24**, 2443 (1968).
6) A. J. McAlees, R. McCrindle, and D. W. Sneddon, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 2030.
7) A. J. McAlees, R. McCrindle, and D. W. Sneddon, *J. Chem. Soc., Perkin Trans. 1*, **1977**, 2037.
8) T. Watanabe, F. Hamaguchi, and S. Ohki, *Chem. Pharm. Bull.*, **20**, 2123 (1972).
9) B. H. Nicolet and J. Sacks, *J. Am. Chem. Soc.*, **47**, 2348 (1925); G. M. Dickinson, L. H. Crosson, and J. E. Copenhaver, *ibid.*, **59**, 1094 (1937).
10) K. Ishizumi, K. Koga, and S. Yamada, *Chem. Pharm. Bull.*, **16**, 492 (1968).
11) W. Borsche, K. Diacont, and H. Hanau, *Chem. Ber.*, **67**, 675 (1934).
12) J. Weinstock, *J. Org. Chem.*, **26**, 3511 (1961).
13) H. Quast, W. Risler, and G. Dollscher, *Synthesis*, **1972**, 558.
14) R. Adams, W. Reifshneider, and M. D. Nair, *Croatica Chem. Acta*, **29**, 277 (1957); R. Adams and A. Ferretti, *J. Am. Chem. Soc.*, **81**, 4927 (1959).
15) G. Born, *Nature* (London), **194**, 927 (1962); G. Born and M. Cross, *J. Physiol.* (London), **168**, 178 (1963).
16) Henriques, *Chem. Ber.*, **21**, 1607 (1888).