Synthesis and Antiplatelet, Antiinflammatory and Antiallergic Activities of 2,3-Disubstituted 1,4-Naphthoquinones

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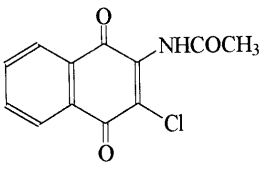
Modification of 2-acetamido-3-chloro-1,4-naphthoquinone, which has potent antiplatelet, antiallergic and antiinflammatory activities, led to a series of 2,3-disubstituted 1,4-naphthoquinones. Some of these compounds showed significant antiplatelet, antiallergic and antiinflammatory activities. Among them, 2-methoxy-3-chloro-1,4-naphthoquinone (15) and 2-ethoxy-3-chloro-1,4-naphthoquinone (17) exhibited potent inhibitory effects on neutrophil and mast cell degranulation. 2-Methoxy-1,4-naphthoquinone (20) and 2-ethoxy-1,4-naphthoquinone (21) exhibited potent inhibitory effect on neutrophil superoxide formation. These four compounds were thus selected for further evaluation.

Key words naphthoquinone; antiplatelet activity; antiinflammatory activity; antiallergic activity; neutrophil degranulation; mast cell degranulation

In a previous paper,[1] a series of 2-substituted 3-chloro-l,4-naphthoquinones were synthesized, and their antiplatelet, antiinflammatory and antiallergic activities were evaluated. We demonstrated that the amide linkage at the 2-position plays an important role in regard to the activities of 2-substituted 3-chloro-1,4-naphthoquinones. Most of the 2-alky/arylcarboxamido derivatives showed potent activities. Among these active compounds, 2-acetamido-3-chloro-1,4-naphthoquinone (**A**) was the most promising agent and showed potent activities in suppressing polymyxin B-induced hind-paw edema, passive cutaneous anaphylactic reaction, and histamine-, serotonin-, bradykinin- or substance P-induced ear edema in mice. Therefore, based on compound **A** as a lead compound, we synthesized various 2,3-disubstituted analogs, and examined their antiplatelet, antiinflammatory and antiallergic activities.

Chemistry

The syntheses of the target 2-alkylcarboxamido-1,4-naphthoquinones (**2**—**8**) and 2-arylcarboxamido-1,4-naphthoquinone (**9**) are summarized in Charts 1 and 2. Acylation of 2-amino-1,4-naphtho-quinone (**1**)[2] with an acyl anhydride or acyl chloride in the presence of concentrated sulfuric acid or dry hydrogen chloride afforded compounds **2**—**8**. The synthesis of **9** was carried out under strongly basic conditions as illustrated in Chart 2. 2-Amino-1,4-naphthoquinone (**1**) was treated with an equimolar ratio of NaH, and then benzoyl chloride was added thereto afford **9**.



(A)

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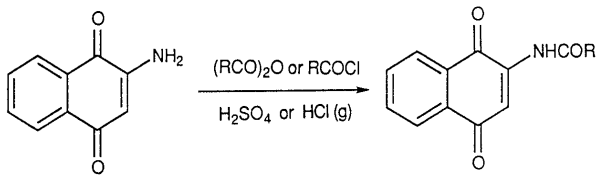
As outlined in Chart 3, reaction of 2-acetamido-3-chloro-1,4-naphthoquinone with ammonia or an (alkyl/aryl)amine afforded 2-acetamido-3-(alkyl/aryl)amino-1,4-naphthoquinones (**10**—**14**).

Chart 4 shows the preparation of 2-alkoxy-3-chloro-1,4-naphthoquinones (**15** and **17**) and 2,3-dialkoxy-1,4-naphthoquinones (**16** and **18**). When 2,3-dichloro-1,4-naphthoquinone was treated with sodium methoxide, 2-methoxy-3-chloro-1,4-naphthoquinone (**15**) and 2,3-dimethoxy-1,4-naphthoquinone (**16**) were formed even though the reactants were used in an equimolar ratio.

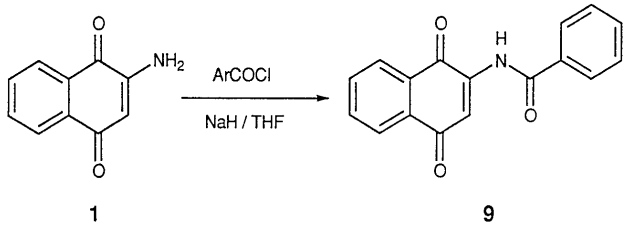
2-Alkoxy-1,4-naphthoquinones (**20** and **21**) were pre-



1

2, R = CH_3
3, R = CH_2CH_3
4, R = $CH_2CH_2CH_3$
5, R = CH_2Cl
6, R = $(CH_2)_2COOCH_3$
7, R = (CH2)3COOCH3
8, R = CH_2-

Chart 1



1 **9**

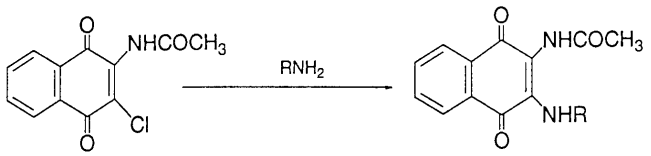
Chart 2

pared according to the previously reported method.[3]

Biological Activity

a. Antiplatelet Activity The antiplatelet activities of the substituted 1,4-naphthoquinone derivatives (**2**—**21**) are summarized in Table 1.

Regarding the inhibitory activities against platelet aggregation induced by thrombin, arachidonic acid (AA), collagen and platelet-activating factor (PAF), 2-acetamido-1,4-naphthoquinone (**2**) showed a poor inhibitory effect compared with the previously synthesized compound **A**.[1] When the methyl group of **2** was replaced with an ethyl group (**3**) and a propyl group (**4**), the potency increased slightly in **3** but decreased in **4** in the AA-induced platelet aggregation assay. In comparison with **2**, the inhibitory effect on the platelet aggregation induced by AA remained unchanged when a chloro group (**5**) was incorporated in the methyl group of **2**. However, the potency decreased significantly when a phenyl group (**8**) was incorporated in the methyl group of **2**. In comparison



A

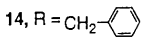
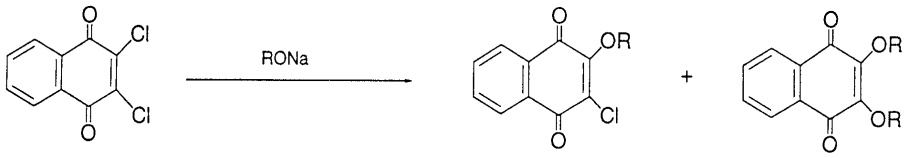
10, R = H
11, R = CH_3
12, R = CH_2CH_3
13, R = 
14, R = CH_2 

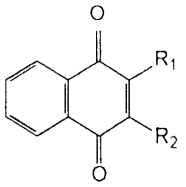
Chart 3



15, R = CH_3
16, R= CH_3
17, R = C_2H_5
18, R= CH_2CH_3

Chart 4

Table 1. The Inhibitory Effect of 2,3-Disubstituted 1,4-Naphthoquinones on Platelet Aggregation Induced by Thrombin, AA, Collagen and PAF



Compound	R_1	R_2	IC_{50} (μg/ml)			
			Thrombin	AA	Collagen	PAF
Aspirin			>100	3.6 ±0.3	>100	>100
A	$NHCOCH_3$	Cl	4.8±0.4	0.30±0.04	2.0±0.3	1.9±0.2
2	$NHCOCH_3$	H	>100	20.9 ±2.1	52.7±4.2	>100
3	$NHCOCH_2CH_3$	H	>100	11.7 ±0.8	54.6±3.2	>100
4	$NHCOCH_2CH_2CH_3$	H	>100	63.1 ±5.2	>100	>100
5	$NHCOCH_2Cl$	H	>100	13.9 ±0.8	70.5±6.5	>100
6	$NHCO(CH_2)_2COOCH_3$	H	>100	14.4 ±0.7	>100	>100
7	$NHCO(CH_2)_3COOCH_3$	H	>100	17.2 ±1.1	17.8±4.3	>100
8	$NHCOCH_2C_6H_5$	H	>100	>100	>100	>100
9	$NHCOC_6H_5$	H	>100	6.1 ±0.3	47.8±3.5	>100
10	$NHCOCH_3$	NH_2	>100	22.4 ±1.8	>100	>100
11	$NHCOCH_3$	$NHCH_3$	>100	>100	>100	>100
12	$NHCOCH_3$	$NHCH_2CH_3$	>100	>100	>100	>100
13	$NHCOCH_3$	NHC_6H_5	>100	>100	>100	>100
14	$NHCOCH_3$	$NHCH_2C_6H_5$	>100	60.4 ±5.2	>100	>100
15	OCH_3	Cl	>100	4.2 ±0.3	7.6±0.8	>100
16	OCH_3	OCH_3	>100	26.2 ±2.1	57.5±4.6	>100
17	OCH_2CH_3	Cl	>100	7.9 ±0.4	>100	7.8±0.2
18	OCH_2CH_3	OCH_2CH_3	>100	29.2 ±1.8	>100	>100
19	OH	H	>100	>100	>100	>100
20	OCH_3	H	>100	10.1 ±0.4	13.7±1.1	28.8±1.6
21	OCH_2CH_3	H	>100	7.6 ±0.2	8.8±0.6	18.0±0.9

Platelets were incubated with a test sample or 0.5% DMSO at 37 °C for 1 min, then thrombin (0.1 unit/ml), AA (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added to trigger the aggregation. Aspirin is a positive control. Values are expressed as mean±S.E. from 4 to 6 separate experiments.

with **3** and **4**, the potency was maintained if a terminal hydrogen of the 2-ethyl/propyl-carboxamido group was replaced by $-COOCH_3$ (**6** and **7**). When the methyl group of **2** was replaced by a phenyl group (**9**), the inhibitory effect on AA-induced platelet aggregation was increased slightly.

The antiplatelet activity of 2-acetamido-3-chloro-1,4-naphthoquinone (**A**) decreased remarkably when the chloro group was replaced by (alkyl/aryl)amino groups (**10**—**14**). In comparison with compound **A**, the potency decreased when the –CONH– (amide linkage) was changed to –O– (ether linkage) (**15** and **17**). The activities also decreased when the chloro group of **15** and **17** was further replaced by alkoxy groups (**16** and **18**). When the amide linkage of **2** was changed to an ether linkage (**20** and **21**), the potency remained unchanged.

These results suggest that the chloro groups of 2-acetamido-3-chloro-1,4-naphthoquinones (**A**) play an important role in the antiplatelet activity in addition to the amide linkage.

b. Antiinflammatory Activity Effect on Neutrophil Degranulation: The effects of **2**—**21** on neutrophil degranulation were examined. As can be seen in Table 2, compound **2** was about 100 times less potent than compound **A** in its effect on the neutrophil degranulation induced by FMLP (formyl–Met–Leu–Phe) (1 μM). When the methyl group of **2** was replaced with an ethyl group (**3**) and a propyl group (**4**), the potency increased slightly in **3** but decreased in **4**. In comparison with **2**, the inhibitory effect increased remarkably when either a chloro group (**5**) or phenyl group (**8**) was incorporated in the methyl group of **2**. In comparison with compounds **3** and **4**, the potency also increased if a terminal hydrogen of the 2-alkylcarboxamido group was replaced by $-COOCH_3$ (**6** and **7**). In the case of **9**, where the 2-alkylcarboxamido group of compounds **2**—**4** was replaced by a phenylcarboxamido group, the activity increased significantly.

The inhibitory effect on neutrophil degranulation induced by FMLP decreased remarkably when the chloro group of 2-acetamido-3-chloro-1,4-naphthoquinone (**A**) was replaced by an (alky/aryl)amino group (**10**—**14**). In comparison with compound A, the inhibitory effect remained unchanged when the –CONH-linkage was changed to an –O-linkage (**15** and **17**); however, the activities decreased when the chloro group of **15** and **17** were further replaced by alkoxy groups (**16** and **18**). When the amide linkage of **2** was changed to an ether linkage (**20** and **21**), the potency remained unchanged. This observation is similar to that found with the antiplatelet activities of **2**—**21**. However, compound **15**, which possesses a methoxy group instead of an acetamido group, has a potency similar to that of compound **A**.

Effect on Neutrophil Superoxide Formation: As shown in Table 3, compared with compound **A**, compound **2** has no significant inhibitory effect on neutrophil superoxide formation induced by FMLP (0.3 μM), but when the methyl group of **2** was replaced by higher alkyl groups (**3** and **4**), the potency was significant. The potency increased slightly when a chloro group (**5**) was incorporated in the methyl group of **2**, while no effect was seen when the incorporated group was phenyl (**8**). In comparison with **3**, the potency was maintained if a terminal hydrogen of the 2-ethyl-

Table 2. The Inhibitory Effect of 2,3-Disubstituted 1,4-Naphthoquinones on the Release of β-Glucuronidase and Lysozyme from Neutrophils

Compound	IC_{50} (μg/ml)	
	β-Glucuronidase	Lysozyme
Trifluoperazine	8.2	7.0
A	0.10±1.5	0.10±0.01
2	10.2 ±1.5	15.7 ±1.7
3	4.2 ±0.3	4.5 ±0.5
4	8.7 ±2.5	34.6 ±4.0
5	1.0 ±0.1	1.5 ±0.2
6	0.60±0.05	0.60±0.06
7	2.9 ±0.3	14.6 ±0.2
8	5.1 ±0.4	4.5 ±0.5
9	0.50±0.04	0.60±0.05
10	>30	>30
11	>30	>30
12	>30	>30
13	>30	>30
14	>30	>30
15	0.10±0.02	0.10±0.02
16	13.4 ±1.5	15.3 ±2.0
17	0.40±0.03	0.40±0.04
18	17.5 ±2.0	14.3 ±2.0
19	>30	>30
20	14.9 ±2.3	19.4 ±2.0
21	17.6 ±1.5	26.2 ±3.0

The neutrophil suspension was preincubated at 37 °C with 0.5% DMSO or a test compound for 3 min in the presence of cytochalasin B 5 μg/ml. Forty-five minutes after the addition of FMLP (1 μM), β-glucuronidase and lysozyme in the supernatant were determined. Trifluoperazine is a positive control. Values are expressed as mean±S.E. from 4 to 6 separate experiments.

Table 3. The Inhibitory Effect of 2,3-Disubstituted 1,4-Naphthoquinones on the Superoxide Formation of Neutrophils

Compound	IC_{50} (μg/ml)
	Superoxide formation/10^6 cells
Trifluoperazine	6.0 ±0.6
A	0.55±0.05
2	>30
3	>30
4	3.94±0.4
5	>30
6	>30
7	>30
8	>30
9	>30
10	>30
11	>30
12	>30
13	>30
14	>30
15	1.0 ±0.2
16	>30
17	2.9 ±0.4
18	1.6 ±0.2
19	>30
20	0.50±0.04
21	0.20±0.03

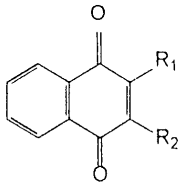
The neutrophil suspension in the presence of tern cytochrome c was preincubated at 37 °C with 0.5% DMSO or a test compound for 3 min. Fifteen minutes after the addition of FMLP (0.3 μM), the absorbance was determined at 550 nm. Trifluoperazine is a positive control. Values are expressed as mean±S.E. from 4 to 6 separate experiments.

carboxamido group was replaced by $-COOCH_3$ (**6**). However, in the case of compound **4**, the potency decreased significantly if a terminal hydrogen of the propylcarboxamido group was replaced by $COOCH_3$ (**7**). When the methyl group of **2** was replaced by a phenyl group (**9**), the activity increased slightly. Compounds **10**—**14**, which are 3-substituted amino derivatives, had essentially no or little or no inhibitory effect on neutrophil superoxide formation. In comparison with compound **A**, the potency decreased slightly when the amide linkage was changed to an ether linkage (**15** and **17**). However, when the amide linkage of **2** was changed to an ether linkage (**20** and **21**), the potency increased significantly in comparison with **2**.

The inhibitory effects of **2**—**14** on neutrophil superoxide formation, as discussed above, show a similar trend to that found on neutrophil degranulation. However, compounds **20** and **21**, which possess an ether linkage instead of an amide linkage, were more potent than compound **A**.

c. Antiallergic Activity Effect on Mast Cell Degranulation: Mast cells, which release various inflammatory mediators during immunological challenge, participate prominently in the passive cutaneous anaphylaxis (PCA) reaction. The effects of **2**—**21** on mast cell degranulation were examined. As shown in Table 4, when compared with compound **A**, compound **2** has a modest inhibitory effect on mast cell degranulation induced by compound 48/80 (10 μg/ml). As the methyl group of **2** was replaced by a higher alkyl group (**3** and **4**), the potency decreased. The potency was maintained when a terminal hydrogen of the 2-ethylcarboxamido group of **2** was substituted with chloro (**5**) or phenyl (**8**). The potency was also maintained if a terminal hydrogen of the 2-alkylcarboxamido groups of **3** and **4** was substituted with $-COOCH_3$ (**6** and **7**). When the 2-methyl group of **2** was replaced by a phenyl group (**9**), the activity increased. Compounds **10**, **11**, **12**, **13** and **14**, which are 3-amino, 3-alkylamino, 3-phenylamino and 3-benzylamino derivatives of 2-acetamido-1,4-naphthoquinone, respectively, had substantially no inhibitory effect on mast cell degranulation. As compared with compound **A**, the inhibitory effect increased significantly when the amide linkage of compound **A** was changed to an ether linkage (**15** and **17**), but decreased when the chloro group of **15** and **17** was further replaced by an additional alkoxy group (**16** and **18**).

Table 4. The Inhibitory Effect of 2,3-Disubstituted 1,4-Naphthoquinones on the Release of β-Glucuronidase and Histamine from Mast Cells



Compound	IC_{50} (μg/ml)	
	β-Glucuronidase	Histamine
Mepacrine	13.5 ±2.0	22.6 ±3.0
A	0.60±0.05	0.50±0.05
2	2.5 ±0.3	3.1 ±0.3
3	2.5 ±0.2	3.3 ±0.4
4	41.9 ±5.2	21.7 ±3.1
5	1.5 ±0.2	1.3 ±0.2
6	1.6 ±0.2	1.4 ±0.3
7	2.8 ±0.3	1.9 ±0.2
8	1.6 ±0.2	3.0 ±0.4
9	0.40±0.03	0.30±0.04
10	>30	>30
11	>30	>30
12	>30	>30
13	>30	>30
14	>30	>30
15	0.20±0.01	0.20±0.01
16	9.9 ±0.8	9.8 ±0.9
17	0.40±0.04	0.40±0.05
18	6.6 ±0.7	7.9 ±0.9
19	>30	>30
20	7.0 ±0.9	7.1 ±0.8
21	4.3 ±0.5	9.2 ±1.0

The mast cell suspension was preincubated at 37°C with 0.5% DMSO or test compound for 3 min. Fifteen minutes after the addition of compound 48/80 (10 μg/ml), β-glucuronidase and histamine in the supernatant were determined. Mepacrine is a positive control. Values are expressed as mean±S.E. from 4 to 6 separate experiments.

Conclusion

In conclusion, the chemical modification of compound **A** has led to two new compounds, **15** and **17**, which are nearly equipotent with compound **A** in terms of effect on neutrophil degranulation and more potent than compound **A** as inhibitors of mast cell degranulation. In addition, compounds **20** and **21** show equipotent and even more potent inhibitory effects, respectively, on neutrophil superoxide formation in comparison with compound **A**. These four compounds (**15**, **17**, **20** and **21**) were thus selected as candidates for pharmacological investigation, and the results will be reported elsewhere.

Experimental

Chemistry All melting points are uncorrected. IR spectra were recorded on Shimadzu IR-440 and Nicolet Impact 400 FT-IR spectrophotometers as KBr pellets. NMR spectra were obtained on Bruker ARX-300 FT-NMR and Varian VXR-300 FT-NMR spectrometers in a suitable deuterated solvent. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad. MS were measured with an HP 5995 GC-MS instrument. The UV spectra were recorded on a Shimadzu UV-160A UV-Visible recording spectrophotometer as alcoholic solutions. Elemental analyses were performed at National Cheng Kung University and National Chung Hsing University, Taiwan.

2-Acetamido-1,4-naphthoquinone (2) Acetic acid (1 ml) was added to a suspension of 2-amino-1,4-naphthoquinone (**1**[2], 5.0 g, 0.03 mol) in acetic anhydride (20.0 g, 0.20 mol). The reaction mixture was stirred at 70 °C for 6 h and cooled to room temperature. Then, an equal amount of diethyl ether was added and the reaction mixture was allowed to stand for 1 d. The resulting precipitate was collected by filtration, washed with diethyl ether, then chromatographed on silica gel with benzene/chloroform to give **2**. Recrystallization from ethanol gave **2** as yellow needles (Table 5).

2-Ethylcarboxamido-1,4-naphthoquinone (3) Compound **1** (5.0 g, 0.03 mol) was reacted with propionic anhydride (20.0 g, 0.15 mol) as described for the preparation of **2** to afford **3** (Table 5).

2-Propylcarboxamido-1,4-naphthoquinone (4) Compound **1** (5.0 g, 0.03 mol) was reacted with butyric anhydride (20.0 g, 0.13 mol) as described for the preparation of **2** to furnish **4** (Table 5).

2-Chloroacetamido-1,4-naphthoquinone (5) A suspension of **1** (10 g, 0.06 mol) in anhydrous xylene was saturated with HCl gas and then chloroacetyl chloride (20.0 g, 0.20 mol) was added. The reaction mixture was refluxed for 40 min under a slow stream of dry HCl then cooled to

Table 5. Physical and Spectral Data of Substituted 1,4-Naphthoquinones

Compd. No.	Yield (%)	mp (°C)	MS (M^+) (m/z)	UV, λ_{max} ($\log\varepsilon$)	IR $\nu_{C=O}$	^{1}H-NMR (ppm)[a]	Analysis (%): C, H, N Calcd (Found)
2	80	270 dec.	215	253 (4.34)	1705 1574	2.29 (3H, s, $-COCH_3$), 7.72—7.79 (2H, m, H-6.7), 7.86 (1H, s, H-3), 8.09—8.12 (2H, m, H-5, 8), 8.36 (1H, br, –NH–)	$C_{12}H_9NO_3$ 66.97 4.22 6.51 (66.86 4.28 6.57)
3	82	179—180	229	251 (4.47)	1705 1674	2.29 (3H, t, J=7.5, $-CH_3$), 2.53 (2H, q, J=7.5, $-CH_2-$), 7.69—7.82 (2H, m, H-6, 7), 7.86 (1H, s, H-3), 8.09—8.12 (2H, m, H-5, 8), 8.37 (1H, br, –NH–)	$C_{13}H_{11}NO_3$ 68.11 4.84 6.11 (67.94 4.88 6.20)
4	80	186—187	243	253 (4.48)	1712 1666	1.02 (3H, t, J=7.5, $-CH_3$), 1.72—1.84 (2H, m, $-CH_2CH_3$), 2.47 (2H, t, J=7.5, $-COCH_2-$), 7.69—7.82 (2H, m, H-6, 7), 7.87 (1H, s, H-3), 8.09—8.12 (2H, m, H-5,8), 8.35 (1H, br, –NH–)	$C_{14}H_{13}NO_3$ 69.12 5.39 5.76 (69.02 5.42 5.81)
5	78	196—197	249	253 (4.24)	1720 1670	4.20 (2H, s, $-COCH_2Cl$), 7.22—7.78 (2H, m, H-6, 7), 7.83 (1H, s, H-3), 8.08—8.13 (2H, m, H-5, 8), 9.44 (1H, br, –NH–)	$C_{12}H_8NO_3$ 57.73 3.23 5.61 (57.64 3.31 5.69)
6	81	176—177	287	253 (4.42)	1736 1705	2.75—2.84 (4H, m, $-CH_2CH_2-$), 3.73 (3H, s, $-OCH_3$), 7.70—7.81 (2H, m, H-6, 7), 7.83 (1H, s, H-3), 8.08—8.12 (2H, m, H-5, 8), 8.60 (1H, br, –NH–)	$C_{15}H_{13}NO_5$ 62.72 4.56 4.88 (62.65 4.62 4.91)
7	83	133—134	301	253 (4.26)	1732 1678	2.00—2.05 (2H, m, $-CH_2C\underline{H}_2CH_2$), 2.42 (2H, t, J=7.1, $CH_2CH_2C\underline{H}_2$), 2.55 (2H, t, J=7.3, $C\underline{H}_2CH_2CH_2-$), 3.67 (3H, s, $-OCH_3$), 7.68—7.75 (2H, m, H-6, 7), 7.81 (1H, s, H-3), 8.05—8.08 (2H, m, H-5, 8), 8.39 (1H, br, –NH–)	$C_{16}H_{15}NO_5$ 63.78 5.02 4.65 (63.67 5.05 4.76)
8	75	165—166	291	253 (4.42)	1705 1670	3.82 (2H, s, $-COCH_2-$), 7.33—7.47 (5H, m, benzene ring proton), 7.66—7.79 (2H, m, H-6, 7), 7.85 (1H, s, H-3), 8.01—8.10 (2H, m, H-5, 8), 8.42 (1H, br, –NH–)	$C_{18}H_{13}NO_3$ 74.22 4.50 4.81 (74.11 4.61 4.88)
9	70	136—137	277	255 (4.44)	1693 1671	7.51—7.65 (3H, m, H-3′, 4′, 5′), 7.71—7.81 (2H, m, H-2′, 6′), 7.92—7.95 (2H, m, H-6, 7), 8.02 (1H, s, H-3), 8.12—8.16 (2H, m, H-5, 8), 9.20 (1H, br, –NH–)	$C_{17}H_{11}NO_3$ 73.64 4.00 5.05 (73.55 4.10 5.12)
10	92	233—234	230	269 (4.22)	1660 1680	2.04 (3H, s, $-COCH_3-$), 6.78 (2H, br, $-NH_2-$), 7.69—7.84 (2H, m, H-6, 7), 7.93–7.97 (2H, m, H-5, 8), 9.06 (1H, br, NHCO–)	$C_{12}H_{10}N_2O_3$ 62.61 4.38 12.17 (62.80 4.32 12.23)
11	85	212—213	244	273 (4.39)	1650 1670	2.24 (3H, s, $-COCH_3$), 3.13 (3H, d, J=5.8, $-CH_3$), 6.25 (1H, br, –NH–), 7.59—7.78 (2H, m, H-6, 7), 7.79—8.12 (2H, m, H-5, 8)	$C_{13}H_{12}N_2O_3$ 63.93 4.95 11.47 (64.02 4.85 11.46)
12	88	198—200	258	273 (4.21)	1650 1670	1.27 (3H, t, J=7.2, $-CH_3$), 2.24 (3H, s, $-COCH_3$), 3.46 (2H, q, J=7.2, $-CH_2-$), 7.58—7.72 (2H, m, H-6, 7), 8.02—8.08 (2H, m, H-5, 8)	$C_{14}H_{14}N_2O_3$ 65.11 5.46 10.85 (64.92 5.60 11.05)
13	80	207—208	306	279 (4.49)	1630 1655	1.59 (3H, s, $-COCH_3$), 6.89—7.37 (5H, m, benzene ring proton). 7.64—7.92 (2H, m, H-6, 7), 8.03—8.14 (2H, m, H-5, 8)	$C_{18}H_{14}N_2O_3$ 70.58 4.61 9.15 (70.59 4.69 9.41)
14	83	207—209	320	272 (4.22)	1665	2.19 (3H, s, $-COCH_3$), 4.63 (2H, d, J=5.8, $-NHCH_2-$), 6.42 (1H, br, –NHCO–), 7.26—7.31 (5H, m, benzene ring proton), 7.58—7.72 (2H, m, H-6, 7), 7.78—8.09 (2H, m, H-5, 8)	$C_{19}H_{16}N_2O_3$ 71.24 5.03 8.74 (71.27 5.11 8.68)
15	55	143—144	222	251 (4.19)	1678	4.27 (3H, s, $-OCH_3$), 7.54—7.69 (2H, m, H-6, 7), 8.02—8.10 (2H, m, H-5, 8)	$C_{11}H_7ClO_3$ 59.35 3.17 (59.12 3.23)
16	30	106—107	218	251 (4.22)	1678	4.06 (6H, s, $-OCH_3$), 7.42 (2H, dd, J=5.7, 3.3, H-6, 7), 7.99 (2H, dd, J=5.7, 3.3, H-5, 8)	$C_{12}H_{10}O_4$ 66.05 4.62 (65.80 4.65)
17	51	89—90	236	246 (4.20)	1674	1.43 (3H, t, J=7.1, OCH_2CH_3), 4.59 (2H, q, J=7.1, $-OCH_2CH_3$), 7.69—7.72 (2H, m, H-6, 7), 8.02—8.11 (2H, m, H-5, 8)	$C_{12}H_9ClO_3$ 60.90 3.83 (60.79 3.91)
18	32	Liquid	246	251 (4.25)	1666	1.36 (3H, t, J=7.1, OCH_2CH_3), 4.32 (2H, q, J=7.1, $-OCH_2CH_3$), 7.60 (2H, dd, J=5.7, 3.3, H-6, 7), 7.96 (2H, dd, J=5.7, 3.3, H-5, 8)	$C_{14}H_{14}O_4$ 68.28 5.73 (68.22 5.80)

a) Compound **10** was dissolved in DMSO-d_6 and others in $CDCl_3$.

room temperature for 6 h. An equal amount of diethyl ether was added, and the solution was allowed to stand for 1 d. The resulting precipitate was collected by filtration and purified by means of chromatography (silica gel, benzene). The product was recrystallized from benzene to give **5** as light yellow needle crystals (Table 5).

2-Methoxycarbonylethylcarboxamido-1,4-naphthoquinone (6) Compound **1** (10.0 g, 0.06 mol) was reacted with methyl succinyl chloride (10 g, 0.08 mol) as described for the preparation of **5** to afford **6** (Table 5).

2-Methoxycarbonylpropylcarboxamido-1,4-naphthoquinone (7) Compound **1** (10.0 g, 0.06 mol) was reacted with methyl glutaryl chloride (10.0 g, 0.08 mol) as described for the preparation of **5** to afford **7** (Table 5).

2-Benzylcarboxamido-1,4-naphthoquinone (8) Compound **1** (10.0 g, 0.06 mol) was reacted with phenylacetyl chloride (20.0 g, 0.08 mol) as described for the preparation of **5** to afford **8** (Table 5).

2-Benzoylamino-1,4-naphthoquinone (9) A solution of compound **1** (3.0 g, 0.02 mol) in tetrahydrofuran (THF) (50 ml) was treated with NaH (0.5 g, 0.02 mol) at room temperature, and the reaction mixture was stirred for 30 min. Then, benzoyl chloride (1.0 g, 0.07 mol) was added and stirring was continued for 5 min. The reaction mixture was poured into ice water, and the whole was extracted with chloroform, then the organic solution was concentrated *in vacuo*. The residue was chromatographed on silica gel with benzene to give **9** (Table 5).

2-Acetamido-3-amino-1,4-naphthoquinone (10) 2-Acetamido-3-chloro-1,4-naphthoquinone (**A**) (8.5 g, 0.34 mol) was dissolved in anhydrous nitrobenzene (300 ml), dry ammonia gas was bubbled through the solution, and the reaction mixture was heated at reflux for 1 h. After cooling, the precipitate was collected and recrystallized from ethanol to give **10** as dark red needles (Table 5).

2-Acetamido-3-methylamino-1,4-naphthoquinone (11) Methylamine (40% in water, 0.6 g, 0.04 mol) was added to a suspension of compound **A** (5.0 g, 0.02 mol) in benzene (100 ml). The reaction mixture was stirred for 30 min at room temperature, then filtered. The precipitate was recrystallized from ethanol, giving **11** as dark red needles (Table 5).

2-Acetamido-3-ethylamino-1,4-naphthoquinone (12) Compound **A** (5.0 g, 0.02 mol) was reacted with ethylamine (70% in water, 0.9 g, 0.04 mol) as described for the preparation of **11** to afford **12** (Table 5).

2-Acetamido-3-anilino-1,4-naphthoquinone (13) Compound **A** (5.0 g, 0.02 mol) was reacted with aniline (3.7 g, 0.04 mol) as described for the preparation of **11** to afford **13** (Table 5).

2-Acetamido-3-benzylamino-1,4-naphthoquinone (14) Compound **A** (5.0 g, 0.02 mol) was reacted with benzylamine (4.3 g, 0.04 mol) as described for the preparation of **11** to afford **14** (Table 5).

2-Methoxy-3-chloro-1,4-naphthoquinone (15) and 2,3-dimethoxy-1,4-naphthoquinone (16) Sodium methoxide (1.1 g, 0.02 mol) was added to a suspension of 2,3-dichloro-1,4-naphthoquinone (5.0 g, 0.02 mol) in anhydrous THF (20 ml). The reaction mixture was stirred for 5 min at room temperature, then poured into ice water, extracted with chloroform, and evaporated. The residue was chromatographed on silica gel with benzene to give **15** and **16** (Table 5).

2-Ethoxy-3-chloro-1,4-naphthoquinone (17) and 2,3-diethoxy-1,4-naphthoquinone (18) 2,3-Dichloro-1,4-naphthoquinone (5.0 g, 0.02 mol) was reacted with sodium ethoxide (1.4 g, 0.02 mol) as described for the preparation of **15** and **16** to yield **17** and **18** (Table 5).

Evaluation of Antiplatelet Aggregation Activity Materials: Collagen (Type 1, bovine Achilles tendon) obtained from Sigma Chem. Co., U.S.A., was homogenized in 25 ml of acetic acid and then stored at −70 °C. Arachidonic acid, bovine serum albumin (BSA), EDTA (disodium salt), sodium citrate, dimethyl sulfoxide (DMSO) and platelet-activating factor (PAF) were purchased from Sigma Chem. Co., U.S.A. Thrombin (bovine) was obtained from Park Davis Co., U.S.A. and dissolved in 50% (v/v) glycerol to give a stock solution of 100 NIH units/ml.

Methods: Platelet Suspension Preparation: Blood was collected from the rabbit marginal ear vein and mixed with EDTA to a final concentration of 6 mM. It was centrifuged at $90 \times g$ for 10 min at room temperature, and the supernatant was obtained as platelet-rich plasma. The latter was further centrifuged at $500 \times g$ for 10 min. The platelet pellets were washed with Tyrode's solution (Ca^{2+}-free) containing 12 mM EDTA and 3.5 mg/ml serum albumin, and centrifuged at $500 \times g$ for 10 min. Then, the pellets were washed with the above Tyrode's solution without EDTA. After centrifugation under the same conditions, 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), $MgCl_2$ (1.1), NaH_2PO_4 (0.33), $CaCl_2$ (1.0) and glucose (11.2). Platelet numbers were counted by a Coulter Counter (Model ZM) and adjusted to 4.5×10^8 platelets/ml.

Platelet Aggregation: Aggregation was measured by the turbidimetric method[4] with a dual-channel Lumiaggregometer (Model 1020, Payton, Canada). All glassware was siliconized. Just 1 min before the addition of the aggregation inducer, the platelet suspension was stirred at 900 rpm. The percentage of aggregation was calculated as described previously.[5]

Evaluation of Antiinflammatory Activity Materials: Sodium pentobarbital, BSA, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), ferricytochrome c, superoxide dismutase (SOD), FMLP, phenolphthalein-β-D-glucuronide and Triton X-100 were purchased from Sigma Chem. Co., U.S.A.

Methods: Isolation of Neutrophils: Rat peripheral neutrophils were isolated by a modification of the procedure described by Boyum.[6] Fresh blood was obtained from the abdominal aorta of pentobarbitone (60 mg/kg, i.p.)-anesthetized rats (Sprague Dawley, 300—350 g) and supplemented with EDTA. Neutrophils were separated from other blood cells by dextran sedimentation and centrifugation on a Ficoll-Hypaque density gradient. Erythrocytes in the pellets were lysed by suspending the cells in 0.05% saline for 15 s followed by washing with 1.75% saline containing 0.25% BSA. Cells were resuspended in Hanks' balanced salt solution containing 4 mM $NaHCO_3$ and 10 mM HEPES, pH 7.4 (HBSS) to a final concentration of 2×10^6 cells/ml. The cell preparations consisted of 90—95% neutrophils (approximately 95% viability by trypan blue exclusion).

Measurement of β-Glucuronidase and Lysozyme Release: The neutrophil suspension was preincubated at 37 °C with DMSO or sample for 3 min, and the release reaction was triggered by the addition of 1 μM FMLP. The reaction was stopped 45 min later by the addition of ice-cold Tyrode solution and the mixture was centrifuged for 10 min at $1000 \times g$, β-glucuronidase activity in the supernatant was determined by spectrophotometry at 550 nm after reaction with phenolphthalein-β-D-glucuronide as a substrate.[7] Lysozyme activity in the supernatant was measured with *Micrococcus Iysodeikticus* as a substrate, by spectrophotometry at 450 nm.[8] The release of β-glucuronidase and lysozyme was expressed as % release = [(release elicited by secretagogue − spontaneous release)/total content] × 100. The total content was measured after treatment of the cell suspension with Triton X-100. Spontaneous release was less than 10%.

Measurement of Superoxide Anion Production: Superoxide anion (O_2^-) production was determined by superoxide dismutase (SOD)-inhibitable ferricytochrome c reduction as previously described[9] with some modifications. Assay mixtures contained 0.2 ml of cell suspension (5×10^6 cells/ml) and 0.9 mg/ml of ferricytochrome c in a final volume of 0.4 ml. The reference tube also received 12.5 μg/ml of SOD. Both reference and sample tubes were incubated at 37 °C for 3 min. The reactions were then started by the addition of 0.3 μM FMLP and incubated at 37 °C for 30 min with occasional agitation. After centrifugation, the supernatant was transferred to a 96-well plate, and the absorbance at 550 nm was recorded with a microplate reader. The amount of O_2^- in the reaction mixture was calculated from the formula[10]; O_2^- (nmol) = 19.08 × absorbance.

Evaluation of Antiallergic Activity Materials: Heparin (Grade I-A; from porcine intestinal mucosa), BSA, compound 48/80, *o*-phthaldialdehyde, phenolphthalein-β-glucuronide and Triton X-100 were purchased from Sigma Chem. Co., U.S.A.

Methods: Rat Peritoneal Mast Cell Preparation: Rat peritoneal mast cells were isolated as previously described.[11] Briefly, heparinized Tyrode solution was injected into the peritoneal cavity of exsanguinated rats (Sprague-Dawley, 250—300 g). After abdominal massage, the cells in the peritoneal fluid were harvested and separated in 38% BSA in glucose-free Tyrode solution. The cell pellet was washed and suspended in Tyrode solution of the following composition (mM): NaCl (137), KCl (2.7), $NaHCO_3$ (12), $MgCl_2$ (1.0), NaH_2PO_4 (0.3), $CaCl_2$ (1.0), glucose (5.6) and BSA 0.1%. The mast cell count was adjusted to $1—1.5 \times 10^6$ cells/ml. Cell viability was assessed with the trypan blue exclusion test.

Measurement of Histamine and β-Glucuronidase Release: The mast cell suspension was preincubated at 37 °C with DMSO or sample for 3 min, and the release reaction was triggered by the addition of 10 mg/ml of compound 48/80. The reaction was stopped 15 min later by the addition of ice-cold Tyrode solution and the mixture was centrifuged for 10 min at $1000 \times g$. Histamine in the supernatant was determined by fluorescence spectrophotometry at 350/450 nm after condensation with *o*-phthaldialdehyde,[12] β-glucuronidase activity in the supernatant was measured, with phenolphthalein-β-glucuronide as substrate, by spectrophotometry at 550 nm.[8] The release of histamine and β-D-glucuronidase was expressed as % release = [(release elicited by secretagogue − spontaneous release)/total content] × 100. The total content was measured after treatment of the cell suspension with Triton X-100. Spontaneous release was less than 10%.

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