Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 12, pp 2111–2120, 1997

PII: S0968-0896(97)00133-8
0968-0896/97 $17.00 + 0.00

Synthesis and Antiplatelet, Antiinflammatory, and Antiallergic Activities of 2-Substituted 3-Chloro-1,4-naphthoquinone Derivatives

Jin-Cherng Lien,[a] Li-Jiau Huang,[a] Jih-Pyang Wang,[b] Che-Ming Teng,[c] Kuo-Hsiung Lee[d] and Sheng-Chu Kuo*,[a]

[a]*Graduate Institute of Pharmaceutical Chemistry, China Medical College, Taichung, Taiwan*
[b]*Department of Medical Research, Taichung Veterans' Hospital, Taichung, Taiwan*
[c]*Pharmacological Institute, College of Medicine, National Taiwan University, Taiwan*
[d]*Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, U.S.A.*

Abstract—A series of 2-substituted 3-chloro-1,4-naphthoquinones was synthesized, and the antiplatelet, antiinflammatory, and antiallergic activities of these compounds were evaluated. The structure–activity relationships in this series were also examined. Most of the 2-alkyl/arylcarboxamido derivatives of 3-chloro-1,4-naphthoquinone showed potent activities with similar trends in each of the activities evaluated. © 1997 Elsevier Science Ltd.

Introduction

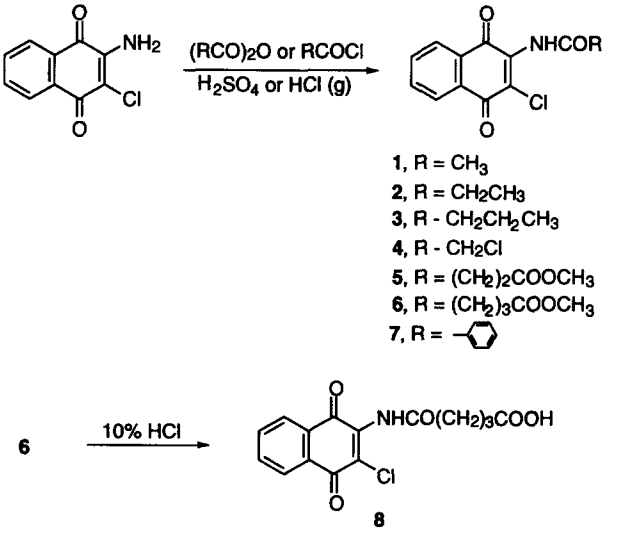
In a previous paper[1] we described the synthesis and cytotoxic structure–activity relationships (SAR) of a series of 1,2-disubstituted naphth[2,3-*d*]imidazole-4,9-diones. 2-Alkyl(aryl)carboxamido-3-chloro-1,4-naphthoquinones were synthetic precursors of these compounds. Since compounds containing a quinone moiety have shown a broad range of biological activities, including enzyme inhibition[2] and antibacterial,[3] antifungal,[4] and anticancer[5] properties, we also screened the parent compound 2-acetamido-3-chloro-1,4-naphthoquinone (**1**) for antiplatelet, antiinflammatory, and antiallergenic activity. The promising activity found in these assays prompted further investigation of additional 2-alkyl(aryl)carboxamido and 2-alkyl(aryl)-amino analogues as described herein.

Chemistry

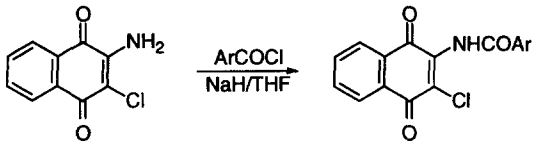
2-Alkylcarboxamido-3-chloro-1,4-naphthoquinones (**1–7**) and 2-arylcarboxamido-3-chloro-1,4-naphthoquinones (**9–12**) were obtained using previously described synthetic methods.[1] Briefly, as shown in Scheme 1, acylation of 2-amino-3-chloro-1,4-naphthoquinone with an acyl anhydride or acyl chloride in the presence of concentrated sulfuric acid or dry hydrogen chloride afforded compounds **1–7**. Furthermore, when 3-chloro-2-methoxycarbonylpropylcarboxamido-1,4-naphthoquinone (**6**) was treated with 10% HCl, the hydrolyzed product 3-chloro-2-hydroxycarbonylpropylcarboxamido-1,4-naphthoquinone (**8**) was obtained.

The synthesis of compounds **9–12** was performed using strongly basic conditions as illustrated in Scheme 2. 2-Amino-3-chloro-1,4-naphthoquinone was treated first with an equimolar ratio of NaH, then with benzoyl chloride or a substituted benzoyl chloride to afford **9–12**.

As outlined in Scheme 3, the reaction of 2,3-dichloro-1,4-naphthoquinone with an alkylamine or arylamine afforded the 2-alkyl(aryl)amino-3-chloro-1,4-naphthoquinones **13–19**.

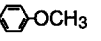


Scheme 1.



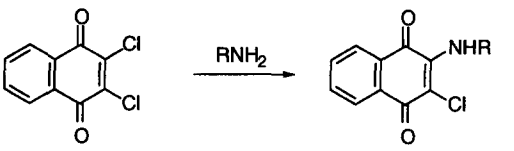
9, Ar = 

10, Ar = 

11, Ar = 

12, Ar = 

Scheme 2.



13, R = CH_3
14, R = CH_2CH_3
15, R = $(CH_2)_2CH_3$
16, R = $(CH_2)_4CH_3$

18, R = 

19, R = CH_2 

Scheme 3.

Antiplatelet activity

The antiplatelet activities of the 2-substituted 3-chloro-1,4-naphthoquinone derivatives **1–19** are summarized in Table 1.

At a concentration of 10–20 μg/mL, 2-alkylcarboxamido derivatives **1–3** completely inhibited the platelet aggregation induced by thrombin, arachidonic acid (AA), collagen, and platelet-activating factor (PAF). At a lower concentration (0.5–1.0 μg/mL), **1–3** still showed a significant effect on the platelet aggregation induced by AA and collagen.

Adding a chlorine group (compound **4**) to the acetamido group of compound **1** did not affect the potency; however, the inhibitory effect on the aggregation induced by collagen decreased significantly when the group added was phenyl (compound **7**). Likewise, addition of a terminal-$COOCH_3$ group (compounds **5** and **6**) to compounds **2** and **3**, respectively, did not affect potency. Hydrolysis of this ester in compound **6** gave the corresponding acid (compound **8**) and dramatically decreased potency.

Comparison of 2-acetamido (compound **1**) and 2-benzamido (compound **9**) groups showed slightly decreased activity with the latter. However, as with the previous results in the cytotoxicity assays,[1] addition of a *p*-F or *p*-OCH_3 group (compounds **10** and **11**) increased antiplatelet activity.

The antiplatelet activities of the above 2-alkyl(aryl)-carboxamido derivative of 3-chloro-1,4-naphthoquinone dropped remarkedly when the –NHCO– (amide linkage, compounds **1–12**) was changed to –NH– (amine linkage, compounds **13–19**). Only three amino compounds (**13–15**) showed any significant inhibitory effect and only in one assay system (platelet aggregation induced by AA) at a higher concentration (100 μg/mL). Therefore, alkylcarboxamido or arylcarboxamido groups at the 2-position of 3-chloro-1,4-naphthoquinones are structurally required for potent antiplatelet activity.

Antiinflammatory activity

Effect on neutrophil degranulation. The effects of compounds **1–19** on neutrophil degranulation also were examined. As can be seen in Table 2, at 0.03–0.1 g/mL, compounds **1–3** had a significant inhibitory effect on neutrophil degranulation induced by FMLP. The SAR results for compounds **4–9**, in general, paralleled those found in the antiplatelet assay. Two slight variations were seen. The phenylacetamido derivative **7** retained the activity of the acetamido compound **1**, and the benzamido compound **9** showed a more significant decrease in activity relative to **1**. The amido linkage was again important for a potent inhibitory effect on neutrophil degranulation; the amino compounds **13–19** were much less potent than their amido counterparts (**1–9**).

Effect on neutrophil superoxide formation. The results in this assay are shown in Table 3. Again, significant inhibitory effects were shown by the 2-alkyl carboxamido compounds **1–3**. This potency was not affected by addition of a terminal carboxylic ester (compounds **5** and **6**), but other additions [chlorine (compound **4**), phenyl (compound **7**), or carboxylic acid (compound **8**)] decreased potency. With the benzamido (compound **9**) and substituted benzamido (compounds **10–12**) derivatives, only the *p*-methoxy benzamido compound **11** had increased activity relative to the acetamido compound **1**. The remaining compounds (**9**, **10**, and **12**) had lower activities.

Compounds **13–19**, the 2-alkylamino or 2-arylamino derivatives of 3-chloro-1,4-naphthoquinone, had essentially no inhibitory effect on neutrophil superoxide formation. Thus, the inhibitory effects of compounds **1–19** on neutrophil superoxide formation, as discussed above, show a similar trend to that found on neutrophil degranulation.

Antiallergic activity

Effect on mast cell degranulation. Mast cells, which release various inflammatory mediators during immunological challenge, participate prominently in the PCA reaction. Therefore, the effects of

Table 1. The inhibitory effects of 2-substituted 3-chloro-1,4-naphthoquinones on platelet aggregation induced by thrombin, AA, collagen and PAF

Compound (μg/mL)		Percentage aggregation			
		Thrombin	**AA**	**Collagen**	**PAF**
Control		94.3 ± 0.3	91.5 ± 2.1***	95.3 ± 1.7***	92.5 ± 2.6
Indomethacin	(7.2)	93.5 ± 1.0	0.0 ± 0.0***	72.3 ± 6.0***	89.4 ± 0.4
1	(100)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	(5)	0.0 ± 0.0	—	10.4 ± 9.0**	0.0 ± 0.0***
	(1)	—	0.0 ± 0.0***	86.6 ± 3.0	90.4 ± 1.6
	(0.5)	—	7.7 ± 6.3***	—	—
2	(10)	0.0 ± 0.0***	—	—	—
	(5)	46.5 ± 6.1***	—	—	0.0 ± 0.0***
	(2)	54.9 ± 13.0**	0.0 ± 0.0***	—	37.1 ± 9.2***
	(1)	91.1 ± 2.0	10.9 ± 8.9***	—	55.4 ± 4.2***
	(0.5)	40.5 ± 16.6**	0.0 ± 0.0***	73.7 ± 7.3*	
3	(20)	0.0 ± 0.0***	—	—	—
	(10)	42.1 ± 13.0***	—	—	0.0 ± 0.0***
	(2)	—	0.0 ± 0.0***	—	60.4 ± 8.7***
	(1)	—	23.2 ± 9.5***	0.0 ± 0.0***	77.7 ± 3.5*
4	(20)	0.0 ± 0.0***	—	—	0.0 ± 0.0***
	(10)	24.1 ± 1.9***	—	—	0.0 ± 0.0***
	(2)	85.6 ± 0.7**	—	—	68.2 ± 4.6***
	(1)	—	0.0 ± 0.0***	0.0 ± 0.0***	83.0 ± 7.4
5	(20)	0.0 ± 0.0***	—	—	—
	(5)	60.7 ± 3.3**	—	—	0.0 ± 0.0***
	(2)	83.8 ± 2.5**	0.0 ± 0.0***	0.0 ± 0.0***	12.2 ± 5.1***
	(1)	—	31.7 ± 13.7***	24.1 ± 12.3***	56.0 ± 6.5***
6	(20)	0.0 ± 0.0***	—	—	0.0 ± 0.0***
	(5)	80.6 ± 5.0**	0.0 ± 0.0***	—	56.7 ± 3.1***
	(2)	—	16.7 ± 10.2***	0.0 ± 0.0***	59.8 ± 2.3***
	(1)	—	88.0 ± 1.2	83.1 ± 1.4*	88.9 ± 3.1
7	(100)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	(50)	—	—	0.0 ± 0.0***	—
	(20)	—	—	13.3 ± 10.9***	—
	(10)	0.0 ± 0.0***	—	—	—
	(5)	61.3 ± 8.7***	—	—	0.0 ± 0.0***
	(1)	—	0.0 ± 0.0***	41.1 ± 4.8***	
8	(100)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	(50)	11.3 ± 5.8***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	(20)	71.8 ± 6.8**	6.5 ± 5.0***	46.1 ± 9.1***	25.9 ± 0.8***
	(10)	89.3 ± 0.8**	71.9 ± 4.5**	81.8 ± 2.8*	58.3 ± 5.1***
9	(100)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	(50)	80.6 ± 4.3**	—	—	75.9 ± 4.2*
	(10)	—	—	0.0 ± 0.0***	—
	(5)	—	0.0 ± 0.0***	10.5 ± 8.6***	—
10	(100)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
	(50)	36.8 ± 16.1***	—	—	—
	(5)	—	—	—	0.0 ± 0.0***
	(2)	—	0.0 ± 0.0***	0.0 ± 0.0***	62.5 ± 6.5***
	(1)	—	26.6 ± 13.6***	21.2 ± 9.1***	79.9 ± 5.3
11	(5)	0.0 ± 0.0***	—	—	0.0 ± 0.0***
	(2)	0.0 ± 0.0***	—	—	29.2 ± 3.1***
	(1)	47.0 ± 6.2***	0.0 ± 0.0***	0.0 ± 0.0***	48.9 ± 0.8***
	(0.5)	90.5 ± 1.1	79.7 ± 1.6***	78.4 ± 4.4**	88.1 ± 0.3

[*Continued overleaf*]

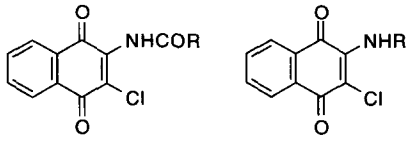
Table 1. *continued*

Compound (μg/mL)		Percentage aggregation			
		Thrombin	AA	Collagen	PAF
12	(10)	0.0 ± 0.0***	—	—	0.0 ± 0.0***
	(5)	81.1 ± 5.5**	0.0 ± 0.0	0.0 ± 0.0***	39.3 ± 4.9***
	(2)	—	76.0 ± 6.8*	10.0 ± 4.7***	79.4 ± 3.9*
13	(100)	85.4 ± 2.6*	2.3 ± 2.1***	67.4 ± 12.1	77.9 ± 2.5***
14	(100)	86.9 ± 1.3*	0.0 ± 0.0***	34.2 ± 13.1***	79.5 ± 3.8**
15	(100)	83.8 ± 3.6*	16.6 ± 14.3***	75.2 ± 3.4***	61.7 ± 6.7***
16	(100)	77.8 ± 4.9***	52.4 ± 15.7**	55.8 ± 10.0**	27.7 ± 11.3***
17	(100)	75.6 ± 2.6***	50.1 ± 15.2**	58.0 ± 5.9***	43.6 ± 16.1**
18	(100)	84.8 ± 1.5***	35.2 ± 14.4***	69.0 ± 5.5***	68.2 ± 9.3*
19	(100)	71.9 ± 3.2***	48.9 ± 15.2***	53.3 ± 1.6***	39.6 ± 15.8**

Platelets were incubated with tested 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. Indomethacin acts as a positive control. Values are presented as mean ± SE.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; — not determined. All values are averages of three to five experiments.

compounds **1–19** on mast cell degranulation were examined, and the data are given in Table 4. The results in this assay again correlated with those found above in the antiplatelet and antiinflammatory assays. Compounds **1–3** at 0.3–1 μg/mL had significant inhibitory effects on mast cell degranulation induced by compound 48/80 (10 μg/mL). Chlorine (compound **4**), phenyl (compound **2**), or carboxylic ester (compounds **5** and **6**) moieties could be added without affecting the potency of the corresponding parent compound, while addition of a carboxylic acid (compound **8**) or conversion of the alkylamido to a benzamido moiety (compounds **9**, **10**, and **12**) decreased activity. Once again, the *p*-methoxy benzamido derivative (compound **11**) had increased potency relative to the acetamido compound **1**.

Compounds **13–17, 18**, and **19**, which are 2-alkylamino, 2-phenylamino, and 2-benzylamino derivatives, respectively, of 3-chloro-1,4-naphthoquinone, had substantially no inhibitory effect on mast cell degranulation except for compound **19**, which showed a significant effect at 10 μg/mL. Thus, compounds **1–19** act similarly in their inhibitory effects on both mast cell and neutrophil degranulation.



Type A Type B

Summary

(1) The tested 2-substituted-3-chloro-1,4-naphthaquinones show similar trends with respect to antiplatelet, antiinflammatory, and antiallergic activities. In other words, if a tested compound is potent in one of these activities, it will also be potent in the other two activities.

(2) The type A compounds (2-amido) exhibit much stronger activity than the type B (2-amino) compounds. Thus, the –NHCO– (amide linkage) at the 2-position plays a key role in the activities of 2-substituted 3-chloro-1,4-naphthoquinones.

In general, the most active 2-amido-3-chloro-1,4-naphthoquinones were significantly more potent than the positive control compounds used in these assays. In view of the interesting SAR and potent activities of some of the tested compounds, we have chosen specific compounds for further studies of structure modification, mechanism of action, and in vivo activity. The results thereof will be presented in a future publication.

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 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–vis recording spectrophotometer as alcoholic solutions. Elemental analyses (C, H, N) (see Table 5) were performed by National Cheng

Table 2. The inhibitory effects of 2-substituted 3-chloro-1,4-naphthoquinones on the release of β-glucuronidase and lysozyme from neutrophils

No. (μg/mL)		Percent release			
		β-glucuronidase	(% inhibition)	lysozyme	(% inhibition)
Control		24.4 ± 0.8	—	34.3 ± 3.8	—
Trifluoperazine	(8.2)	10.5 ± 1.0**	55.8 ± 2.7	10.1 ± 2.2**	69.2 ± 3.2
1	(0.1)	19.9 ± 0.8*	43.9 ± 4.2	26.7 ± 2.1*	39.2 ± 4.0
	(0.03)	29.1 ± 1.6	18.7 ± 1.5	43.5 ± 1.0	0.8 ± 0.7
2	(0.1)	7.3 ± 1.2**	79.3 ± 3.6	15.4 ± 0.8**	64.9 ± 2.1
	(0.03)	25.6 ± 1.4	27.5 ± 3.2	33.9 ± 4.7	22.5 ± 11.0
3	(0.1)	10.3 ± 0.8**	70.8 ± 2.4	18.5 ± 4.1**	58.1 ± 7.7
	(0.03)	20.4 ± 1.4**	42.3 ± 3.7	34.1 ± 3.6	21.8 ± 10.1
4	(0.3)	4.5 ± 0.8**	87.3 ± 3.0	2.0 ± 1.6**	95.3 ± 4.0
	(0.1)	13.4 ± 1.0**	61.9 ± 5.3	32.8 ± 2.0	24.9 ± 5.8
5	(1)	6.2 ± 1.2**	77.8 ± 4.0	24.9 ± 5.7*	51.9 ± 6.8
	(0.3)	8.7 ± 1.0**	68.8 ± 2.8	32.1 ± 5.9**	37.3 ± 4.8
	(0.1)	24.8 ± 1.7	10.6 ± 4.8	49.3 ± 5.3	1.3 ± 4.4
6	(1)	0.3 ± 0.9**	99.0 ± 3.5	−1.5 ± 0.7**	103.3 ± 1.6
	(0.3)	2.9 ± 0.4**	89.2 ± 1.6	14.1 ± 1.7**	72.3 ± 2.0
7	(0.1)	3.3 ± 0.5**	88.7 ± 2.2	16.1 ± 9.0**	71.9 ± 13.4
	(0.03)	17.5 ± 1.9**	41.2 ± 3.1	41.9 ± 7.5	20.8 ± 6.0
8	(10)	9.8 ± 2.0**	64.9 ± 7.4	25.5 ± 11.2**	42.5 ± 13.5
	(3)	18.6 ± 1.3**	33.7 ± 5.7	35.0 ± 8.2	14.6 ± 4.1
	(1)	25.6 ± 1.3	8.9 ± 2.9	38.5 ± 6.7	4.0 ± 3.5
9	(1)	22.3 ± 1.8	21.3 ± 1.5	41.9 ± 1.3	2.8 ± 5.5
	(0.3)	22.6 ± 4.0	21.5 ± 9.5	38.3 ± 3.8	12.3 ± 3.7
10	(0.1)	8.1 ± 0.3**	72.7 ± 0.3	11.7 ± 6.1**	79.5 ± 8.9
	(0.03)	24.2 ± 0.6	17.5 ± 6.1	45.2 ± 4.2	13.2 ± 2.1
11	(0.3)	1.6 ± 0.3**	95.4 ± 1.0	−4.2 ± 0.2**	109.7 ± 0.5
	(0.1)	7.6 ± 0.1**	78.4 ± 0.1	14.6 ± 30.**	66.4 ± 7.6
12	(1)	4.0 ± 0.8**	88.7 ± 2.6	2.1 ± 1.1**	95.1 ± 2.8
	(0.3)	15.6 ± 1.6**	55.9 ± 4.2	29.5 ± 5.7*	33.3 ± 12.0
13	(30)	17.7 ± 2.7	10.6 ± 4.6	—	—
14	(30)	16.0 ± 2.6	19.6 ± 3.6	—	—
15	(30)	12.9 ± 1.8	31.4 ± 11.2	15.3 ± 5.1**	63.9 ± 14.7
16	(30)	16.2 ± 2.9	24.1 ± 6.6	22.6 ± 4.5	24.5 ± 12.0
17	(30)	13.4 ± 3.9	38.1 ± 3.1	16.6 ± 2.7	42.1 ± 14.9
18	(30)	15.8 ± 1.7	17.2 ± 7.5	13.8 ± 2.9**	54.3 ± 11.7
19	(10)	10.6 ± 1.9	49.7 ± 7.9	17.8 ± 0.1	34.2 ± 9.2

The neutrophil suspension was preincubated at 37 °C with 0.5% DMSO or 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 acts as a positive control and the control is not shown. Values are presented as mean ± SE.

*$p < 0.05$, **$p < 0.01$; — not determined. All values are the averages of three to five experiments.

Table 3. The inhibitory effects of 2-substituted 3-chloro-1,4-naphthoquinones on superoxide formation in neutrophils

No.(μg/mL)		Superoxide formation nmol/10^6 cell	(% inhibition)
Control		2.98 ± 0.29	—
Trifluoperazine	(4.1)	0.21 ± 0.08*	76.4 ± 9.5
		0.58 ± 0.08	45.0 ± 10.2
1	(1)	0.21 ± 0.08*	76.4 ± 9.5
	(0.3)	0.58 ± 0.08	45.0 ± 10.2
2	(1)	0.28 ± 0.09**	78.4 ± 6.1
	(0.3)	0.56 ± 0.23**	57.0 ± 11.8
3	(1)	0.18 ± 0.03**	84.3 ± 5.7
	(0.3)	0.48 ± 0.09**	60.2 ± 11.7
4	(30)	1.39 ± 0.28	42.4 ± 9.6
5	(1)	0.19 ± 0.12*	82.9 ± 9.8
	(0.3)	2.14 ± 0.22	8.7 ± 12.7
6	(1)	0.07 ± 0.14*	97.9 ± 6.4
	(0.3)	1.75 ± 0.12	29.3 ± 4.1
7	(1)	0.15 ± 0.21**	89.1 ± 17.5
	(0.3)	1.17 ± 0.19	25.6 ± 12.7
8	(10)	0.93 ± 0.30	55.6 ± 6.2
	(3)	1.97 ± 0.68	2.1 ± 8.9
9	(3)	1.30 ± 0.44	47.1 ± 17.1
	(1)	1.65 ± 0.20	30.4 ± 4.3
10	(10)	0.04 ± 0.24**	100.0 ± 16.9
	(3)	0.32 ± 0.28*	57.4 ± 9.9
11	(1)	0.14 ± 0.18**	89.6 ± 13.2
	(0.3)	0.97 ± 0.15	18.2 ± 8.8
12	(10)	0.04 ± 0.02**	96.9 ± 1.9
	(3)	0.76 ± 0.14*	53.7 ± 4.9
13	(30)	3.26 ± 0.43	−44.3 ± 0.6
14	(10)	2.85 ± 0.18	−18.7 ± 7.4
15	(30)	3.56 ± 0.35*	−58.6 ± 8.7
16	(30)	3.68 ± 0.17*	−66.0 ± 12.4
17	(30)	2.98 ± 0.33	−32.5 ± 6.5
18	(30)	2.66 ± 0.38	−18.4 ± 12.3
19	(30)	3.15 ± 0.18	−29.7 ± 5.4

The neutrophil suspension in the presence of ferricytochrome c was preincubated at 37 °C with 0.5% DMSO or test compound for 3 min. Fifteen minutes after the addition of FMLP (0.3 μM) the absorbance was determined at 550 nm. Trifluoperuzine acts as a positive control. Values are presented as mean SE.
*$p < 0.05$, **$p < 0.01$. All values are the averages of three to five experiments.

Kung University and National Chung Hsing University, Taiwan and were within 0.4 ppm.

2-Acetamido-3-chloro-1,4-naphthoquinone (1). To a suspension of 2-amino-3-chloro-1,4-naphthoquinone (52 g, 0.25 mol) in acetic anhydride (75 mL) were added five drops of concentrated H_2SO_4. The reaction mixture was stirred at room temperature for 20 min and then filtered. The precipitate was washed with Et_2O and recrystallized from EtOH giving golden needle crystals of **1**. Yield, mp, and spectral data are given in Table 6. Compounds **2** and **3** were prepared by the same method from the appropriate acid anhydride. Yields, mps, and spectral data are given in Table 6.

2-Chloroacetamido-3-chloro-1,4-naphthoquinone (4). To a suspension of 2-amino-3-chloro-1,4-naphthoquinone (10.4 g, 0.05 mol) in anhydrous xylene (100 mL) were added 50 mL of chloroacetyl chloride and dry HCl. The reaction mixture was refluxed for 40 min; during this period, a slow stream of dry HCl was passed into the solution. After reflux, the reaction mixture was cooled to room temperature for 6 h. An equal amount of Et_2O was added, and the solution sat for one day. The solution was then filtered, and the precipitate was recrystallized from benzene to give **4** as light yellow needle crystals. Yield, mp, and spectral data are given in Table 6. Compounds **5–7** were prepared using the same method from the appropriate acyl chloride. Yield, mp, and spectral data are given in Table 6.

3-Chloro-2-hydroxycarbonylpropylcarboxamido-1,4-naphthoquinone (8). 3-Chloro-2-methoxycarbonylpropylcarboxamido-1,4-naphthoquinone (**6**) (10 g, 0.03 mol) was suspended in 10% HCl (50 mL) and stirred at room temperature for 30 min. The mixture was then poured into water and filtered. The precipitate was washed with water and recrystallized from EtOH giving yellow powdery crystals of **8** in a 52% yield. Spectral data are shown in Table 6.

2-Benzoylamino-3-chloro-1,4-naphthoquinone (9). To a solution of 2-amino-3-chloro-1,4-naphthoquinone (1.0 g, 4.8 mmol) in THF (50 mL) was added 0.2 g (0.3 mmol) NaH at room temperature, and the reaction mixture was stirred for 30 min. Then, 1 g (7.1 mmol) of benzoyl chloride was added and stirring continued for 5 min. The reaction mixture was then poured into ice water, extracted with $CHCl_3$, and evaporated. The residue was chromatographed on silica gel with benzene as eluent to give **9**. Yield, mp, and spectral data are given in Table 6. Compounds **10–12** were prepared in an analogous manner from the appropriate acyl chloride. Yield, mp, and spectral data are given in Table 6.

3-Chloro-2-methylamino-1,4-naphthoquinone (13). To a suspension of 2,3-dichloro-1,4-naphthoquinone (5 g, 0.02 mol) in benzene was added an excess of methylamine. The reaction mixture was stirred for 30

Table 4. The inhibitory effects of 2-substituted 3-chloro-1,4-naphthoquinones on the release of β-glucuronidase and histamine from mast cells

No. (μg/mL)		Percent release			
		β-glucuronidase	(% inhibition)	Histamine	(% inhibition)
Control		28.9 ± 1.3	—	41.3 ± 5.6	—
Mepacrine	(12.0)	15.8 ± 2.0**	43.1 ± 9.4	28.4 ± 5.4**	30.0 ± 3.1
1	(1)	3.2 ± 2.0**	90.3 ± 5.8	3.7 ± 0.8**	92.9 ± 2.1
	(0.3)	29.1 ± 3.8	9.7 ± 6.6	42.8 ± 2.0	22.0 ± 4.2
2	(1)	3.3 ± 0.9**	89.4 ± 3.5	2.8 ± 3.0**	95.7 ± 5.6
	(0.3)	20.7 ± 1.5**	34.1 ± 8.9	42.5 ± 2.4	22.4 ± 5.9
3	(1)	2.7 ± 2.1**	90.5 ± 7.9	12.4 ± 13.1**	102.0 ± 6.2
	(0.3)	29.0 ± 3.0	8.9 ± 6.5	44.1 ± 2.0	19.5 ± 4.3
4	(1)	5.8 ± 0.1**	81.6 ± 1.3	4.7 ± 0.4**	91.3 ± 1.0
	(0.3)	34.8 ± 1.1	−9.7 ± 5.3	52.9 ± 1.7	3.7 ± 4.9
5	(1)	6.5 ± 0.5**	82.7 ± 2.4	5.8 ± 2.4**	88.1 ± 4.3
	(0.3)	20.0 ± 5.7**	48.2 ± 13.7	27.5 ± 7.0**	41.9 ± 14.0
	(0.1)	34.7 ± 2.0	9.3 ± 0.9	45.3 ± 3.6	3.4 ± 4.5
6	(1)	7.5 ± 2.8**	81.2 ± 6.0	8.7 ± 5.7**	81.1 ± 12.9
	(0.3)	29.4 ± 3.6**	23.4 ± 7.1	37.6 ± 3.2**	19.2 ± 7.9
	(0.1)	36.7 ± 1.0	3.5 ± 3.5	49.2 ± 6.1	−4.4 ± 7.9
7	(1)	15.3 ± 2.9**	51.7 ± 1.9	14.1 ± 5.1**	75.4 ± 7.8
	(0.3)	27.9 ± 1.5	12.3 ± 1.5	48.3 ± 4.8	12.2 ± 7.0
8	(10)	12.5 ± 4.0**	68.2 ± 8.2	8.9 ± 4.2**	80.2 ± 9.7
	(3)	34.8 ± 1.5	8.6 ± 3.7	47.3 ± 3.3	−2.2 ± 12.2
	(1)	39.7 ± 2.6	−4.1 ± 5.2	46.2 ± 4.3	1.3 ± 7.2
9	(10)	23.7 ± 5.6	25.4 ± 16.6	38.3 ± 10.0**	32.9 ± 13.7
	(3)	27.7 ± 2.6	12.3 ± 9.5	43.9 ± 7.8	21.9 ± 8.7
10	(10)	7.6 ± 1.8**	75.4 ± 6.7	14.3 ± 0.7**	73.8 ± 7.8
	(3)	19.8 ± 1.1**	37.6 ± 3.6	37.9 ± 1.6**	29.8 ± 9.5
11	(1)	6.0 ± 0.9**	80.5 ± 4.7	9.7 ± 1.9**	82.6 ± 2.6
	(0.3)	31.9 ± 1.3	-1.2 ± 10.4	51.6 ± 3.8	6.1 ± 5.1
12	(10)	9.7 ± 2.9**	69.9 ± 7.8	4.7 ± 4.4**	92.6 ± 7.7
	(3)	6.7 ± 4.1**	79.8 ± 12.2	8.9 ± 2.7**	82.8 ± 6.7
13	(30)	15.5 ± 2.4*	44.9 ± 4.7	16.2 ± 2.7*	53.6 ± 6.1
14	(30)	17.3 ± 1.9	26.1 ± 5.5	21.6 ± 5.2	36.3 ± 2.2
15	(30)	21.4 ± 3.3	9.1 ± 8.9	27.2 ± 8.2	22.2 ± 2.6
16	(30)	23.9 ± 4.5	24.3 ± 8.5	28.9 ± 6.7	24.4 ± 9.9
17	(30)	11.4 ± 0.2**	48.5 ± 4.1	22.2 ± 10.3	42.1 ± 13.3
18	(30)	16.5 ± 4.0	30.7 ± 13.1	28.0 ± 8.5	20.0 ± 3.1
19	(10)	9.6 ± 1.0**	58.5 ± 5.1	14.8 ± 5.6	58.9 ± 9.25

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 acts as a positive control. Values are presented as mean ± SE.

*$p < 0.05$, **$p < 0.01$. All values are averages of three to five experiments.

Table 5. Elemental analyses

Compound		Calculated			Found		
		C	H	N	C	H	N
1	$C_{12}H_8ClNO_3$	57.73	3.23	5.61	57.59	3.22	5.66
2	$C_{13}H_{10}ClNO_3$	59.22	3.82	5.31	58.93	3.90	5.35
3	$C_{14}H_{12}ClNO_3$	60.55	4.36	5.04	60.61	4.30	5.01
4	$C_{12}H_7ClNO_3$	50.73	2.48	4.93	50.80	2.44	4.85
5	$C_{15}H_{12}ClNO_5$	56.00	3.76	4.35	56.09	3.71	4.38
6	$C_{16}H_{14}ClNO_5$	57.24	4.20	4.17	57.14	4.23	4.18
7	$C_{18}H_{12}ClNO_3$	66.37	3.71	4.30	66.11	3.74	4.59
8	$C_{12}H_{12}ClNO_5$	56.00	3.76	4.35	55.93	3.81	4.40
9	$C_{17}H_{10}ClNO_3$	65.50	3.23	4.49	65.44	3.25	4.51
10	$C_{17}H$-$ClFNO_3$	61.93	2.75	4.25	62.01	2.67	4.30
11	$C_{18}H_{12}ClNO_4$	62.26	3.54	4.10	63.33	3.51	4.18
12	$C_{19}H_{14}ClNO_5$	61.38	3.79	3.77	61.42	3.83	3.74
13	$C_{11}H_8ClNO_2$	59.61	3.64	6.32	59.45	3.70	6.38
14	$C_{12}H_{10}ClNO_2$	61.16	4.28	5.94	61.05	4.33	5.99
15	$C_{13}H_{12}ClNO_2$	62.53	4.84	5.61	62.46	4.90	5.70
16	$C_{14}H_{14}ClNO_2$	63.76	5.35	5.31	63.63	5.40	5.36
17	$C_{15}H_{16}ClNO_2$	64.86	5.81	5.04	64.71	5.85	5.08
18	$C_{16}H_{10}ClNO_2$	67.74	3.55	4.94	67.68	3.58	4.98
19	$C_{17}H_{12}ClNO_2$	68.58	4.06	4.70	68.46	4.10	4.85

min at room temperature, then filtered. The precipitate was recrystallized from EtOH giving dark-red needle crystals of **13**. Yield, mp, and spectral data are given in Table 6. Compounds **14–17** and **19** were prepared using the same method from the appropriate amine. Yield, mp, and spectral data are given in Table 6.

2-Anilino-3-chloro-1,4-naphthoquinone (18). To a suspension of 2,3-dichloro-1,4-naphthoquinone (5 g, 0.02 mol) in benzene was added an excess of aniline. The reaction mixture was stirred for 30 min at 50–60 °C, then cooled and filtered. The precipitate was recrystallized from EtOH giving dark red needle crystals of **18**. Yield, mp, and spectral data are given in Table 6.

Evaluation of antiplatelet aggregation activity

Materials. Collagen (type 1, bovine achilles tendon) obtained from Sigma Chemical Co., was homogenized in 25 mL acetic acid and then stored at −70 °C. Adachidonic acid, bovine serum albumen (BSA), EDTA (disodium salt), sodium citrate, dimethylsulfoxide (DMSO), and platelet-activating factor (PAF) were purchased from Sigma Chemical Co. Thrombin (bovine) was obtained from Park Davis Co. 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 was mixed with EDTA to a final concentration of 6 mM. It was centrifuged at 90*g* for 10 min at room temperature, and the supernatant was obtained as platelet-rich plasma. The latter was further centrifuged at 500*g* for 10 min. The platelet pellets were washed with Tyrode's solution without EDTA. After centrifugation at 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 Coulter Counter (Model ZM) and adjusted to 4.5×10^8 platelets/mL.

Platelet aggregation. Aggregation was measured by the turbidimetric method[2] with a dual-channel Lumi-aggregometer (Model 1020, Payton, Canada). All glassware was siliconized. One minute before the addition of the aggregaton inducer, the platelet suspension was stirred at 900 rpm. The percentage of aggregation was calculated as described previously.[3]

Evaluation of antiinflammatory activity

Materials. Sodium pentobarbital, bovine serum albumin (BSA), *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES), ferricytochrome-*c*, superoxide dismutase (SOD), formyl-Met-Leu-Phe (FMLP), phenolphthalein-β-D-glucuronide and Triton X-100 were purchased from Sigma Chemical Co.

Methods

Isolation of neutrophils. Rat peripheral neutrophils were isolated by a modification of the procedure described by Boyum.[4] EDTA-mixed fresh blood was obtained from the abdominal aorta of pentobarbitone (60 mg/kg, ip) anesthetized rats (Sprague–Dawley, 300–350 g). 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%

Table 6. Physical and spectral data of 2-substituted 3-chloro-1,4-naphthoquinones[a]

No.	Yield (%)	Mp (°C)	MS (M^+) (*m/z*)	UV, λ_{max} (log ε)	IR $\nu_{C=O}$	^{1}H NMR (ppm)[b]
1	98	219–220	249.5	253 (4.26)	1670 1700	2.31 (3H, s, $-COCH_3$), 7.71 (1H, br, –NH), 7.73–7.82 (2H, m, H-6, 7), 8.10–8.13 (1H, m, H-5), 8.18–8.20 (1H, m, H-8)
2	92	173–175	263.5	253 (4.36)	1665 1700	1.28 (3H, t, J = 7.3, $-CH_3$), 2.55 (2H, q, J = 7.3, $-CH_2-$), 7.71–7.86 (2H, m, H-6, 7), 8.05–8.23 (2H, m, H-5, 8)
3	90	137–138	277.5	252 (4.22)	1670 1700	1.04 (3H, t, J = 7.1, $-CH_3$), 1.57–1.99 (2H, m, $-CH_2CH_3$), 2.50 (2H, t, J = 7.6, $-COCH_2-$), 7.69–7.77 (2H, m, H-6, 7), 8.02–8.20 (2H, m, H-5, 8)
4	85	167–169	284	270 (4.32)	1665 1700	4.24 (2H, s, $-COCH_2$), 7.73–7.82 (2H, m, H-6, 7), 8.09–8.12 (1H, m, H-5), 8.13–8.18 (1H, m, H-8)
5	81	174–175	321.5	254 (4.33)	1740	2.74–2.82 (4H, m, $-CH_2CH_2-$), 3.70 (3H, s, $-OCH_3$), 7.69–7.77 (2H, m, H-6, 7)
6	83	127–128	335.5	254 (4.11)	1730	2.00–2.10 (2H, m, $-CH_2\mathit{CH_2}CH_2-$), 2.45 (2H, t, J = 7.0, $-\mathit{CH_2}CH_2CH_2-$), 2.57(2H, t, J = 7.0, $-CH_2CH_2\mathit{CH_2}-$), 3.67 (3H, s, $-OCH_3$), 7.71–7.75 (2H, m, H-6, 7), 8.04–8.07 (1H, m, H-5), 8.12–8.15 (1H, m, H-8)
7	70	207–208	325.5	252 (4.31)	1650 1700	3.83 (2H, s, $-COCH_2-$), 7.24–7.41 (5H, m, benzene ring proton), 7.71–7.75 (2H, m, H-6, 7), 8.04–8.07 (1H, m, H-5), 8.12–8.15 (1H, m, H-8)
8	52	203–204	286.0 (M^+–Cl)	253 (4.24)	1710	1.76–1.86 (2H, m, $-CH_2CH_2\mathit{CH_2}-$), 2.28 (2H, t, J = 7.5, $-\mathit{CH_2}CH_2CH_2-$), 2.47 (2H, t, J = 7.5, $-CH_2CH_2\mathit{CH_2}-$), 7.86–7.9 (2H, m, H-6, 7), 7.99–8.07 (2H, m, H-5, 8)
9	80	254–256	311.5	253 (4.50)	1665 1690	7.25–7.45 (5H, m, benzene ring proton), 7.71–7.83 (2H, m, H-6, 7), 8.08–8.25 (2H, m, H-5, 8)
10	65	212–214	329.5	253 (4.67)	1660 1690	7.18 (2H, d, J = 8.9, H-3′, 5′), 7.24 (2H, d, J = 8.9, H-2′, 6′), 7.75–7.79 (2H, m, H-6, 7), 8.10–8.13 (1H, m, H-5), 8.10–8.13 (1H, m, H-8)
11	61	162–164	341.5	207 (4.40)	1665 1690	3.88 (3H, s, $-OCH_3$), 6.98 (2H, d, J = 8.8, H-3′, 5′), 7.71–7.81(2H, m, H-6, 7), 7.94 (2H, d, J = 8.8, H-2′, 6′), 8.05–8.24 (2H, m, H-5, 8)
12	63	193–194	371.5	209 (4.33)	1660 1690	3.84 (6H, s, $-OCH_3$), 6.67 (1H, s, H-4′), 7.06 (2H, s, H-2′6′), 7.74–7.79 (2H, m, H-6, 7), 8.10–8.21 (2H, m, H-5, 8)
13	92	160–161	221.0	275 (4.32)	1682	3.41 (3H, d, J = 5.4, $-CH_3$), 6.07 (1H, br, –NH–), 7.55–7.71 (2H, m, H-6, 7), 7.79–7.99 (1H, m, H-5), 8.09–8.12 (1H, m, H-8)
14	93	129–131	235.0	275 (4.41)	1678	1.30 (3H, t, J = 7.2, $-CH_3$), 3.82–3.91 (2H, m, $-CH_2-$), 5.98 (1H, br, –NH–), 7.55–7.68 (2H, m, H-6, 7), 7.96–7.99 (1H, m, H-5), 8.08–8.11 (1H, m, H-8)
15	92	109–110	249.0	276 (4.36)	1682	0.97 (3H, t, J = 7.5, $-CH_3$), 1.61–1.73 (2H, m, $-\mathit{CH_2}CH_3$), 3.73–3.79 (2H, m, $-NHCH_2-$), 6.04 (1H, br, –NH–), 7.55–7.66 (2H, m, H-6, 7), 7.94–7.97 (1H, m, H-5), 8.06–8.09 (1H, m, H-8)
16	91	105–106	263.0	276 (4.43)	1674	0.93 (3H, t, J = 7.3, $-CH_3$), 1.33–1.46 (2H, m, $-\mathit{CH_2}CH_3$), 1.58–1.68 (2H, m, $-\mathit{CH_2}CH_2CH_3-$), 3.77–3.83 (2H, m, $-NHCH_2-$), 6.02 (1H, br, –NH–), 7.55–7.66 (2H, m, H-6, 7), 7.94–7.97 (1h, m, H-5), 8.07–8.10 (1H, M, H-8)
17	92	86–87	277.0	276 (4.37)	1674	0.89 (3H, t, J = 7.3, $-CH_3$), 1.34–1.35 (4H, m, $-\mathit{CH_2CH_2}CH_3$), 1.65–1.67 (2H, m, $-NHCH_2\mathit{CH_2}-$), 3.76–3.83 (2H, m, $-NHCH_2-$), 6.02 (1H, br, –NH–), 7.54–7.69 (2H, m, H-6, 7), 7.96–7.99 (1H, m, H-5), 8.08–8.11 (1H, m, H-9)
18	81	207–208	283.0	277 (4.22)	1674	7.05–7.36 (5H, m, benzene ring proton), 7.64–7.78 (2H, m, –NH-& H-6, 7), 8.08–8.12 (1H, m, H-5), 8.10–8.19(1H, m, H-8)
19	90	111–112	297.0	274 (4.42)	1682	5.00 (2H, s, $-CH_2-$), 6.21 (1H, br, –NH–), 7.28–7.35 (5H, m, benzene ring proton), 7.56–7.67 (2H, m, H-6, 7), 7.95–7.97 (1H, m, H-5), 8.07–8.10 (1H, m, H-8)

[a]All compounds gave satisfactory CHN analyses.
[b]Compound **8** was dissolved in DMSO-d_6, all others in $CDCl_3$.

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 Na_2CO_3 and 10 mM *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] (HEPES), pH 7.4 (HBSS) to a final concentration of 2×10^6 cells/mL. The cell preparations consisted of 90–95% neutrophils (viability approximately 95% 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's solution and the mixture was centrifuged for 10 min at 1000*g*. β-Glucuronidase activity in the supernatant was determined by spectrophotometry at 550 nm after reaction with phenolphthalein-β-D-glucuronide as substrate.[5] Lysozyme activity in the supernatant was measured, with *Micrococcus lysodeikticus* as substrate, by spectrophotometry at 450 nm.[6] The release of β-glucuronidase and lysozyme was expressed as percentage 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[7] with modifications. Assay mixtures contained 0.2 mL 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 incubation 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:[8] O_2^- (nmol) = 19.08 × absorbance.

Evaluation of antiallergic activity

Materials. Heparin (grade I-A; from porcine intestinal mucosa), bovine serum albumin, compound 48/80, *o*-phthaldialdehyde, phenolphthalein-β-D-glucuronide and Triton X-100 were purchased from Sigma Chemical Co.

Methods

Rat peritoneal mast cell preparation. Rat peritoneal mast cells were isolated as previously described.[9] Briefly, heparinized Tyrode's 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% bovine serum albumin in glucose-free Tyrode's solution. The cell pellet was washed and suspended in Tyrode's 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 bovine serum albumin 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's solution and the mixture was centrifuged for 10 min at 1000*g*. Histamine in the supernatant was determined by fluorescence spectrophotometry at 350/450 nm after condensation with *o*-phthaldialdehyde.[10] β-Glucuronidase activity in the supernatant was measured, with phenolphthalein-β-D-glucuronide as substrate, by spectrophotometry at 550 nm.[7] The release of histamine and β-glucuronidase was expressed as percentage release = [(release elicited by secretagogue − spontaneous release)/total content] × 100. The total content was measured after treatment of cell suspension with Triton X-100. Spontaneous release was less than 10%.

Acknowledgements

This work was supported by a research grant from the National Science Council of the Republic of China (NSC 82-0412-B039-006).

References

1. Kuo, S. C.; Lien, J. C.; Huang, L. J.; Wang, J. P.; Teng, C. M.; Lee, K. H. *J. Med. Chem.* **1996**, *39*, 1447.

2. O'Brien, J. R. *J. Clin. Path.* **1962**, *15*, 450.

3. Teng, C. M.; Chen, W. Y.; Ko, W. C.; Ouyang, C. *Biochem. Biophys. Acta* **1987**, *924*, 375.

4. Boyum, A. *Scand. J. Clin. Invest.* **1968**, *97 Supp.*, 77.

5. Barret, J. P. In *Lysosomes, A Laboratory Handbook*; Dingle, J. T., Ed.; Elsevier/North-Holland: Amsterdam, 1972; p 118.

6. Absolom, D. R. *Methods Enzymol.* **1986**, *132*, 95.

7. Cohen, H. J.; Chovaniec, M. E. *J. Clin. Invest.* **1978**, *61*, 1088.

8. Markert, M.; Andrews, P. C.; Babior, B. M. *Methods Enzymol.* **1984**, *105*, 358.

9. Wang, J. P.; Hsu, M. F.; Ouyang, C.; Teng, C. M. *Eur. J. Pharmacol.* **1989**, *161*, 143.

10. Hakanson, R.; Ronnberg, A. L. *Anal. Biochem.* **1974**, *60*, 560.

(*Received in U.S.A.* 28 *March* 1997; *accepted* 17 *June* 1997)