Synthesis of 2-Alkoxy 1,4-Naphthoquinone Derivatives as Antiplatelet, Antiinflammatory, and Antiallergic Agents

Jin-Cherng LIEN,*[,a] Li-Jiau HUANG,[a] Che-Ming TENG,[b] Jih-Pyang WANG,[c] and Sheng-Chu KUO[a]

[a] *Graduate Institute of Pharmaceutical Chemistry, China Medical College; 91 Hsueh-Shih Road, Taichung, Taiwan, R.O.C.:* [b] *Pharmacological Institute, College of Medicine, National Taiwan University; Taiwan, R.O.C.: and* [c] *Department of Education and Research, Taichung Veterans General Hospital; Taiwan, R.O.C.*

Received November 30, 2001; accepted January 21, 2002

In our continuing search for novel antiplatelet, antiallergic, and antiinflammatory agents, 2-alkoxy derivatives of 1,4-naphthoquinone were prepared. Some of these compounds showed significant antiplatelet, antiallergic, and antiinflammatory activities. Among them, 2-propoxy-1,4-naphthoquinone and 2-butoxy-1,4-naphthoquinone exhibited potent inhibitory effect on neutrophil superoxide anion formation. These two compounds are worthy of further exploration.

Key words naphthoquinone; antiplatelet activity; antiallergic activity; antiinflammatory activity

In a previous paper,[1,2] a series of 2,3-disubstituted 1,4-naphthoquinones were synthesized and their antiplatelet, antiinflammatory, and antiallergic activities were evaluated. Among them, compounds **1**—**4** (Chart 1) showed potent inhibitory effect on neutrophil and mast cell degranulation. In particular, 2-chloro-3-methoxy-1,4-naphthoquinone (**1**) had impressive IC_{50} values of 0.5 and 0.9 μM against neutrophil and mast cell degranulation, respectively. Encouraged by this preliminary result, in this investigation we explored further the chain length of compounds **1** and **3**. During the screening for antiplatelet, antiinflammatory, and antiallergic activities, we found that some of the target compounds demonstrated improved and more potent activity in comparison with **1** and **3**. Our findings are summarized in this report.

Chemistry

The target compounds, 2-alkoxy-3-chloro-1,4-naphthoquinones (**5**—**9**) and 2-alkoxy-1,4-naphthoquinones (**10**—**14**), were synthesized as shown in Charts 2 and 3. The preparation of two typical compounds, 2-chloro-3-propoxy-1,4-naphthoquinone (**5**) and 2-propoxy-1,4-naphthoquinones (**10**), is described to illustrate the general procedures. As shown in Chart 2, the starting 2,3-dichloro-1,4-naphthoquinone was treated with sodium propoxide in propanol to form **5**. As illustrated in Chart 3, the starting 2-hydroxy-1,4-naphthoquinone was treated with propyl iodide in the presence of silver oxide to afford **10**.

Antiplatelet Activity[3,4] As indicated in Table 1, all of the synthesized compounds significantly inhibited arachidonic acid (AA)-, collagen-, and platelet-activating factor (PAF)-induced platelet aggregation. On the contrary, they did not inhibit thrombin-induced aggregation at concentrations up to 100 μg/ml (not shown in Table 1). In other words, these compounds showed similar patterns of preferential inhibition toward platelet aggregation to that of compound **1**. Their inhibitory potency is greatest against AA- and collagen-induced platelet aggregation, whereas their potency is only moderate against PAF-induced aggregation, and the weakest toward thrombin-induced aggregation.

Among compounds **5**—**7**, the inhibitory potency against AA-, collagen-, and PAF-induced platelet aggregation was found to increase with increasing 2-alkoxy chain length. Alternatively, the phenoxy group was introduced into the 2-position to obtain **9**. Such modification resulted in greatly enhanced potency against AA-, collagen-, and PAF-induced platelet aggregation.

Replacing the 3-chloro groups of compounds **5**—**8** with hydrogens yielded compounds **10** and **12**—**14**, with slightly lower potency against AA- and collagen-induced platelet aggregation. These results suggest that the presence of a chloro group at the 3-position of the tested compounds plays an effective role, as does the increase in the alkoxy chain length at 2-position. In general, the better inhibitory effect on the AA- and collagen-induced response of this series of compounds implies the inhibition of prostaglandin production, although confirmation of this awaits further investigation.

Antiinflammatory Activity Effect on Neutrophil Degranulation[5,6]: The inhibitory effects of compounds **5**—**14** on neutrophil degranulation were examined. As can be seen in Table 2, the potency of compounds **5**—**8** were about 2—4-

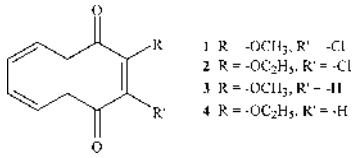

Chart 1

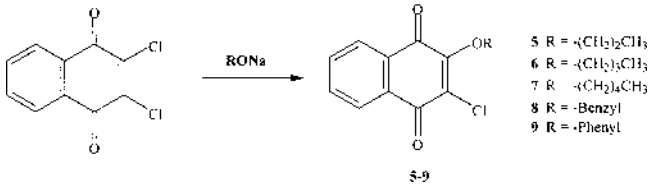

Chart 2

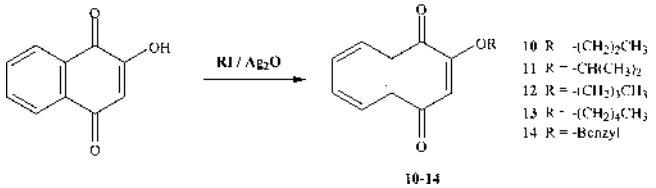

Chart 3

* To whom correspondence should be addressed. e-mail: jclien@mail.cmc.edu.tw

Table 1. Inhibitory Effect of 2-Alkoxy-(3-chloro) 1,4-Naphthoquinones on Platelet Aggregation Induced by Thrombin, Arachidonic Acid, Collagen, and Platelet-activating Factor

Compound	IC_{50} (μM)[a]			
	Thrombin	AA	Collagen	PAF
1	>150	18.9	34.2	>150
2	>150	33.5	>150	33.1
3	>150	53.9	73.2	>150
4	>150	38.1	43.8	89.2
5	>150	20.5	24.6	106
6	>150	19.8	21.8	133
7	>150	8.8	10.6	32.4
8	>150	10.8	11.9	26.8
9	>150	1.6	1.9	5.8
10	>150	26.1	26.0	75.8
11	>150	36.3	34.8	72.5
12	122	23.3	22.8	42.6
13	119	17.0	30.2	54.5
14	>150	15.2	21.7	>150
Aspirin	>150	20.0	>150	>150

Platelets were incubated with test compounds at 37 °C for 1 min, then thrombin (0.1 unit/ml), arachidonic acid (AA, 100 μM), collagen (10 μg/ml), or platelet-activating factor (PAF, 2 ng/ml) was added to trigger the aggregation. Values are expressed as the concentration (μM) at which 50% inhibition of platelet aggregation occurred (IC_{50}). Aspirin was used as a positive control. *a*) The accuracy of IC_{50} values are within ±10%.

Table 2. Inhibitory Effect of 2-Alkoxy-(3-chloro) 1,4-Naphthoquinones on the Release of β-Glucuronidase and Lysozyme from Neutrophils

Compound	IC_{50} (μM)[a]	
	β-Glucuronidase	Lysozyme
1	0.5	0.5
2	1.7	1.7
3	79.3	>100
4	87.2	>100
5	1.0	1.3
6	1.5	2.1
7	2.1	2.5
8	1.9	1.9
9	14.7	15.1
10	60.2	58.4
11	18.8	24.6
12	14.6	25.7
13	3.7	4.8
14	55.6	>100
Trifluoperazine	20.1	17.2

The neutrophil suspension was preincubated at 37 °C with 0.5% DMSO or a test compound for 10 min in the presence of cytochalasin B (5 μg/ml). Forty-five minutes after the addition of fMLP (1 μM), β-glucuronidase and lysozyme levels in the supernatant were determined. Values are expressed as the concentration (μM) at which 50% inhibition of neutrophil degranulation occurred (IC_{50}). Trifluoperazine was used as a positive control. *a*) The accuracy of IC_{50} values are within ±10%.

Table 3. Inhibitory Effect of 2-Alkoxy-(3-chloro) 1,4-Naphthoquinones on the Superoxide Anion Formation of Neutrophils

Compound	IC_{50} (μM)[a]
	Superoxide anion formation
1	4.5
2	12.3
3	2.8
4	0.8
5	2.8
6	5.5
7	4.7
8	4.1
9	6.9
10	0.6
11	2.1
12	0.6
13	ND
14	ND
Trifluoperazine	14.7

The neutrophil suspension in the presence of tern cytochrome c was preincubated at 37 °C with 0.5% DMSO or a test compound for 10 min in the presence of cytochalasin B (5 μg/ml). Fifteen minutes after the addition of fMLP (0.3 μM), the absorbance was determined at 550 nm. Values are expressed as the concentration (μM) at which 50% inhibition of neutrophil superoxide anion formation occurred (IC_{50}). Trifluoperazine was used as a positive control. ND, not determined. *a*) The accuracy of IC_{50} values are within ±10%.

Table 4. Inhibitory Effect of 2-Alkoxy-(3-chloro) 1,4-Naphthoquinones on the Release of β-Glucuronidase and Histamine from Mast Cells

Compound	IC_{50} (μM)[a]	
	β-Glucuronidase	Histamine
1	0.9	0.9
2	1.7	1.7
3	37.3	37.6
4	21.5	45.4
5	1.2	1.6
6	1.0	2.0
7	1.6	1.6
8	2.6	2.0
9	1.0	1.1
10	9.1	10.1
11	5.7	10.2
12	3.0	9.1
13	1.8	3.3
14	2.1	2.8
Mepacrine	33.7	56.5

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 levels in the supernatant were determined. Values are expressed as the concentration (μM) at which 50% inhibition of mast cell degranulation occurred (IC_{50}). Mepacrine was used as a positive control. *a*) The accuracy of IC_{50} values are within ±10%.

fold less potent than compound **1** in terms of their inhibitory effect on the neutrophil degranulation induced by formyl-Met-Leu-Phe (fMLP). The inhibitory potency was reduced markedly when a phenoxy group was placed at the 2-position (compound **9**). On the other hand, the potency of compounds **10**—**13** was found to increase over compound **3** in proportion with the length of their 2-alkoxy chains.

Effect on Neutrophil Superoxide Formation[7,8]: The screening results in the neutrophil superoxide formation assay are shown in Table 3. All tested compounds demonstrated significant inhibitory effect on neutrophil superoxide anion formation induced by fMLP. Among them, the 3-chloro analogues **5**—**9** were nearly equipotent in comparison with lead compound **1**, while the 3-unsubstituent analogues **10**—**12** were more potent than their parent compound **3**. Most importantly, both compounds **10** and **12**, with IC_{50} values of 0.6 μM, are 25 times more potent than the positive control (trifluoperazine).

Antiallergic Activity Effect on Mast Cell Degranulation[9,10]: The inhibitory effects of compounds **5**—**14** on mast cell degranulation were examined. As shown in Table 4, all 3-chloro analogues (**5**—**9**) exhibited almost the same in-

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

Compound	Yield (%)	mp (°C)	MS (M^+) (m/z)	IR (cm^{-1})	^{1}H-NMR (ppm)
5	85	Liquid	250	1674	1.06 (3H, t, J=7.5, –$CH_2\underline{CH_3}$); 1.78—1.87 (2H, m, –$\underline{CH_2}CH_3$); 4.53 (2H, t, J=6.6, –OCH_2–); 7.72—7.75 (2H, m, H-6, 7); 8.06—8.12 (2H, m, H-5, 8)
6	83	Liquid	264	1674	0.98 (3H, t, J=7.5, –$CH_2\underline{CH_3}$); 1.46—1.58 (2H, m, –$\underline{CH_2}CH_3$); 1.74–1.84 (2H, m, –$OCH_2\underline{CH_2}$–); 4.57 (2H, t, J=6.6, –OCH_2–); 7.72—7.75 (2H, m, H-6, 7); 8.04—8.13 (2H, m, H-5, 8)
7	82	Liquid	278	1674	0.93 (3H, t, J=7.2, –$CH_2\underline{CH_3}$); 1.34—1.49 (4H, m, –$\underline{CH_2}\underline{CH_2}CH_3$); 1.76—1.84 (2H, m, –$OCH_2\underline{CH_2}$–); 4.56 (2H, t, J=6.6, –OCH_2–); 7.72—7.75 (2H, m, H-6, 7); 8.05—8.14 (2H, m, H-5, 8)
8	75	115—116	298	1678	5.64 (2H, s, –OCH_2–); 7.32—7.40 (3H, m, H-3′, 4′, 5′); 7.45—7.48 (2H, m, H-2′, 6′); 7.72—7.78 (2H, m, H-6, 7); 8.06—8.21 (2H, m, H-5, 8)
9	78	142—143	284	1674	6.98—7.35 (5H, m, H-2′, 3′, 4′, 5′, 6′); 7.75—7.78 (2H, m, H-6, 7); 8.02—8.03 (1H, m, H-5); 8.18—8.21 (1H, m, H-8)
10	82	106—107	216	1705	1.09 (3H, t, J=7.4, –$CH_2\underline{CH_3}$); 1.89—1.97 (2H, m, –$\underline{CH_2}CH_3$); 4.09 (2H, t, J=6.4, –OCH_2–); 5.93 (1H, s, H-3); 7.55—7.67 (2H, m, H-6, 7); 7.84—7.87 (1H, m, H-5); 8.07—8.10 (1H, m, H-8)
11	85	119—120	216	1698	1.46 (6H, d, J=6.1, –$CH(\underline{CH_3})_2$); 4.69—4.73 (1H, m, –$O\underline{CH}$–); 5.92 (1H, s, H-3); 7.54—7.66 (2H, m, H-6, 7); 7.84—7.87 (1H, m, H-5); 8.07—8.10 (1H, m, H-8)
12	81	97—98	230	1701	0.67 (3H, t, J=7.2, –$CH_2\underline{CH_3}$); 0.98—1.05 (2H, m, –$\underline{CH_2}CH_3$); 1.34—1.39 (2H, m, –$OCH_2\underline{CH_2}$–); 4.03 (2H, t, J=6.5, –OCH_2–); 5.94 (1H, s, H-3); 7.55—7.66 (2H, m, H-6, 7); 7.82—7.85 (1H, m, H-5); 8.07—8.10 (1H, m, H-8)
13	82	80—81	244	1701	0.94 (3H, t, J=7.2, –$CH_2\underline{CH_3}$); 0.98—1.05 (4H, m, –$\underline{CH_2}\underline{CH_2}CH_3$); 1.87—1.94 (2H, m, –$OCH_2\underline{CH_2}$–); 4.11 (2H, t, J=7.2, –OCH_2–); 5.91 (1H, s, H-3); 7.54—7.66 (2H, m, H-6, 7); 7.82—7.85 (1H, m, H-5); 8.06—8.09 (1H, m, H-8)
14	75	182—183	264	1698	5.19 (2H, s, –OCH_2–); 6.05 (1H, s, H-3); 7.42—7.45 (5H, m, H-2′, 3′, 4′, 5′, 6′); 7.56—7.66 (2H, m, H-6, 7); 7.86—7.87 (1H, m, H-5); 8.09—8.12 (1H, m, H-8)

hibitory effect as their parent compound **1** on mast cell degranulation induced by compound 48/80 (10 μg/ml). Meanwhile, regardless of the higher potency than their lead compound **3**, the 3-unsubstituted compounds **10**—**12** were less potent than their 3-chloro counterparts **5** and **6**. However, compounds **7** and **8** exhibited potency similar to that of their 3-unsubstituted counterparts **13** and **14**.

Experimental

IR spectra were recorded on a Nicolet Impact 400 FT-IR spectrophotometer as KBr pellets. NMR spectra were obtained on a Bruker Avance DPX-200 FT-NMR. MS were measured with HP 5995 GC-MS and VG PLATFORM II GC-MS instruments. Elemental analyses of C, H, and N were carried out on a Perkin-Elmer 2400 Series II CHNS/O Analyzer and were accurate within ±0.4% of theoretical values.

2-Alkoxy-3-chloro-1,4-naphthoquinones 5—9 Sodium alkoxide (20 mmol) was added to a suspension of 2,3-dichloro-1,4-naphthoquinone (20 mmol) in anhydrous THF (20 ml). The reaction mixture was stirred for 5 min at room temperature, then poured into ice water, and extracted with chloroform. After drying and removal of the solvent, chromatographic purification on silica gel afforded the desired naphthoquinones **5**—**9** (Table 5).

2-Alkoxy-1,4-naphthoquinones 10—14 A mixture of 2-hydroxy-1,4-naphthoquinone (15 mmol), alkyl iodide (20 mmol), silver oxide (20 mmol), and chloroform (50 ml) was heated under reflux for 2 h. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel to give **10**—**14** (Table 5).

References

1) Lien J. C., Huang L. J., Wang J. P., Teng C. M., Lee K. H., Kuo S. C., *Chem. Pharm. Bull.*, **44**, 1181—1187 (1996).
2) Lien J. C., Huang L. J., Wang J. P., Teng C. M., Lee K. H., Kuo S. C., *Bioorg. Med. Chem.*, **5**, 2111—2120 (1997).
3) O'Brien J. R., *J. Clin. Pathol.*, **15**, 452—455 (1962).
4) Teng C. M., Chen W. Y., Ko W. C., Ouyang C., *Biochem. Biophys. Acta*, **924**, 375—382 (1987).
5) Boyum A., *Scand. J. Clin. Invest.*, **97** (Suppl.), 77—89 (1968).
6) Barret J. P., "Lysosomes, A Laboratory Handbook," ed. by Dingle J. T., Elsevier, Amsterdam, 1972, pp. 118—120.
7) Cohen H. J., Chovaniec M. E., *J. Clin. Invest.*, **61**, 1088—1096 (1978).
8) Markert M., Andrews P. C., Babior B. M., *Methods Enzymol.*, **105**, 358—365 (1984).
9) Wang J. P., Hsu M. F., Ouyang C., Teng C. M., *Eur. J. Pharmacol.*, **161**, 143—149 (1989).
10) Hakanson R., Ronnberg A. L., *Anal. Biochem.*, **60**, 560—567 (1974).