CHAPTER III
RESULTS AND DISCUSSION

3.1 Chemistry

3.1.1 Synthesis of benzoquinones, halobenzoquinones and halohydroquinones

From literature search, it was obviously revealed that benzoquinones are organic compounds which could be isolated from both nature and chemical manipulations. Most of them exhibited interesting biological activities. Therefore, in recent years, there has been reported various alternatives to synthesize this class of compounds. In this research, two important steps to synthesize halobenzoquinones and related compounds were employed. Step 1 was bromination of various substituted phenols to give bromophenols or bromination of hydroquinones to give bromohydroquinones. The other step involved the oxidation of bromophenols by chromic trioxide in acetic acid or the oxidation of bromohydroquinones by chromic trioxide at low temperature to give haloquinones, except for Compound (**8b**) which was oxidized by nitric acid.[29]

Thirty-two compounds were synthesized as above mentioned methods and could be categorized into five types. They were seven bromophenols, four bromohydroquinones, eleven haloquinones, four benzoquinones and six alkyl-benzoquinones. Five synthesized compounds: (**5a**), (**14a**), (**15a**), (**15b**) and (**16**), have never been reported in chemical literature; thus they are new compounds. All compounds were fully characterized by spectroscopic evidence including IR, ^{1}H- and ^{13}C-NMR spectra. The comparative results of the synthesized benzoquinones, halobenzoquinones and halohydroquinones are tabulated in Table 3.1 and their structures are shown in Figs. 3.1-3.5.

Table 3.1 The physical properties and %yield of synthesized compounds

Type of compound	Compound	physical properties		%yield
		Appearance	m.p. (°C)	
bromo phenol	**1a**	white crystal	54-55	93%
	2a	white needle	80-81	99%
	3a	white needle	120	64%
	6a	white crystal	50-51	93%
	7a	white crystal	195	48%
	8a	pale yellow needle	88-89	68%
	15a	white crystal	164-165	76%
bromo hydro quinone	**4a**	yellow plate	210 (dec.)	63%
	5a	white needle	165	98%
	13a	white crystal	189-190	42%
	14a	white powder	205-206	30%
halo benzo quinone	**1b**	orange needle	97	90%
	2b	yellow plate	115-116	60%
	3b	yellow crystal	151-152	94%
	4b	yellow powder	223 (dec.)	48%
	5b	yellow plate	150-152	91%
	6b	yellow plate	215	79%
	8b	yellow powder	134-135	50%
	12	yellow plate	56-58	59%
	13b	reddish-brown needle	188-189	33%
	14b	reddish-brown needle	201-202	82%
	15b	rectangle crystal	173-174	86%
benzo quinone	**7b**	reddish-brown powder	220 (dec.)	85%
	9	yellow needle	109-111	46%
	10	yellow plate	65-66	34%
	11	yellow plate	54	97%

Table 3.1 (cont.)

Type of compound	Compound	physical properties		%yield
		Appearance	m.p. (°C)	
alkyl benzo quinone	16	reddish-brown semisolid	*	25%
	17	brown semisolid	*	86%
	18	brown semisolid	*	69%
	19	brown semisolid	*	51%
	20	reddish-brown semisolid	*	10%
	21	reddish-brown semisolid	*	15%

Note: * semisolid

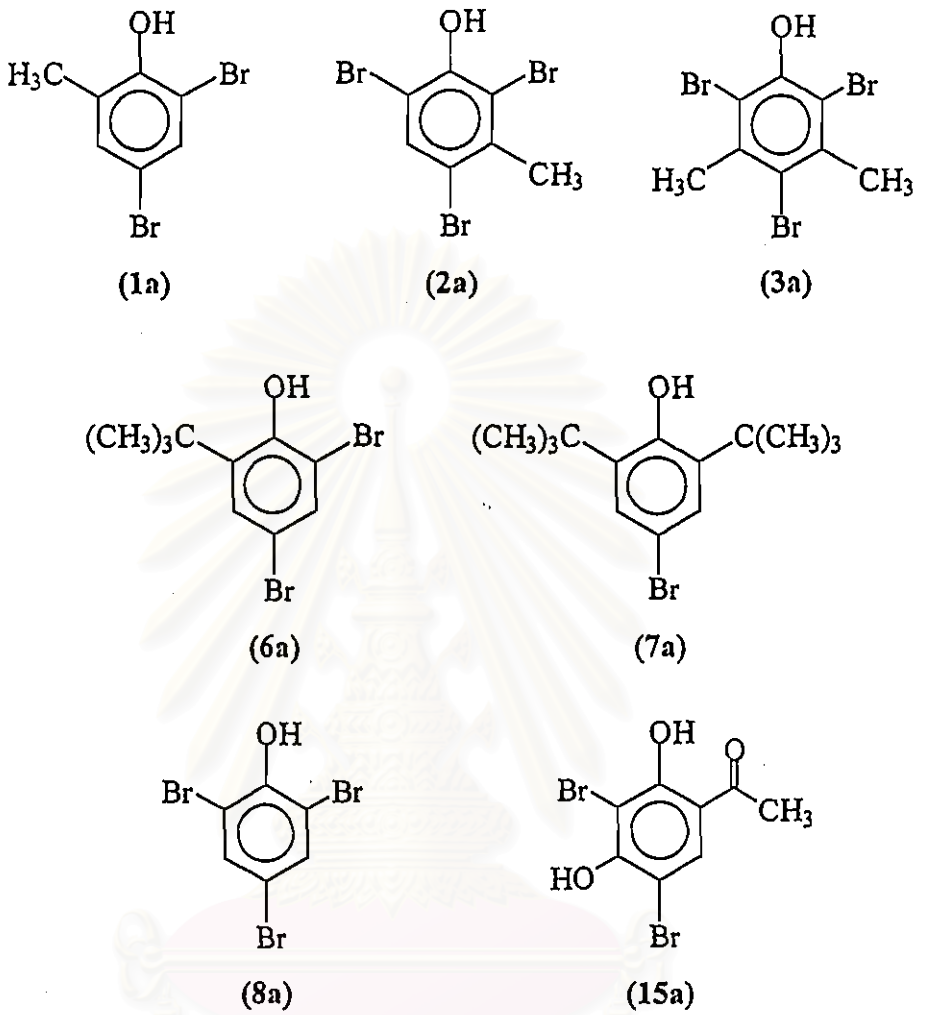


Fig. 3.1 The structures of bromophenols

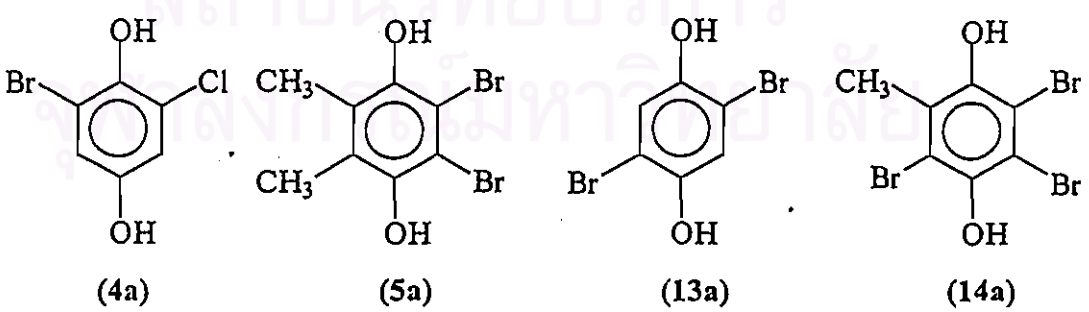


Fig 3.2 The structures of bromohydroquinones

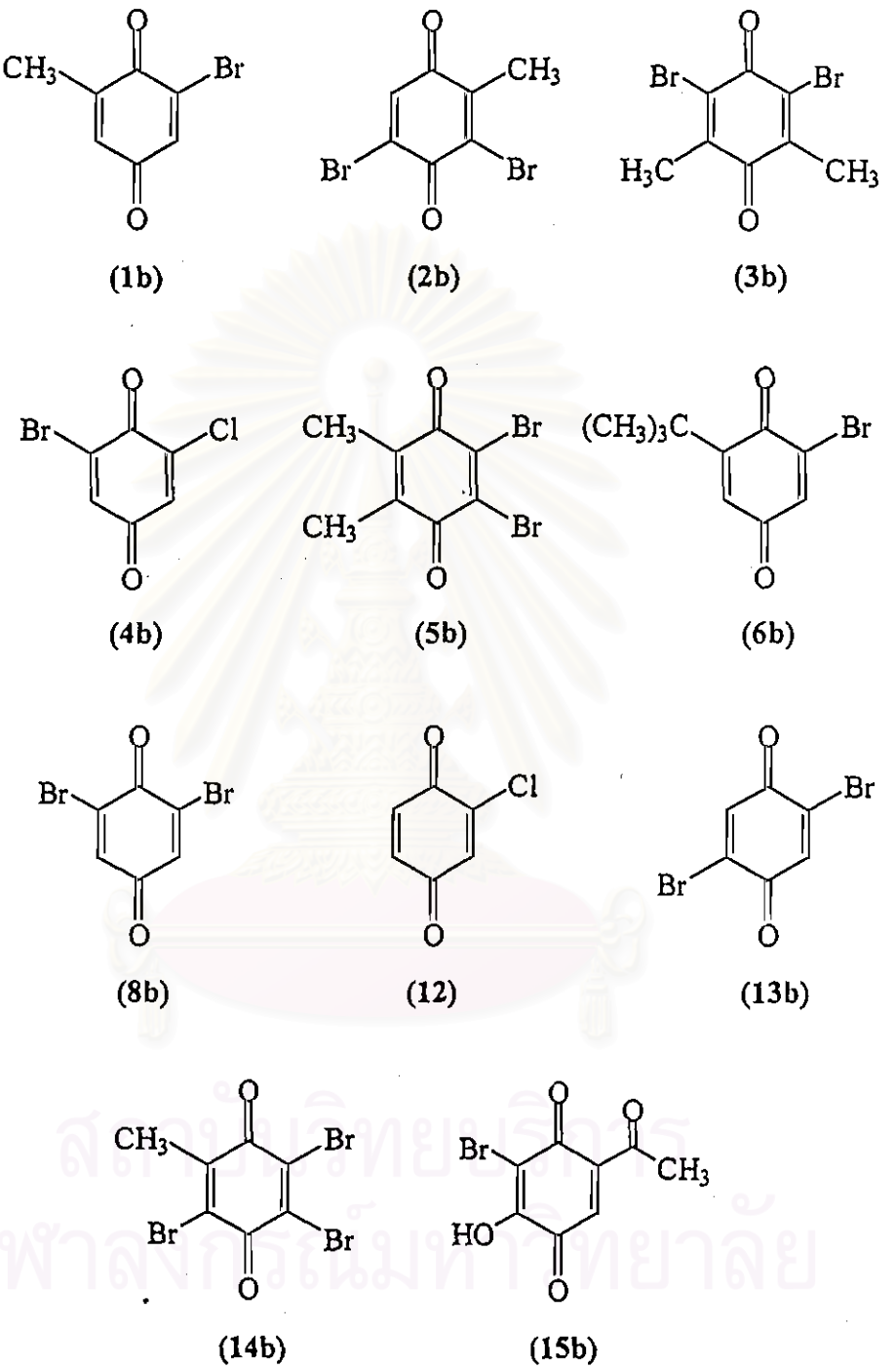
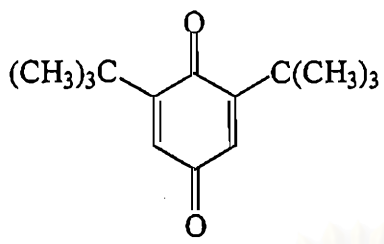
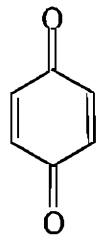


Fig. 3.3 The structures of halobenzoquinones



(7b)



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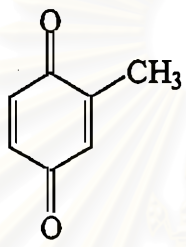

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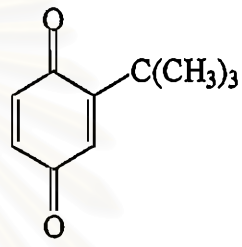
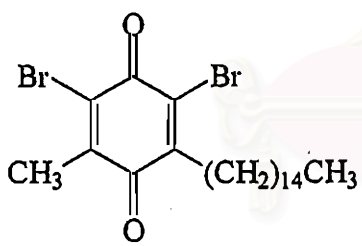


(11)

Fig. 3.4 The structures of benzoquinones



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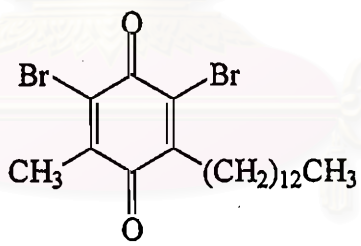

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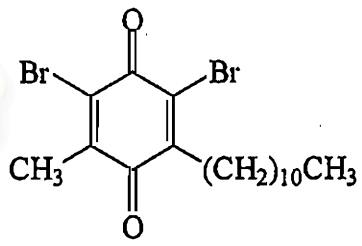

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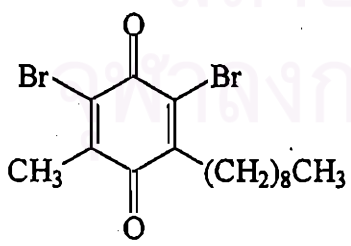

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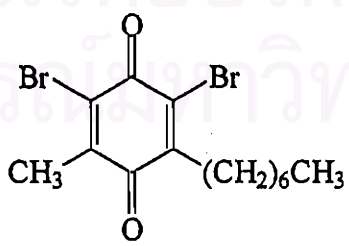

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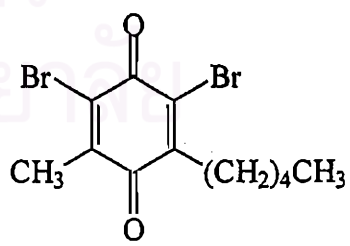


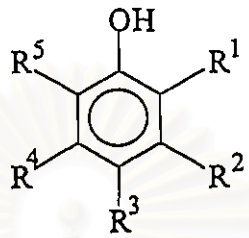
(21)

Fig. 3.5 The structures of alkyl-benzoquinones

3.1.2 Spectroscopy

Infrared Spectroscopy (IR)

Phenols

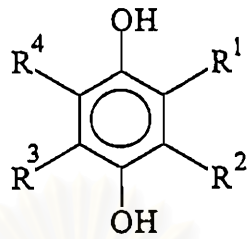


The FT-IR spectra of phenols generally revealed the absorption band of O-H stretching vibration at 3600-3200 cm^{-1} (br.), C-H aromatic stretching vibration at 2900-3100 cm^{-1} (w), C=C ring stretching vibration at 1500-1400 cm^{-1} and the C-O stretching vibration at 1200-1000 cm^{-1} (w). The FT-IR absorption band assignments of phenols are tabulated in Table 3.2.

Table 3.2 The FT-IR absorption band assignments of phenols

Cpd	wave number (cm^{-1})				
	O-H	Ar-H	C=C (benzene)	CH_3	C-O
1a	3500-3250 (br.)	3083 (w)	1465 (s)	1398 (m)	1137 (s)
2a	3500-3200 (br.)	3073 (w)	1444 (s)	1368 (m)	1173 (s)
3a	3500-3200 (br.)	3070 (w)	1450 (s)	1370 (m)	1145 (s)
6a	3529-3447 (br.)	2960 (w)	1440 (s)	1388 (m)	1168 (m)
7a	3600-3237 (br.)	2955 (w)	1467 (s)	1368 (m)	1096 (m)
8a	3493-3390 (br.)	3067 (w)	1460 (s)	-	1198 (m)
15a	3500-3100 (br.)	2970 (w)	-	-	-

Hydroquinones

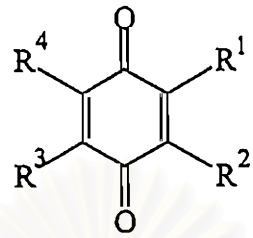


The FT-IR spectra of hydroquinones normally displayed the absorption band of O-H stretching vibration at 3700-3100 cm^{-1} (br.), C=C ring stretching vibration at 1600-1300 cm^{-1}, the C-O stretching vibration at 1300-1200 cm^{-1} and the absorption band of C-X (X= Br, Cl) at 900-600 cm^{-1}. The FT-IR absorption band assignments of hydroquinones are presented in Table 3.3.

Table 3.3 The FT-IR absorption band assignments of hydroquinones

compound	wave number (cm^{-1})			
	O-H	C=C (benzene)	C-O	C-X (X= Br, Cl)
4a	3656-3375 (br.)	1567 (w)	1265 (w)	881 (w)
5a	3631-3155 (br.)	1434 (m)	1214 (w)	830 (w)
13a	3498-3100 (br.)	1424 (m)	1235 (w)	799 (m)
14a	3500-3100 (br.)	1393 (m)	1296 (m)	676 (w)

Quinones



The FT-IR spectra of quinones generally showed the presence of C=O stretching vibration of diketone around 1600-1700 cm^{-1} (s) and C=C ring stretching approximately 1600-1500 cm^{-1} (w or m) were observed. Other absorption peaks of C-X (X= Br, Cl) were found in the range of 900-600 cm^{-1}. The FT-IR absorption band assignments of quinones are tabulated in Table 3.4.

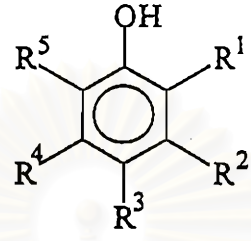
Table 3.4 The FT-IR absorption band assignments of quinones

compound	wave number (cm^{-1})		
	C=O	C=C ring	C-X (X=Br,Cl)
1b	1669 (s)	1557 (m)	681 (m)
2b	1659 (s)	1588 (m)	681 (m)
3b	1685 (s)	1603 (m)	702 (m)
4b	1675 (s)	1568 (w)	886 (w)
5b	1670 (s)	1583 (m)	702 (m)
6b	1624 (s)	1547 (w)	738 (w)
7b	1655 (s)	1550 (m)	-
8b	1660 (s)	1583 (m)	748 (m)
9	1675 (s)	1590 (m)	-
10	1654 (s)	1560 (m)	-
11	1660 (s)	1593 (w)	-
12	1670 (s)	1540 (w)	714 (m)
13b	1675 (s)	1588 (s)	681 (m)
14b	1660 (s)	1577 (m)	676 (m)
15b	1650 (s)	-	-
16	1654 (s)	1583 (w)	-
17	1650 (s)	1589 (w)	-
18	1656 (s)	1590 (w)	-
19	1655 (s)	1598 (s)	-
20	1659 (s)	1587 (w)	-
21	1660 (s)	1592 (w)	-

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR

Phenols

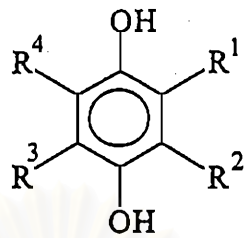


The ^{1}H-NMR spectra of 1 Ar-OH generally exhibited a singlet signal around 5.51-6.02 ppm except for (**15a**) which showed at 13.32 ppm. The aromatic protons detected around 7.19-7.88 ppm and methyl protons were appeared in the range of 1.38-2.61 ppm as a singlet signal. The ^{1}H-NMR spectra assignments of phenols are presented in Table 3.5.

Table 3.5 The ^{1}H-NMR spectral assignments of phenols

Cpd	R					chemical shift (ppm)					
							H of R				
	R^1	R^2	R^3	R^4	R^5	Ar-OH	R^1	R^2	R^3	R^4	R^5
1a	Br	H	Br	H	CH_3	5.51 (s)	-	7.42 (d)	-	7.19 (d)	2.26 (s)
2a	Br	CH_3	Br	H	Br	5.93 (s)	-	2.53 (s)	-	7.67 (s)	-
3a	Br	CH_3	Br	CH_3	Br	6.02 (s)	-	2.60 (s)	-	2.60 (s)	-
6a	Br	H	Br	H	t-Bu	5.78 (s)	-	7.47 (d, J=2.44 Hz)	-	7.31 (d, J=2.44 Hz)	1.38 (s)
7a	t-Bu	H	Br	H	t-Bu	5.79 (s)	1.42 (s)	7.71 (s)	-	7.71 (s)	1.42 (s)
8a	Br	H	Br	H	Br	5.90 (s)	-	7.59 (s)	-	7.59 (s)	-
15a	C(O) CH_3	H	Br	OH	Br	13.32 (s)	2.61 (s)	7.88 (s)	-	-	-

Hydroquinones

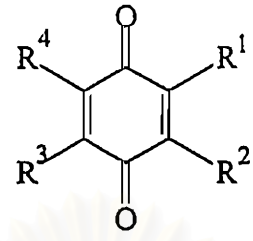


The ^{1}H-NMR spectra of hydrquinones normally revealed 2H of Ar-OH around 5.17-5.69 ppm and the aromatic protons at 7.17-7.47 ppm as siglet signals. The other signal, methyl protons exhibited around 2.19-2.40 ppm. The ^{1}H-NMR spectra assignments of hydroquinones are displayed in Table 3.6.

Table 3.6 The ^{1}H-NMR spectral assignments of hydroquinones

Cpd	R				chemical shift (ppm)					
					H of	H of	R			
	R^1	R^2	R^3	R^4	C-1	C-2	R^1	R^2	R^3	R^4
4a	Br	H	H	Cl	-	-	-	7.47 (s)	7.47 (s)	-
5a	Br	Br	CH_3	CH_3	5.24 (s)	5.24 (s)	-	-	2.19 (s)	2.19 (s)
13a	Br	H	Br	H	5.17 (s)	5.17 (s)	-	7.17 (s)	-	7.17 (s)
14a	Br	Br	Br	CH_3	5.44 (s)	5.69 (s)	-	-	-	2.40 (s)

Quinones



The ^{1}H-NMR spectra of quinones exhibited as singlet, doublet, quartet and multiplet with various coupling constants around 6.57-8.26 ppm and the methyl protons appeared in the range of 1.34-2.61 ppm as singlet or triplet signals. The ^{1}H-NMR spectra assignments of quinones are recorded in Table 3.7.

Table 3.7 The ^{1}H-NMR spectral assignments of quinones

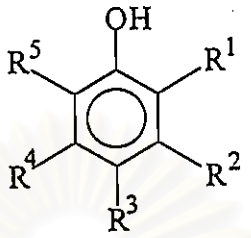
Cpd	R				chemical shift (ppm)			
					H of R			
	R^1	R^2	R^3	R^4	R^1	R^2	R^3	R^4
1b	Br	H	H	CH_3	-	7.45 (s)	6.86 (q)	2.17 (d)
2b	CH_3	Br	Br	H	2.25 (s)	-	-	7.33 (s)
3b	Br	CH_3	CH_3	Br	-	2.27 (s)	2.27 (s)	-
4b	Br	H	H	Cl	-	7.26 (s)	7.26 (s)	-
5b	Br	Br	CH_3	CH_3	-	-	2.13 (s)	2.13 (s)
6b	Br	H	H	t-Bu	-	8.26 (d)	7.68 (d)	1.39 (s)
7b	t-Bu	H	H	t-Bu	1.37 (s)	7.71 (s)	7.71 (s)	1.37 (s)
8b	Br	H	H	Br	-	7.25 (s)	7.25 (s)	-
9	H	H	H	H	6.67 (s)	6.67(s)	6.67 (s)	6.67 (s)
10	CH_3	H	H	H	2.00 (s)	6.57-6.69 (m)	6.57-6.69 (m)	6.57-6.69 (m)
11	t-Bu	H	H	H	1.34 (s)	6.65-6.73 (m)	6.65-6.73 (m)	6.65-6.73 (m)
12	Cl	H	H	H	-	6.78-7.00 (m)		
13b	Br	H	Br	H	-	7.47 (s)	-	7.47 (s)
14b	Br	Br	Br	CH_3	-	-	-	2.33 (s)
15b	$C(O)CH_3$	H	OH	Br	2.61 (s)	7.89 (s)	13.32 (s)	-
16	Br	CH_3	pentadecyl	Br	-	2.26 (s)	0.89 (t), 1.17-1.46 (m)	-
17	Br	CH_3	tridecyl	Br	-	2.24 (s)	0.90 (t), 1.18-1.24 (m)	-
18	Br	CH_3	undecyl	Br	-	2.30 (s)	0.90 (t), 1.18-1.25 (m)	-

Table 3.7 (cont.)

Cpd	R				chemical shift (ppm)			
					H of R			
	R^1	R^2	R^3	R^4	R^1	R^2	R^3	R^4
19	Br	CH_3	nonyl	Br	-	2.25 (s)	0.90 (t), 1.17-1.30 (m)	-
20	Br	CH_3	heptyl	Br	-	2.30 (s)	0.90 (t), 1.20-1.46 (m)	-
21	Br	CH_3	pentyl	Br	-	2.28 (s)	0.90 (t), 1.18-1.26 (m)	-

^{13}C-NMR

Phenols

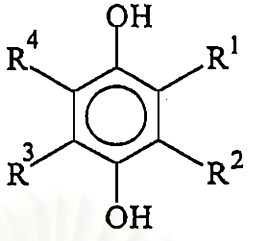


The ^{13}C-NMR spectra of phenols revealed the signals around 106.8-160.3 ppm, a C-OH signal showed around 115.2-150.5 ppm. The chemical shifts of alkyl groups were observed around 16.5-36.0 ppm except for the methyl group on R^1 which showed at the signal 202.0 ppm. The ^{13}C-NMR spectra assignments of phenols are exhibited in Table 3.8.

Table 3.8 The ^{13}C-NMR spectral assignments of phenols

Cpd	R					chemical shift (ppm)										
						C-1	C-2	C-3	C-4	C-5	C-6	C of R				
	R^1	R^2	R^3	R^4	R^5							R^1	R^2	R^3	R^4	R^5
1a	Br	H	Br	H	CH_3	149.8	127.7	131.3	112.0	133.1	110.4	-	-	-	-	16.5
2a	Br	CH_3	Br	H	Br	148.9	113.1	137.7	115.0	134.1	106.8	-	24.0	-	-	-
3a	Br	CH_3	Br	CH_3	Br	148.4	109.7	137.3	117.9	137.3	109.7	-	25.2	-	25.2	-
6a	Br	H	Br	H	t-Bu	149.7	112.2	131.5	112.5	129.8	139.4	-	-	-	-	29.1, 35.6
7a	t-Bu	H	Br	H	t-Bu	150.5	138.2	129.8	126.0	129.8	138.2	29.6, 36.0	-	-	-	29.6, 36.0
8a	Br	H	Br	H	Br	149.0	110.4	134.2	112.7	134.2	110.4	-	-	-	-	-
15a	C(O) CH_3	H	Br	OH	Br	115.2	155.6	98.9	160.3	99.4	133.3	26.3, 202.0	-	-	-	-

Hydroquinones

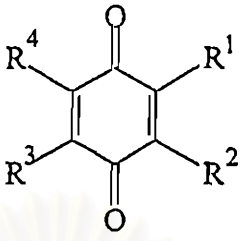


The ^{13}C-NMR spectra of hydroquinones showed a signal around 108.2-126.5 ppm and 2C of Ar-OH were observed at 144.4-153.2 ppm as C-1 and C-4. The remaining signals were methyl carbons as substituent which showed the signal approximately 17.2-13.11 ppm. The ^{13}C-NMR spectra assignments of hydroquinones are tabulated in Table 3.9.

Table 3.9 The ^{13}C-NMR spectral assignments of hydroquinones

Cpd	R				chemical shift (ppm)							
					C-1	C-2	C-3	C-4	C-5	C-6	C of R	
	R^1	R^2	R^3	R^4							R^3	R^4
4a	Br	H	H	Cl	150.8	112.4	120.2	153.2	115.8	126.5	-	-
5a	Br	Br	CH_3	CH_3	145.1	108.3	108.3	145.1	124.8	124.8	13.1	13.1
13a	Br	H	Br	H	146.7	109.8	118.5	146.7	109.8	118.5	-	-
14a	Br	Br	Br	CH_3	145.4	113.1	112.2	144.4	128.8	108.2	-	17.2

Quinones



The ^{13}C-NMR spectra of carbonyl signals of quinones exhibited in the range of 134.2-188.4 ppm and the remaining carbons were observed around 98.9-175.1 ppm. Alkyl carbons revealed around 13.5-36.7 ppm. The ^{13}C-NMR spectra of quinones are shown in Table 3.10.

Table 3.10 The ^{13}C-NMR spectral assignments of quinones

Cpd	R				chemical shift (ppm)									
					C-1	C-2	C-3	C-4	C-5	C-6	C of R			
	R^1	R^2	R^3	R^4							R^1	R^2	R^3	R^4
1b	Br	H	H	CH_3	177.8	132.7	139.6	177.2	137.0	146.1	-	-	-	16.6
2b	CH_3	Br	Br	H	172.3	146.5	135.6	181.8	134.3	137.9	17.1	-	-	-
3b	Br	CH_3	CH_3	Br	172.1	134.2	146.1	181.4	146.1	134.2	-	17.4	17.4	-
4b	Br	H	H	Cl	169.0	110.5	134.2	171.1	136.2	132.3	-	-	-	-
5b	Br	Br	CH_3	CH_3	177.7	139.2	139.2	177.7	141.3	141.3	-	-	13.5	13.5
6b	Br	H	H	t-Bu	150.8	127.5	132.3	178.8	133.1	136.0	-	-	-	-
7b	t-Bu	H	H	t-Bu	150.5	136.1	126.0	186.5	126.0	136.1	29.6, 36.0	-	-	29.6, 36.0
8b	Br	H	H	Br	134.2	109.6	127.9	155.0	127.9	109.6	-	-	-	-
9	H	H	H	H	186.2	134.5	134.5	186.2	134.5	134.5	-	-	-	-
10	CH_3	H	H	H	187.5	145.8	133.3	187.7	136.6	136.5	15.8	-	-	-
11	t-Bu	H	H	H	187.4	156.0	131.5	188.4	138.6	134.9	29.1,35.2	-	-	-
12	Cl	H	H	H	184.9	144.1	133.7	179.2	136.8	136.1	-	-	-	-
13b	Br	H	Br	H	176.9	137.8	137.1	176.9	137.8	137.1	-	-	-	-
14b	Br	Br	Br	CH_3	170.6	137.8-139.5	175.1	134.7	146.1	-	-	-	-	18.2
15b	$C(O)CH_3$	H	OH	Br	160.3	99.4	115.3	155.6	133.4	98.9	26.3, 202.0	-	-	-
16	Br	CH_3	pentadecyl	Br	172.4	133.8-134.0	146.1-149.9	181.2	146.1-149.9	133.8-134.0	-	14.1-37.8		-
17	Br	CH_3	tridecyl	Br	175.5	133.8-134.0	146.0-149.9	181.0	146.0-149.9	133.8-134.0	-	14.1-31.9		-
18	Br	CH_3	undecyl	Br	172.4	132.3-135.2	145.9-149.9	180.5	145.9-149.9	132.3-135.2	-	14.2-36.8		-
19	Br	CH_3	nonyl	Br	169.0	131.6-134.0	146.0-150.0	180.5	146.0-150.0	131.6-134.0	-	14.1-34.9		-
20	Br	CH_3	heptyl	Br	176.2	131.5-133.9	146.0-148.9	180.8	146.0-148.9	131.5-133.9	-	14.0-37.8		-
21	Br	CH_3	pentyl	Br	172.6	131.5-134.0	145.9-150.0	179.7	145.9-150.0	131.5-134.0	-	14.2-35.0		-

3.2 Biology

The major objective of this research is to figure out the relationship between structures of benzoquinones, halobenzoquinones, halohydroquinones and halobenzoquinones containing various numbers of carbon on side chains (alkyl-quinone) and Brine Shrimp Lethality Test (BSLT). LC_{50} value was reported as indicator of this test.

3.2.1 Brine shrimp lethality test against *Artemia salina* Leach.

Basically, lethality test bioassays depend on the ability to measure the amount survived brine shrimp from test samples. If LC_{50} value is high, it means that those compounds have low lethality efficiency. On the other hand, if LC_{50} value is low, it implies that those compounds have high lethality efficiency. The LC_{50} values of tested compounds are presented as shown in Table 3.11 and Fig. 3.6.

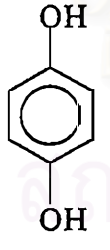
Table 3.11 The LC_{50} value at 24 h of tested compounds

Compound	LC_{50} (μg/mL)	Bioactivity	Type of compound
1b	0.39994	High	halobenzoquinone
2b	1.19669	High	halobenzoquinone
3b	3.00263	High	halobenzoquinone
4a	23.4510	Medium	bromohydroquinone
4b	12.6080	Medium	halobenzoquinone
5a	3.56320	High	bromohydroquinone
5b	0.24241	High	halobenzoquinone
6b	1478.99	No activity	halobenzoquinone
7b	12003.4	No activity	benzoquinone
8b	17.4008	Medium	halobenzoquinone
9	6.84589	High	benzoquinone
10	8.78579	High	benzoquinone
11	20.4979	Medium	benzoquinone
13a	18.8579	Medium	bromohydroquinone

Table 3.11 (cont.)

Compound	LC_{50} (μg/mL)	Bioactivity	Type of compound
13b	19.6714	Medium	halobenzoquinone
14a	1.96083	High	bromohydroquinone
14b	1.52098	High	halobenzoquinone
15b	2.14170	High	halobenzoquinone
16	14.7906	Medium	alkyl-benzoquinone
17	64.6224	Medium	alkyl-benzoquinone
18	151.218	Low	alkyl-benzoquinone
19	239.684	Low	alkyl-benzoquinone
22*	9.59957	High	hydroquinone
23*	3.14993	High	hydroquinone
24*	8.09325	High	hydroquinone

* commercial available



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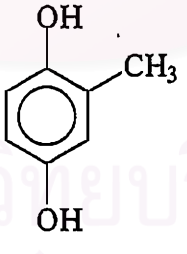

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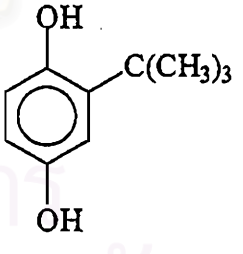

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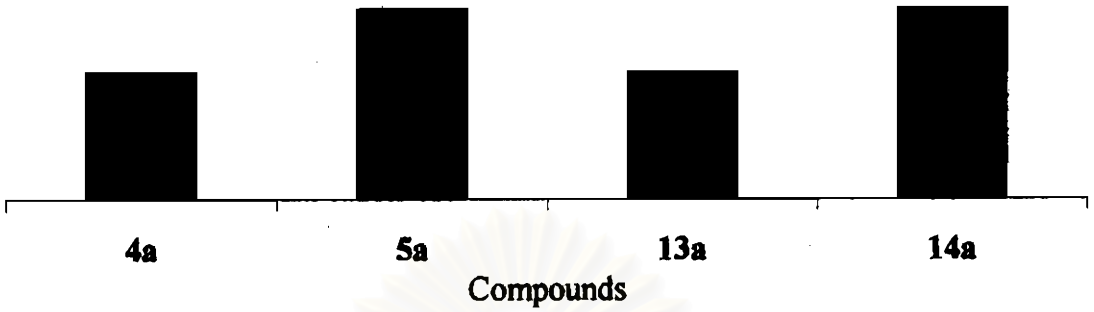


Fig. 3.6 The BSLT activity of bromohydroquinones

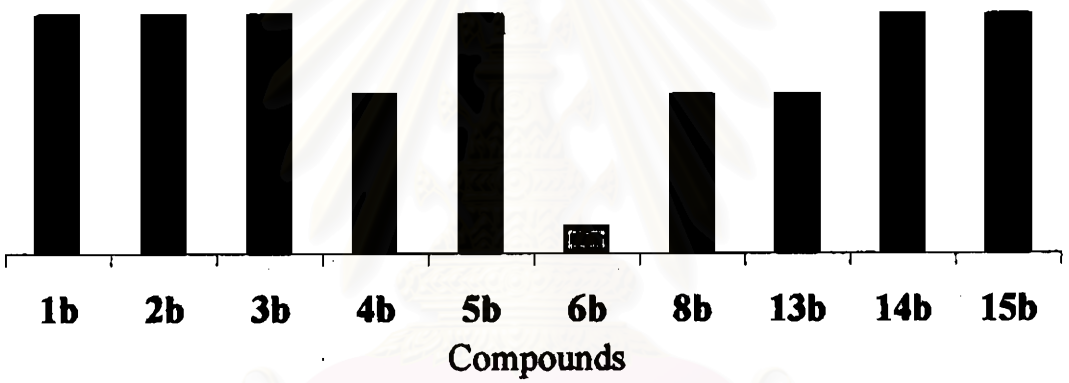


Fig. 3.7 The BSLT activity of halobenzoquinones

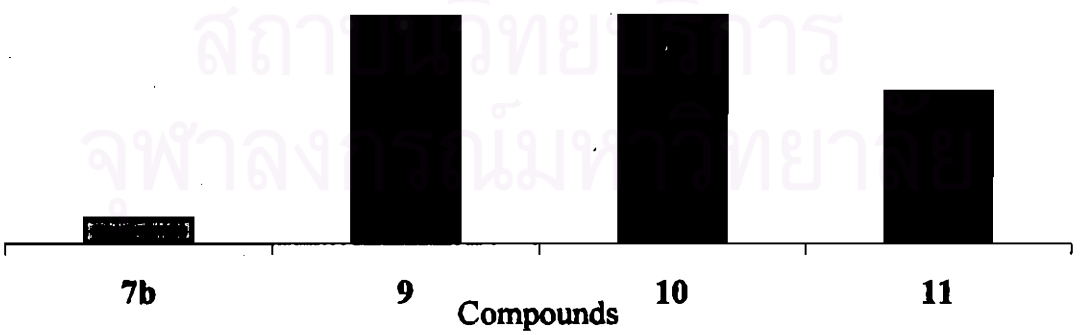


Fig. 3.8 The BSLT activity of benzoquinones

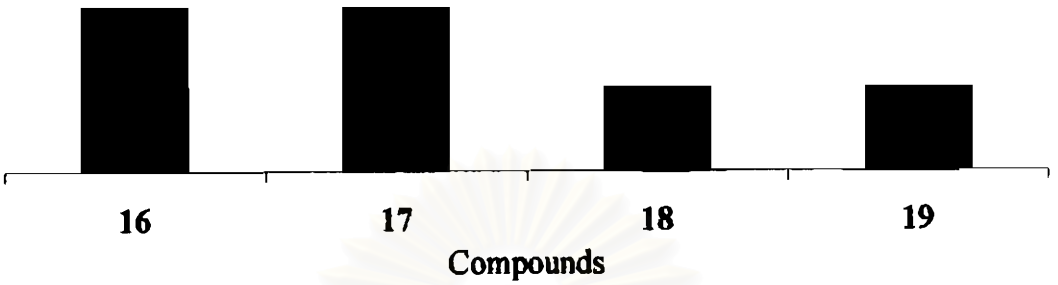


Fig. 3.9 The BSLT activity of alkyl-benzoquinones

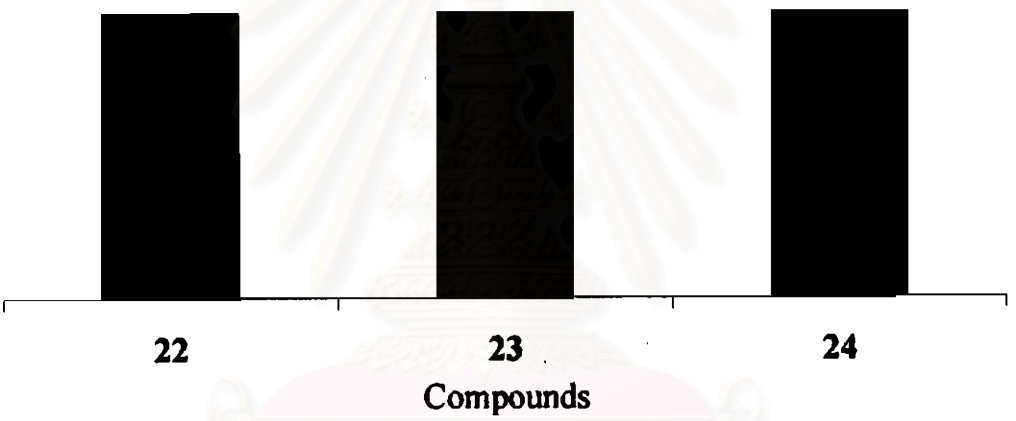


Fig. 3.10 The BSLT activity of hydroquinones

Note: = High activity (LC_{50} < 10 μg/mL)

= Medium activity (LC_{50} < 100 μg/mL)

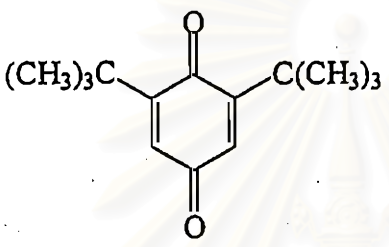
= Low activity (LC_{50} < 1000 μg/mL)

= No activity (LC_{50} > 1000 μg/mL)

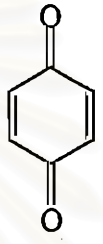
From Table 3.11, all compounds could be classified into three categories according to their structures.

The first group was halobenzoquinones. This group was consisted of thirteen compounds: **1b, 2b, 3b, 4b, 5b, 6b, 7b, 8b, 9, 10, 11, 13b** and **14b.**

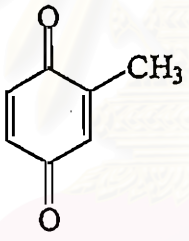
Considering the structures and activity relationship, it was found that there were some remarkable points that could be observed from this study.



(7b)



(9)



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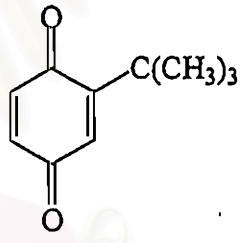
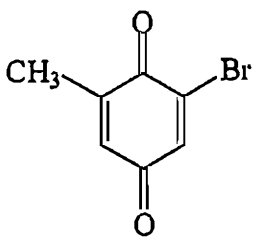
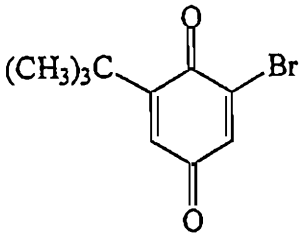


(11)

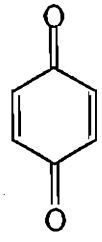
For instance, when Compounds **9** and **10** were compared, it was observed that the LC_{50} value of both compounds were almost the same. This implied that a methyl group attached to a benzoquinone moiety did not make any difference in terms of brine shrimp lethality. However, when Compounds **9** and **11** were focused, it was disclosed that the LC_{50} of Compound **9** was far less than that of Compound **11**. This perhaps stated that a bulky alkyl group attached to a benzoquinone moiety decreased the biological activity. Another interesting point arose from the information derived from the comparison of Compounds **9, 11** and **7b**, the LC_{50} value of Compound **7b** was extremely higher than those of Compounds **9** and **11**. This observation clearly confirmed the previous statement that the more bulky groups attached to the benzoquinone moiety, the less activity exhibited.



(1b)



(6b)



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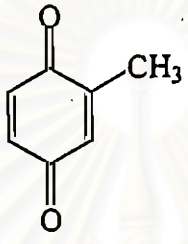

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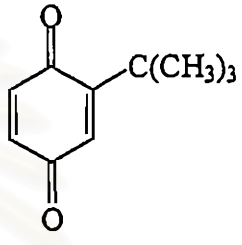
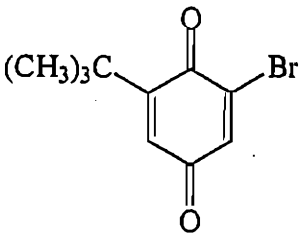
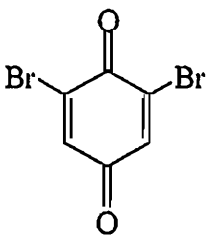


(11)

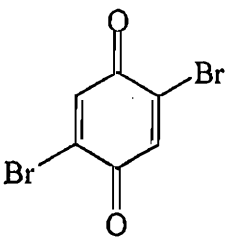
The effect of the introduction of a bromine atom to the benzoquinone nucleus was clearly seen from the comparison of Compounds **1b**, **9** and **10**. It was clearly found that the LC_{50} value of Compound **1b** was extremely less than that of Compound **10**. This revealed that the presence of bromine bearing to a methylbenzoquinone made the degree of lethality be significantly increased. Nevertheless, the trend that the introduction of bromine atom increased the activity did not seem to be true when alkyl group present in the benzoquinone was a sterically hindered group such as a *tert*-butyl group. This observation could be seen from a series of Compounds **9**, **11** and **6b**; *i.e.*, the LC_{50} of Compound **6b** was higher than that of Compound **11**.



(6b)

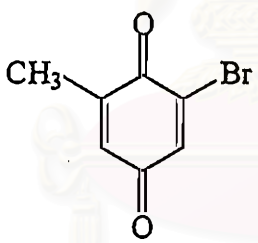


(8b)



(13b)

Further studies on the effects of the number of bromine atoms present in the benzoquinone molecules were conducted. From the results of the LC_{50} of Compounds **6b** and **8b**, it could be obviously stated that the more bromine atoms attaching to the benzoquinone moiety, the higher activity was obserevd. The effects of the positions of two bromine atoms on the benzoquinone ring were also examined using Compounds **8b** and **13b** as chemical models. It was found that there was no difference in activity regarding to the position of bromine atoms present, either at 2,6-position in Compound **8b** or at 2,5-position in Compound **13b**.



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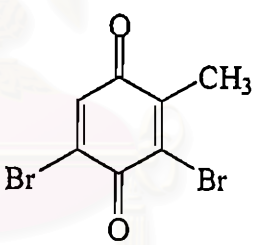
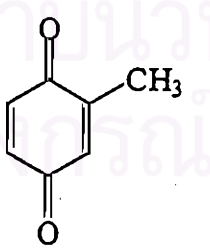


(2b)



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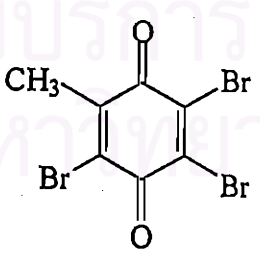
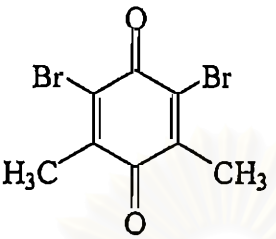


(14b)

The variation of the numbers of bromine atoms in a benzoquinone ring was investigated. It was found that in a series of Compounds **1b**, **2b**, **10** and **14b**. Compound **10** gave the highest LC_{50}, followed by those of **14b**, **2b** and **1b**, respectively. The results clearly displayed that methylbenzoquinones bearing a

bromine atom provided better activity. However, the more bromine atoms introduced to the benzoquinone moiety, the less activity was observed.



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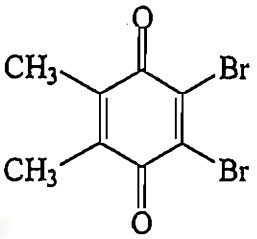
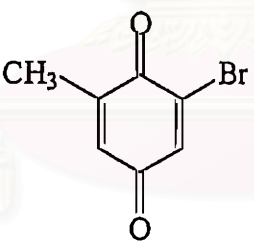


(5b)

The comparison between Compounds **3b** and **5b** was set up in order to answer posed question whether the positions of methyl groups and bromines affect the activity. The results clearly demonstrated that if two bromine atoms were present at 2,3-position, while the other two positions were fully occupied by two methyl groups, the lethality observed was very far higher than that of two bromine atoms present in 2,6-positions.



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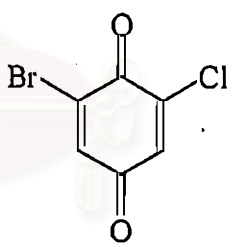

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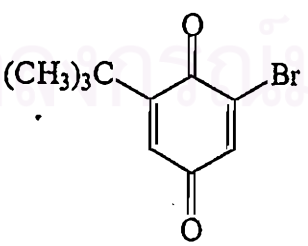

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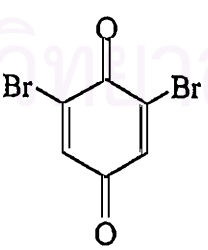
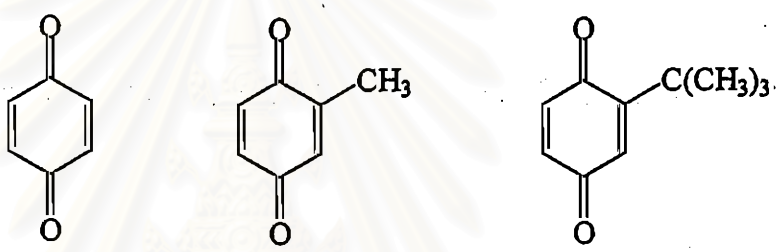


(8b)

When Compounds **1b**, **4b**, **6b** and **8b** were taken into account of consideration, it was observed that Compound **6b** gave the highest value of LC_{50} followed by

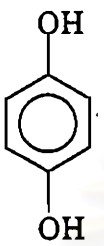
Compounds **8b**, **4b** and **1b**, respectively. This obviously implied that a substituent type attached to a bromobenzoquinone did matter with some extent to the lethal activity. For example, the introduction of a *tert*-butyl and a bromine group did not show any significant effect on the activity, whereas the co-occurance of one bromine and one chlorine groups greatly increased the activity. The more extent was found when two bromine groups were introduced into a benzoquinone moiety. However, the most preeminent result was observed for Compound **1b** where 1 methyl and 1 bromine groups present in the molecule.



(9) **(10)** **(11)**

The examination of the relationship of benzoquinones **9**, **10** and **11** and their activity was carried out. It clearly stated that the activity was downwards from Compounds **11**, **10** and **9**, respectively. This result revealed that the more the number of carbon on side chains were added, the less lethality was observed. In this particular case, it was found that the benzoquinone containing *tert*-butyl group gave the lowest brine shrimp lethality following by benzoquinones attaching with a methyl group and that without alkyl substitutent, respectively.

The second group was hydroquinone. This group was composed of seven compounds: **4a**, **5a**, **13a**, **14a**, **22**, **23** and **24**. The former four compounds were synthesized, while the latter three were commercially purchased.



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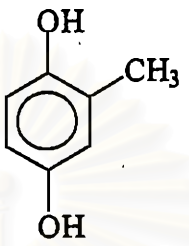
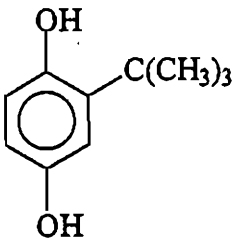
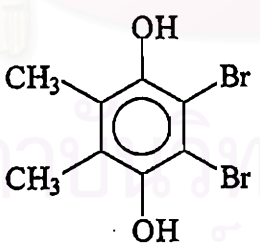


(23)

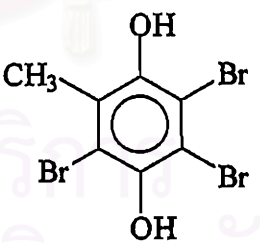


(24)

Considering Compounds **22**, **23** and **24**, it was observed that the LC_{50} value of **22** was higher than those of **23** and **24**. This clearly demonstrated that a methyl group essentially increased lethality of brine shrimp. On the other hand, the LC_{50} value of Compounds **22** and **24** did not display significantly different. This result implied that the *tert*-butyl group did not play any important role in hydroquinone moiety to this activity. This result clearly exhibited that the bulkier substituents present in the molecule, the less biological activity observed.

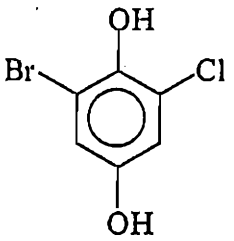


(5a)

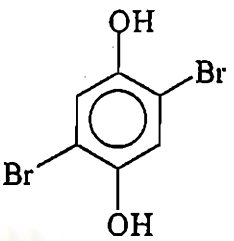


(14a)

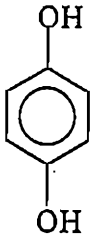
The numbers of bromine atoms in hydroquinones also displayed significant outcome. When Compounds **5a** and **14a** were compared, it was found that the activity of **14a** was superb around twice. This result implied that the substitution of a methyl group with a bromine atom would increase this interested activity.



(4a)



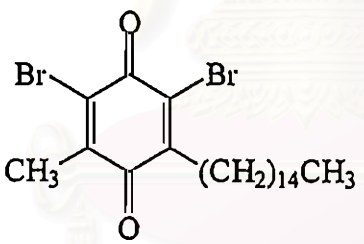
(13a)



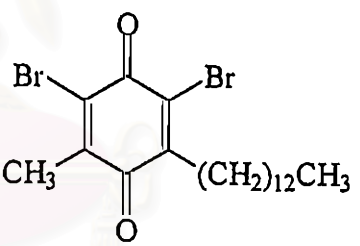
(22)

When the comparison of Compounds **13a**, **4a** with Compound **22** was made, it was clearly observed that various substituents bearing in hydroquinone molecule rendered the activity. Among them, hydroquinone (Compound **22**) itself displayed the most intriguing activity in this group.

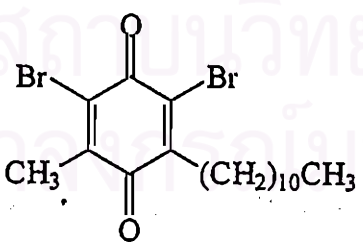
The final group was alkyl-halobenzoquinone. This group was composed of four compounds: **16**, **17**, **18** and **19**.



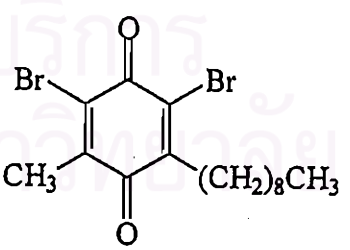
(16)



(17)



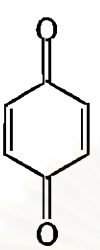
(18)



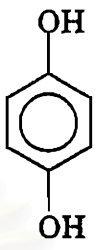
(19)

Comparing all substrates in this group, it was found that Compound **16** gave the lowest value of LC_{50} following by those of Compounds **17**, **18** and **19**, respectively. This implied that the numbers of carbon atoms of side chain had great

effect on biological activity. The more carbons on side chain were, the more lethality was observed.

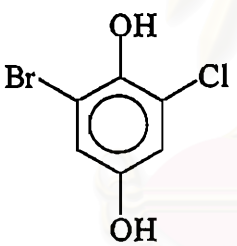


(9)

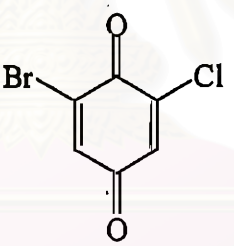


(22)

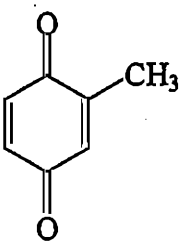
When the classes of halobenzoquinones and hydroquinones were compared, it was found to be a general trend that the lethal efficiency of benzoquinones was basically higher than that of hydroquinones. For instance, the LC_{50} value of Compound 9 was found to be lower than that of Compound 22.



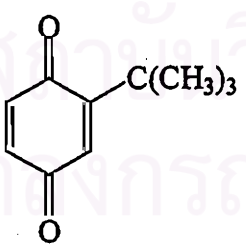
(4a)



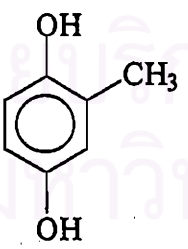
(4b)



(10)



(11)



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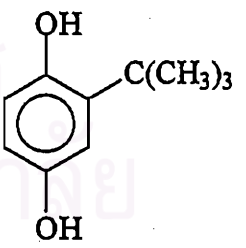

(24)

An interesting point was detected when Compounds **10** and **23** were compared. It was observed that Compound **10** gave nearly three times higher of LC_{50} value than that of Compound **23**. This means that the activity of such compounds did depend on the structure of parent compounds and their substitutent(s). In this case, it was observed that the introduction of a methyl group to hydroquinone moiety

increased the activity markedly, whereas greatly decreased the activity in the case of quinones. This trend was also observed in the case of the introduction of a *tert*-butyl, and Br and Cl groups (Compounds **11** and **24**, and Compounds **4a** and **4b**), respectively.