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Synthesis and evaluation of novel 1,4-naphthoquinone derivatives as antiviral, antifungal and anticancer agents

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Abstract—The synthesis and evaluation of some 2-substituted-1,4-naphthoquinones **2**, *S*-(1,4-naphthoquinon-2-yl)-mercaptoalkanoic acid amides **4**, related benzoquinone and naphthoquinone derivatives **6–9** and 2,3-disubstituted 1,4-naphthoquinones **10–11** were carried out. The antifungal, antibacterial, antiviral and anticancer activities were determined by using the standard assay. The results show that compounds **2b** and **10a** showed in vitro antiviral activity against *Inflenza-A Virus* and *Herpes Simplex Virus* and possess pronounced antifungal profile whereas **4a** showed anticancer activites against *Lymphoid Leukaemia P 388*.

The 1,4-naphthoquinone structure is common in numerous natural products associated with antifungal, antibacterial, antiviral and antitumour activities.[1] A number of 1,4-naphthoquinones having sulfur atom present in them have been shown to possess antifungal activity.[2–5] 1,4-naphthoquinone pharmacophore is known to impart cytotoxity in a number of drugs, for example, streptonigrin,[6] actinomycins,[7] mitomycins,[8] alkannins,[9] 2-hydroxynaphthoquinone derivatives[10] and 1,4-furanonaphthoquinones.[11] In addition to imparting antifungal and cytotoxic activity, 1,4-naphthoquinones have also exhibited significant antimicrobial activity.[12]

The antifungal, antitumour and antimicrobial profile of compounds mentioned above prompted us to synthesize 1,4-naphthoquinones **2–11** possessing sulfur atom in them.

The evaluation of antifungal properties of **2–11** against various strains of pathogenic fungi, for example, *C. albicans*, *C. neoformans*, *T. mentagraphytes*, *A. fumigatus* and *M. cannis* was carried out according to the method of Dhar et al.[13] The antifungal activity was compared with Miconazole, Nystatin and Amphotericin B. The compounds having minimum inhibitory concentration (MIC) of 50 μg/mL or less were considered active and compared with standard drugs referred to above and summarized in Table 1. Comparison of activity of compounds with the antifungal drug Miconazole showed that compound **2b** had better activity against fungi *C. albicans* and *C. neoformans*. Compound **10a** too showed better antifungal activity against *C. albicans* and *C. neoformans*. Compound **10a** had same activity against *T. mentagraphytes* when compared with Miconazole. On comparison of antifungal activity of compounds referred to in Table 1 with Nystatin, the antifungal drug, the compound **2b** showed better activity against *C. albicans* and *C. neoformans*. Compound **10a** also showed better activity when compared with Nystatin against *C. neoformans*. On comparison of antifungal activity of **10a** with Amphotericin B, the most active antifungal drug, it was observed that compound **10a** had better antifungal activity against *T. mentagraphytes* and *M. cannis*. Compounds **2a**, **2c**, **6**, **8c**, **9b**, **10a** ($R^2 = OH$) and **11c** exhibited moderate antifungal activity. Other compounds whose MIC was >75 μg/mL are not reported in Table 1 as these were considered inactive compounds.

Antibacterial activities of naphthoquinone derivatives **2(a–b)**, **6**, **8c**, **10a** and **11a** were evaluated and the results are reported in Table 2. Table 2 shows MIC values of **2(a–b)**, **6**, **8c**, **10a** and **11a** against four strains of the bacteria, *S. aureus* methicillin resistance, *T. tumefaciens*, *P. aueruginosa* and *K. pneumoniae*. Gentamycin was used as a positive control in all the tests and its MIC value is expressed in μg/mL.

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Table 1. Structure and in vitro antifungal activity for compounds **2**, **6**, **8** and (**9**–**11**)

Compds	*n*	R	R^1	R^2	Ar	MIC (μg/mL)				
						C. albicans	*C. neoformans*	*T. mentagraphytes*	*A. fumigatus*	*M. cannis*
2a	3	H	H	OH	b	>50	>50	25	>50	>50
2b	3	OH	OH	OH	b	3.125	1.56	3.125	>50	3.125
2c	2	OH	CI	OH	b	>50	>50	25	>50	>50
6	b	b	b	b	b	>50	50	>50	>50	>50
8c	b	OH	CI	H	b	>50	>50	25	>50	>50
9b	b	OH	OH	H	b	>50	>50	25	>50	>50
10a	b	H	H	H	Ph	12.5	1.56	<0.78	25	<0.98
10a	b	H	H	OH	Ph	25	>50	6.25	>50	25
11c	b	OH	Cl	b	Ph	>50	>50	>50	50	50
11c	b	OH	CI	b	Np	50	>50	50	>50	>50
Miconazole						25	12.5	<0.78	12.5	<0.78
Nystatin						7.8–7.9	3.5–3.9	a	a	a
Amphotericin-B						0.39	0.78	1.56	a	1.56

a: Activity not reported; b: not required; Np = naphthyl; Ph = phenyl.

Table 2. Structure and in vitro antibacterial activity for compounds **2**, **6**, **8**, **10** and **11**

Compds	*n*	R	R^1	R^2	Ar	MIC (μg/mL)			
						S. aureus	*T. tumefaciens*	*P. aeruginosa*	*K. pneumoniae*
2a	2	H	H	OH	b	12.5	25.0	12.5	50
2a	3	H	H	OH	b	12.5	12.5	50	25
2b	3	OH	OH	OH	b	>50	12.5	>50	50
6	b	b	b	b	b	>50	>50	25	25
8c	b	OH	Cl	H	b	>50	>50	50	25
10a	b	H	H	H	Ph	25	>50	>50	>50
11a	b	H	H	b	Np	50	>50	>50	>50
Gentamycin						0.78	a	0.78	0.39

a: Activity not reported; b: not required; Np = naphthyl; Ph = phenyl.

Compound **2a** ($n = 2$ and 3) showed significant activity against *S. aureus*, *T. tumefaciens*, *P. aueruginosa* and *K. pneumoniae*. Compound **2b** showed significant activity against *T. tumefaciens* and had no antibacterial activity against other strains of bacteria tested. The series of 1,4-naphthoquinones having mercaptobenzoic acid moiety present in them viz. Compounds **6** and **8c** analogues showed significant activity against *P. aueruginosa* and *K. pneumoniae* and had no antibacterial activity against other strains of bacteria referred in Table 2. Arylthio substituted 1,4-naphthoquinones **10a** and **11a** showed significant activity against *S. aureus* only. However the compounds referred in Table 2 and discussed above were less active than the standard drug Gentamycin.

The antiviral activity of compounds **2b** and **10a** against *Influenza-A* virus are given in Table 3. The antiviral assay was done according to Sidwell and Hoffman.[14] Only compounds **2b** and **10a** showed significant activity against *Influenza-A* virus and *Herpes Simplex Virus* (HSV-1).

The anticancer activity was carried out against *Lymphoid Leukaemia P 388* and *L 1210*. The anticancer activities of **4a** are shown in Table 4. Compound **4a** ($n = 2$, N< = NH_2) exhibited marked anticancer activity having T/C 49% whereas **4a** ($n = 2$, N< = piperidino) exhibited better anticancer activity having T/C 30%. Compound **2a** on evaluation of anticancer activity against *Lymphoid Leukaemia L 1210* was found to be inactive. The results are shown in Table 5.

The synthesis of *S*-(1,4-naphthoquinon-2-yl)-mercapto-alkanoic acid amides **4(a–c)** is shown in Scheme 1. Compound **4** were synthesized by condensation of 1,4-naphthoquinone **1** with mercaptoalkanoic acid to form

Table 3. In vitro antiviral activity of compounds against Influenza-A and Herpes Simplex Virus-1

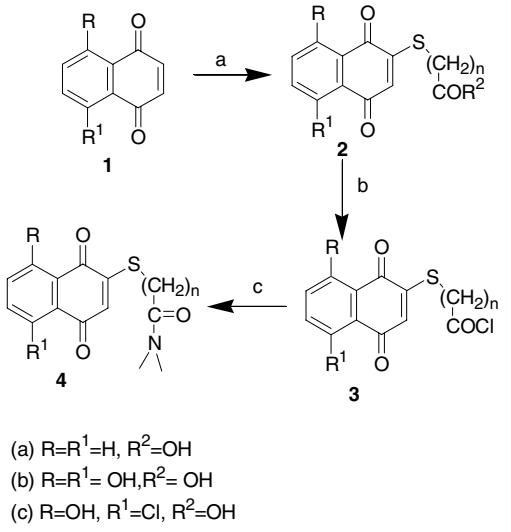
Compds	Influenza-A inhibition (%)	Herpes Simplex Virus (HSV) protection (%)
10a (R^2 = OH, Ar = Ph)	50	20
2b ($n = 2$)	52	18

Table 4. Anticancer activity against Lymphoid Leukaemia P 388 in rats

Compds $n = 2$	Dose (mg/kg)	Survivors	T/C (%)	Remark
4a N< = NH_2	10	4/4	49	Active
4a N< = piperidino	10	4/4	30	Active

Table 5. Anticancer activity against Lymphoid Leukaemia L-1210

Compds	Dose (mg/kg)	Survivors	Tumour evaluation test control		T/C	Remark
			Test	Control		
2a ($n = 2$)	25.0	1/6	4.0	12.0	Toxic	Inactive
	12.5	6/6	13.3	12.0	110	Inactive
	6.25	6/6	13.8	12.0	115	Inactive
2a ($n = 3$)	6.25	6/6	10.8	10.8	100	Inactive
	3.12	6/6	10.4	10.8	96	Inactive
	1.56	6/6	9.7	10.8	89	Inactive



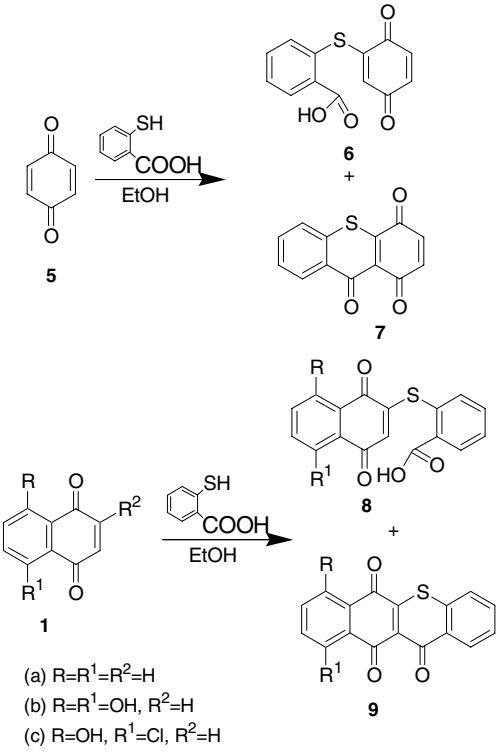
Scheme 1. Reagents and conditions: (a) $HS(CH_2)_nCOOH$, EtOH; (b) $SOCl_2$, benzene; (c) N<.

S-(1,4-naphthoquinon-2-yl)-mercaptoalkanoic acid **2**, which in turn were converted to amides **4** by reaction with $SOCl_2$ and primary or secondary amines.[15]

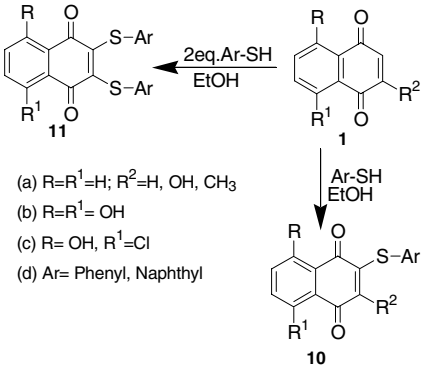
Reaction of 1,4-benzoquinone **5** and 1,4-naphthoquinones **1** with thiosalicylic acid resulted in the formation of *S*-(1,4-benzoquinon-2-yl) mercaptobenzoic acid **6** and 7,10-dihydrothioxanthene-6,7,10-trione **7**, *S*-(1,4-naphthoquinon-2-yl)-mercaptobenzoic acid 8 and 7,8-dihydrobenzo[*b*]thioxanthene-6,7,12(H)-trione **9** as shown in Scheme 2.[16]

Scheme 3 describes about reactions of 1,4-naphthoquinones **1** with arylthiol leading to the formation of 2-arylthio-1,4-naphthoquinone **10** and 2,3-bis-(phenylthio)-1,4-naphthoquinone **11**.[17]

In conclusion we have synthesized a series of 2-substituted 1,4-naphthoquinones containing sulfur atom in them and carried out their biological activities. Amongst the promising compounds **4a** ($N< = NH_2$, piperidino) has shown significant anticancer activity against *Lymphoid Leukaemia P 388*. Compound **2b** ($n = 2$) and **10b** ($R^2 = OH$, Ar = phenyl) have shown in vitro antiviral activity against *Influenza-A* virus and *Herpes Simplex Virus* (HSV-1). Compounds **2a**, **2b**, **6**, **8c**, **10a** and **11a** have shown some antibacterial activity. Compounds **2b** and **10a** are lead compounds for antifungal activity. Further work on compounds **2b** and **10a** is in progress.



Scheme 2.



Scheme 3.

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15. General procedure for the preparation of *S*-(1,4-naphthoquinone-2-yl)-mercaptoalkanoic acid amides **4(a–c)**. Thionyl chloride (1.42 g, 12 mmol) was added to a stirred solution of 1,4-naphthoquinone derivatives **2(a–c)** (10 mmol) in benzene (20 mL). The reaction mixture was refluxed for 4 h and concentrated in vacuo. Compounds **4(a–c)** were obtained as oil in quantitative yield and was further used without purification. To a stirred solution of **4(a–c)** (10 mmol) in benzene (20 mL) was added 5 mL of concentrated solution of NH_4OH and the mixture was stirred at 0 °C for 3 h. The solid thus obtained were filtered and crystallized from methanol. Compound **4a** ($n = 2$, $N<=NH_2$); 78% yield; mp 168 °C; IR: 3258 (NH_2), 1658 and 1630 (quinone –C=O) cm^{-1}; MS; M^+ (m/e) 261. Anal. Calcd for $C_{13}H_{11}NO_3S$ (261): C, 59.77; H, 4.21; N, 5.36; S, 12.26. Found: C, 60.12; H, 4.30; N, 5.48; S, 12.42.
16. General procedure for the synthesis of *S*-(1,4-naphthoquinone-2-yl)-mercaptobenzoic acid **8a** and 7,8-dihydrobenzo [*b*] thioxanthene-6,7,12 (H)-trione **9a**. Thiosalicylic acid (1.54 g, 10 mmol) was added to a solution of 1,4-naphthoquinone **1** (1.58 g, 10 mmol) in absolute alcohol and stirred at room temperature for 4 h. The dark red solution was concentrated in vacuo. The residue was dissolved in ether and extracted with aqueous $NaHCO_3$. The $NaHCO_3$ extract was acidified with 3 N HCl, when the mixture of **8a** and **9a** was obtained as an orange solid. It was washed with water. Compound **8a** was obtained after two crystallizations with $CHCl_3$–hexane; orange crystals, yield 2.78 g (90%); mp 205–207 °C; IR (KBr): 1700 (>C=O of acid), 1650 (>C=O of quinone carbonyl); ^1H NMR ($CDCl_3$): δ 7.5–8.1 (m, 8H, aromatic protons); MS: M^+ (m/e) 310. Anal. Calcd for $C_{17}H_{10}O_4S$ (310): C, 65.80; H, 3.32; S, 10.32. Found: C, 66.08; H, 3.34; S, 10.54. Compound **9a**: Mother liquor on crystallization yielded **9a** as orange needles; 116 mg (4%), mp 190–192 °C; IR (KBr): 1665 (>C=O of keto carbonyl) cm^{-1}; MS: M^+ (m/e) 292. Anal. Calcd for $C_{17}H_8O_3S$ (292): C, 69.84; H, 2.73; S, 10.95. Found: C, 69.98; H, 2.86; S, 11.12.
17. General procedure for the prepration of 2-arylthio-1,4-naphthoquinones **10(a–c)**. Arylthiol (10 mmol) was added to a solution of 1,4-naphthoquinone **1** (10 mmol) in anhydrous ethanol (40 mL). The reaction mixture was refluxed for 10 h on steam bath. The dark coloured mixture was cooled and poured in ice cooled water. The solid product was filtered and crystallized with methanol. Compound **10a** ($R^2 = H$) was obtained in 83% yield; mp 142–143 °C; IR: 1656 and 1568 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.12 (s, 1H, C_3H), 7.52 (s, 5H, thiophenyl protons), 7.77 (m, 2H, C_6–H and C_7–H), 8.05 (m, 1H, C_8–H), 8.15 (m, 1H, C_5–H); MS: M^+ (m/e) 266. Anal. Calcd for $C_{16}H_{10}O_2S$ (266): C, 72.18; H, 3.78; S, 12.03. Found: C, 72.30; H, 3.82; S, 12.28.