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Synthesis and biological evaluation of novel 1,4-naphthoquinone derivatives as antibacterial and antiviral agents

Vishnu K. Tandon,[a,*] Dharmendra B. Yadav,[a] Ravindra V. Singh,[b] Meenu Vaish,[a] Ashok K. Chaturvedi[c] and Praveen K. Shukla[c]

[a]*Department of Chemistry, Lucknow University, Lucknow 226007, India*
[b]*GEITC, Corporation, Bangalore 37, India*
[c]*Division of Fermentation Technology, Central Drug Research Institute, Lucknow 226001, India*

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Abstract—A series of 1,4-naphthoquinone derivatives were synthesized and evaluated for antibacterial and antiviral activities. The structure–activity relationships of these compounds were also studied. The results suggest that compounds **9**–**22** showed in vitro marked antibacterial activity. Compounds **4c** and **7a** showed inhibitory effect against RNA dependent RNA polymerase induced poliovirus type 2 infected *HeLa cells*.

1,4-Naphthoquinone pharmacophore is known to impart pronounced biological effects in 1,4-naphthoquinone derivatives, leading to antitumor,[1,2] antiproliferative,[3] antimycobacterial,[4] antiplatelet, anti-inflammatory, antiallergic,[5] antimalarial,[6] and antileishmanial[7] activities. The incorporation of sulfur atom in 1,4-naphthoquinone derivatives has led to antifungal, antibacterial, antiviral, and anticancer activities.[8–12]

The interesting biological profile resulting due to the presence of a sulfur atom in 1,4-naphthoquinones prompted us to synthesize 1,4-naphthoquinone derivatives **2**–**24** possessing a sulfur atom in the side chain or inside the ring.

Antibacterial activities of 1,4-naphthoquinone derivatives **2**–**24** against various strains of the bacteria, for example, *Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were carried out according to the broth microdilution technique described by NCCLS.[13,14] The minimum inhibitory concentration (MIC) of each compound was determined against test isolates using this technique. The MICs of a standard antibacterial activity (Gentamycin) and of the compounds were determined in 96-well tissue culture plates using Muellor–Hinton broth.

Table 1 shows antibacterial activity of compounds **2**–**24**. The antibacterial activity was compared with that of Gentamycin, which was used as a positive control in all the tests with MIC values expressed in μg/mL. Compound **11** (Ar = *p*-anisyl) showed significant activity against *S. faecalis*, *K. pneumoniae*, and *E. coli*. Compound **12** (Ar = *o*-anisyl) showed marked activity against *E. coli* and *S. faecalis*. Compound **15** ($R^2 = CH_3$, Ar = phenyl) exhibited significant activity against *E. coli* and *S. faecalis*. Compounds **9** (Ar = phenyl) and **10** (Ar = *o*-tolyl) showed significant activity against *S. aureus*, whereas **7b**, **7e**, **11** (Ar = *p*-anisyl), **13** (Ar = *p*-nitrophenyl), **15** (Ar = phenyl), **16** (Ar = *p*-anisyl), and **21** (Ar = *p*-nitrophenyl) had moderate activity against *S. aureus*.

However, none of the compounds referred to in Table 1 showed activity against *P. aeruginosa* and on comparison of antibacterial activity of compounds **2**–**24** referred to in Table 1, it was observed that none of the compounds showed better activity than Gentamycin.

The study of structure–activity relationship in **9**–**22** revealed that replacement of R^3 by H enhances antibacterial activity, whereas substitution of R^3 by CH_3 leads to

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* Corresponding author. Tel.: +91 522 2322283; fax: +91 522 2326665; e-mail: vishnutandon@yahoo.co.in

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Table 1. Structure and in vitro antibacterial activity of compounds **2**, **5**, **7**, and **9**–**24**

Compound	R	R^1	R^2	R^3	Ar	MIC (μg/mL)				
						S. aureus	*S . faecalis*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*
2	b	b	b	b	b	>50	>50	>50	>50	>50
5a	H	H	b	b	b	>50	>50	>50	>50	>50
5b	OH	OH	b	b	b	>50	>50	>50	>50	>50
7b	H	H	H	CH_3	b	25	50	50	50	>50
7d	H	H	OH	CH_3	b	50	50	50	50	>50
7g	OH	OH	H	CH_3	b	>50	25	50	50	>50
7e	H	H	CH_3	CH_3	b	25	50	>50	50	>50
7h	OH	OH	H	H	b	>50	>50	>50	>50	>50
9	H	H	H	H	Ph	12.5	50	50	50	>50
10	H	H	H	H	*o*-Tolyl	12.5	50	>50	50	>50
11	H	H	H	H	*p*-Anisyl	25	6.25	6.25	12.5	50
12	H	H	H	H	*o*-Anisyl	>50	12.5	25	6.25	>50
13	H	H	H	H	*p*-Nitro-Ph	25	>50	>50	>50	>50
14	H	H	H	H	α-Naphthyl	>50	12.5	25	12.5	>50
15	H	H	H	CH_3	Ph	25	12.5	>50	6.25	>50
16	H	H	H	CH_3	*p*-Anisyl	25	>50	>50	50	>50
17	H	H	H	CH_3	*o*-Anisyl	>50	>50	12.5	>50	>50
18	H	H	H	CH_3	*o*-Tolyl	>50	>50	>50	>50	>50
19	H	H	H	CH_3	*p*-Tolyl	>50	>50	>50	>50	>50
20	H	H	H	CH_3	*m*-Tolyl	>50	>50	>50	>50	>50
21	H	H	H	CH_3	*p*-Nitro-Ph	25	50	>50	50	>50
22	H	H	H	CH_3	α-Naphthyl	>50	>50	>50	>50	50
24a	H	H	H	H	b	>50	>50	25	>50	>50
Gen.						0.78	a	0.39	a	0.78

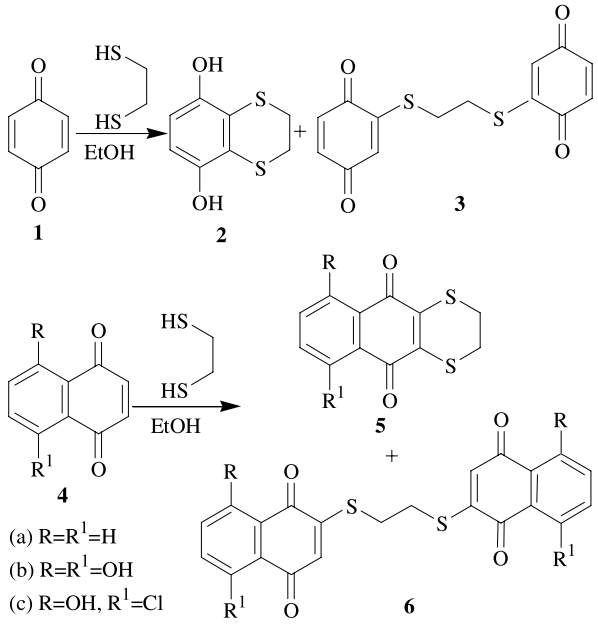
a, Activity not reported; b, not required; Ph, phenyl; Gen., Gentamycin.

a decrease in antibacterial activity. The introduction of OCH_3 group in the ring of Ar causes a marked increase in antibacterial activity.

The antiviral activity of compounds **4c** and **7a** against poliovirus type 2 infected *HeLa cells* is given in Table 2. The assay measures ^3H uridine triphosphate incorporation. Amongst compounds of the series only compounds **4c** and **7a** showed significant activity.

The synthesis of 1,4-naphthoquinono[2,5-*c*]-dithianes **5a**–**c** and 1,2-(bis-2-mercapto-1,4-naphthoquinonyl)-ethane and analogs **6a**–**c** was accomplished by condensation of 1,4-naphthoquinones with ethane 1,2-dithiol. Related analogs 1,4-dihydroxybenz[2,5-*c*]-dithiane **2** and 1,2-(bis-2-mercapto-1,4-benzoquinonyl)-ethane **3** were synthesized by a similar procedure using 1,4-benzoquinone **1** and ethane 1,2-dithiol according to Scheme 1.[15]

The synthesis of *S*-(1,4-naphthoquinon-2-yl)-mercaptoalkanoic acid amides **9**–**22** is exhibited in Scheme 2. Compounds **9**–**22** were synthesized by condensation of 1,4-naphthoquinone **4** with mercaptoalkanoic acids to yield *S*-(1,4-naphthoquinon-2-yl)-mercaptoalkanoic acids **7**, which were converted to amides **9**–**22** by reaction with $SOCl_2$ and aromatic amines.[16]



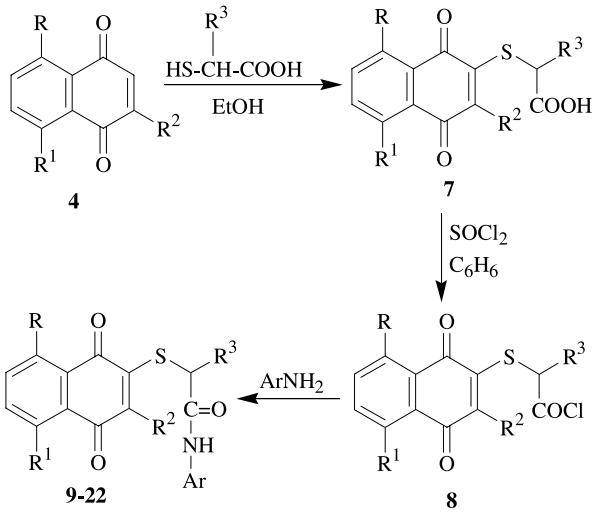
Scheme 1.

Table 2. In vitro antiviral activity of compounds against poliovirus type 2 infected *HeLa cells*

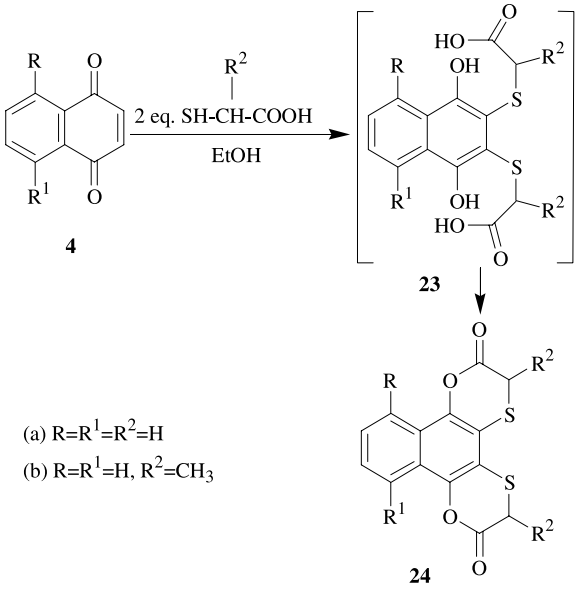
Compound	DPM	% control
Control	340 ± 25	25
4c	375 ± 15	55
7a	675 ± 71	—

The reaction of 1,4-naphthoquinone **4** with one equivalent thiolactic acid results in the formation of *S*-(1,4-naphthoquinon-2-yl)-mercaptolactic acids **7b**,**d**,**e**,**g**.[17]

Scheme 3 describes the reaction of 1,4-naphthoquinone **4a** with two equivalent amounts of thiolactic acid lead-



Scheme 2.



Scheme 3.

ing to the formation of tetracyclic 1,4-naphth[1,2]oxathiino[5,6-*c*]-8,11-dimethyl-1,4-oxathiin-7,12-dione **24b**. Analogous reaction of 1,4-naphthoquinones **4a** with two equivalent amounts of thioglycolic acid resulted in the formation of 1,4-naphth[1,2]oxathiino[5,6-*c*]-1,4-oxathiin-7,12-dione **24a** in low yield.[18] The precedence of cyclization of benzoquinone propionic acid derivative to a spirolactone in the presence of water, which is conformational and pH dependent, has already been accomplished by Borchardt and Cohen.[19]

In conclusion, we have synthesized a series of 1,4-naphthoquinone derivatives possessing sulfur atom in them and biological activities of these compounds have been carried out. Amongst the promising compounds **11** (Ar = *p*-anisyl) has shown in vitro significant antibacterial activity against *S. faecalis* and *K. pneumoniae*. Compounds **12** (Ar = *o*-anisyl) and **15** (Ar = phenyl, $R^3 = CH_3$) have shown in vitro antibacterial activity against *E. coli*. Compounds **4c** and **7a** have shown significant antiviral activity against *poliovirus* type 2 infected *HeLa cells*. Further work on these compounds is in progress.

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References and notes

1. Lee, E. J.; Lee, H. J.; Park, H. J.; Min, H. Y.; Suh, M. E.; Chung, H. J.; Lee, S. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5175.
2. Song, G. Y.; Kim, Y.; Zheng, X. G.; You, Y. J.; Cho, H.; Chung, J. H.; Sok, D. E.; Ahn, B. Z. *Eur. J. Med. Chem.* **2000**, *35*, 291.
3. Shchekoikhin, A. E.; Buyanov, V. N.; Preobrazhenskaya, M. N. *Bioorg. Med. Chem.* **2004**, *12*, 3923.
4. Tran, T.; Saheba, E.; Arcerio, A. V.; Chavez, V.; Li, Q. Y.; Martinez, L. E.; Primm, T. P. *Bioorg. Med. Chem.* **2004**, *12*, 4809.
5. Lien, J. C.; Huang, L. J.; Wang, J. P.; Teng, C. M.; Lee, K. H.; Kuo, S. C. *Bioorg. Med. Chem.* **1997**, *12*, 2111.
6. dos Santos, E. V. M.; Carneiro, J. W. D. M.; Ferreira, V. F. *Bioorg. Med. Chem.* **2004**, *12*, 87.
7. Valderramma, J. A.; Banites, J.; Cortes, M.; Mahana, H. P.; Prina, E.; Fournet, A. *Bioorg. Med. Chem.* **2003**, *11*, 4713.
8. Ruy, C. K.; Choi, K. U.; Shim, J. Y.; You, H. J.; Choi, I. H.; Chae, M. J. *Bioorg. Med. Chem.* **2003**, *11*, 4003.
9. Huang, S. T.; Kuo, H. S.; Hsiao, C. L.; Lin, Y. L. *Bioorg. Med. Chem.* **2002**, *10*, 1947.
10. Tandon, V. K.; Chhor, R. B.; Singh, R. V.; Rai, S.; Yadav, D. B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1079.
11. Tandon, V. K.; Singh, R. V.; Yadav, D. B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2901.
12. Mariso, J. W., Jr.; Goldman, L. *PCT Int. Appl.* **1975**, 5.
13. National Committee for Clinical Laboratory Standard, **1997**. Reference method for broth dilution antifungal susceptibility testing of yeast, approved standard. Document M27-A. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.
14. National Committee for Clinical Laboratory Standard, **1998**. Reference method for broth dilution antifungal susceptibility testing of conidium forming filamentous fungi: proposed standard. Document M38-P. National Committee for Clinical Laboratory Standard, Wayne, PA, USA.
15. General procedure for the preparation of 1,4-naphthoquinono[2,5-*c*]dithianes **5a**–**c** and 1,2-(bis-2-mercapto-1,4-naphthoquinonyl)-ethanes **6a**–**c**. Ethane 1,2-dithiol (1.13 g, 12 mmol) was added to a stirred solution of 1,4-naphthoquinone derivatives **4a**–**c** (10 mmol) in abs EtOH (25 mL). The reaction mixture was refluxed for 3 h and concentrated in vacuo. The mixture of compounds **5a**–**c** and **6a**–**c** thus obtained was separated by extraction with $CHCl_3$ (20 mL). **5a**–**c** were obtained from the mother liquor, while **6a**–**c** were obtained as insoluble residues in

$CHCl_3$. Compound **5a** was obtained as reddish brown crystals after crystallization with $CHCl_3$–hexane; yield 992 mg (40%); mp >250 °C; IR (KBr): 1645 (>C=O of quinone carbonyl) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.30 (s, 4H, $(CH_2)_2$), 7.76 (m, 2H, C_6–H and C_7–H), 8.06 (m, 2H, C_5–H and C_8–H); MS: (M^++2) 250, M^+ (*m/e*) 248. Anal. Calcd for $C_{12}H_8O_2S_2$ (248): C, 58.06; H, 3.22; S, 25.80. Found: C, 58.25; H, 3.08; S, 25.68. Compound **6a** was obtained as yellow crystals after crystallization with acetone; yield 1.21 g (30%); mp >280 °C; IR (KBr): 1640 (>C=O of quinone carbonyl) cm^{-1}; ^1H NMR ($CDCl_3$ + acetone d_6): δ 2.69 (s, 4H, $(CH_2)_2$), 7.0 (s, 2H, C_3–H), 7.64 (m, 4H, C_6–H and C_7–H), 8.06 (m, 4H, C_5–H, and C_8–H); MS: (M^++2) 408, M^+ (*m/e*) 406. Anal. Calcd for $C_{22}H_{14}O_4S_2$ (406): C, 65.02; H, 3.44; S, 15.76. Found: C, 65.34; H, 3.50; S, 15.92. 1,4-Dihydroxybenz[2,5-*c*]dithane **2** and 1,2-(bis-2-mercapto-1,4-benzoquinonyl)-ethane **3** were prepared by analogous procedure reported above for **5** and **6**.

16. General procedure for the preparation of *S*-(1,4-naphthoquinon-2-yl)-mercaptoalkanoic acid arylamides **9**–**22**. Thionyl chloride (1.42 g, 12 mmol) was added to a stirred solution of 1,4-naphthoquinone derivatives **7a**–**h** in benzene (25 mL). The reaction mixture was refluxed for 4 h and concentrated in vacuo. Compounds **8a**–**h** were obtained as viscous syrup in almost quantitative yield and were further used without purification. To a stirred solution of **8a**–**h** (10 mmol) in benzene (25 mL) was added $ArNH_2$ (12 mmol) in benzene (5 mL) dropwise and the mixture was stirred at room temperature for 3–5 h. Benzene was distilled off from the solution in vacuo. The solids thus obtained were filtered and crystallized from $CHCl_3$/MeOH. Compound **21** ($R^3 = CH_3$, Ar = *p*-nitrophenyl) was obtained in 72% yield; mp 148–150 °C; IR: 1697 (>C=O of amide), 1654 (>C=O of quinone carbonyl) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO d_6): δ 1.71 (d, J = 7.0 Hz, 3H, CH_3), 3.49 (q, J = 7.0 Hz, 1H, CH), 6.65 (s, 1H, NH), 6.81 (s, 1H, C_3–H), 7.26–8.23 (m, 8H, Ar–H); MS: M^+ (*m/e*) 382. Anal. Calcd for $C_{19}H_{14}N_2O_5S$ (382): C, 59.68; H, 3.66; N, 7.32; S, 8.37. Found: C, 60.02; H, 3.88; N, 7.44; S, 8.56.
17. General procedure for the preparation of *S*-(1,4-naphthoquinon-2-yl)-mercaptolactic acids **7b**,**d**,**e**,**g**. Thiolactic acid (1.27 g, 12 mmol) was added to a stirred solution of 1,4-naphthoquinone derivatives **4a**–**c** (10 mmol) in abs EtOH (20 mL). The reaction mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was dissolved in EtOAc and extracted with aqueous $NaHCO_3$ solution (10%, 20 mL). The bicarbonate extract was acidified with 3 N HCl (20 mL) at 0 °C. The solid products were filtered and crystallized with ethanol. Compound **7b** was obtained in 72% yield; mp 134–135 °C; IR (KBr): 1725 (>C=O of COOH), 1641 (>C=O of quinone carbonyl), 3431 (OH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.68 (d, J = 7.0 Hz, 3H, CH_3), 3.94 (q, J = 7.0 Hz, 1H, C–H), 5.94 (s, 1H, COOH), 6.89 (s, 1H, C_3–H), 7.75 (m, 2H, C_6–H and C_7–H), 8.09 (m, 2H, C_5–H and C_8–H); MS: M^+ (*m/e*) 262. Anal. Calcd for $C_{13}H_{10}O_4S$ (262): C, 59.54; H, 3.81; S, 12.21. Found: C, 59.80; H, 3.66; S, 12.34.
18. Procedure for the preparation of 1,4-naphth[1,2]oxanthiino[5,6-*c*]-8,11-dimethyl-1,4-oxathiin-7,12-dione **24b**. Thiolactic acid (2.12 g, 20 mmol) was added to a stirred solution of 1,4-naphthoquinone **4a** (1.58 g, 10 mmol) in abs EtOH (50 mL). The reaction mixture was stirred at room temperature for 1.5 h and the resulting brown colored solution was concentrated in vacuo to yield a sticky mass, which was crystallized with $CHCl_3$–hexane. Compound **24b** was obtained in 63% yield; mp 170 °C; IR (KBr): 1760 (>C=O of lactone ring) cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD): δ 1.46 and 1.58 (2d, J = 7.0 Hz, 6H, 8-CH_3 and 11-CH_3), 3.85 and 4.56 (2q, J = 7.0 Hz, 2H, C_8–H and C_{11}–H), 7.65 (m, 2H, C_2–H and C_3–H), 7.95 (m, 2H, C_1–H and C_4–H); MS: M^+ (*m/e*) 332. Anal. Calcd for $C_{16}H_{12}S_2O_4$ (332): C, 57.83; H, 3.61; S, 19.27. Found: C, 57.58; H, 3.88; S, 19.40.
19. Borchardt, R. T.; Cohen, L. A. *J. Am. Chem. Soc.* **1972**, *94*, 9175.