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Synthesis and biological evaluation of novel (L)-α-amino acid methyl ester, heteroalkyl, and aryl substituted 1,4-naphthoquinone derivatives as antifungal and antibacterial agents

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Abstract—A series of (*S*)-*N*-(1,4-naphthoquinon-2-yl)-α-amino acid methyl esters **3**–**9**, 2-*N*,*N*-dialkylamino-1,4-naphthoquinones **10**–**11** and 2-hydroxy-3-(2′-mercaptoimidazolyl)-1,4-naphthoquinones and their cyclic analogs **12**–**15** were synthesized and evaluated for antifungal and antibacterial activities. The structure–activity relationships of these compounds were studied and the results show that the compounds **9b** and **13c** exhibited in vitro antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Sporothrix schenckii*, whereas compound **6a** showed in vitro antibacterial activity against *Streptococcus faecalis*, *K. pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*.

1,4-Naphthoquinone structure is common in various natural products[1] and is associated with biological activities including enzyme inhibition and antifungal, antibacterial, anticancer, anti-proliferative, antiplatelet, anti-inflammatory, antiallergic, and antimalarial activities.[2–11] The biological activity imparted by 1,4-naphthoquinones in most cases relies upon their ability to accept one and/or two electrons to form radical anion or dianion species.[12] The presence of electron-donating or -attracting substituents in 1,4-naphthoquinones modulates the generation of radical anion and the redox property which is further responsible for compounds to catalytically cycle and generate oxidative radicals, such as hydrogen peroxide and superoxide which damage the cells.[13]

Many amino and heterocyclic 1,4-naphthoquinones have been used for the construction of numerous biologically important compounds.[14,15] The interesting biological profile resulting from the presence of heteroatom, sulfur or nitrogen, in 1,4-naphthoquinones prompted us to synthesize 1,4-naphthoquinone derivatives **3**–**15** possessing nitrogen and sulfur atoms at the 2 or 3 position in the side chain or inside the ring.

The evaluation of antifungal activities of **3**–**15** against various strains of pathogenic fungi, for example, *Candida albicans*, *Cryptococcus neoformans*, *Sporothrix schenckii*, *Trichophyton mentagraphytes*, *Aspergillus fumigatus*, and *Candida parapsilosis* (ATCC 22019), was carried out according to the broth microdilution technique described by NCCLS.[16,17] The minimum inhibitory concentration (MIC) of each compound was determined against test isolates using this technique.

The antifungal activity was compared with standard drugs miconazole, nystatin, fluconazole, and amphotericin B. MIC of these standard drugs are referred to in Table 1 and the compounds were determined in 96-well tissue culture plates using RPMI 1640 media buffered with MOPS (3-[*N*-morpholino]-propanesulfonic acid) (Sigma Chemical).

Comparison of activity of compounds **3**–**15** referred to in Table 1 with antifungal drug miconazole showed that compound **9b** had better activity against fungi *C. albicans* and had same antifungal profile against

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Table 1. Structures and in vitro antifungal activities for compounds **3**–**15** (MIC, μg/mL)

Compound	R	R^1	R^2	R^3	MIC (μg/mL)					
					C. albicans	*C. neoformans*	*S. schenckii*	*T. mentagraphytes*	*A. fumigatus*	*C. parapsilosis*
3a	H	H	H	b	50	50	50	>50	25	50
4b	H	OH	CH_3	b	>50	25	>50	>50	>50	50
6a	H	H	$CH_2CH(CH_3)_2$	b	>50	25	>50	>50	>50	50
9a	H	H	$(CH_2)_2SCH_3$	b	>50	25	>50	>50	>50	>50
9b	H	OH	$(CH_2)_2SCH_3$	b	12.5	12.5	25	12.5	12.5	25
11c	OH	H	b	*i*-Pr	>50	>50	>50	50	>50	a
13a	H	b	b	b	50	12.5	25	25	25	a
13c	OH	b	b	b	25	<12.5	<12.5	25	>50	a
15b	H	OH	b	b	>50	50	>50	>50	>50	>50
Mic.					25	12.5	a	<0.78	12.5	a
Nys.					7.8	3.5	13.2	a	a	a
Flu.					1.0	1.0	2.0	0.5	2.0	2.0
Amp.					0.39	0.78	a	1.56	a	a

a, activity not reported; b, not required; Mic., miconazole; Nys., nystatin; Flu., fluconazole; Amp., amphotericin B.

C. neoformans and *A. fumigatus*. Compounds **13a** and **13c** had the same activity against *C. neoformans* and *C. albicans*, respectively, when compared with miconazole. Compound **13c** also exhibited enhanced activity against fungi *S. schenckii* in comparison with antifungal drug nystatin. In addition to a promising antifungal profile of compound **9b** when compared with miconazole, it was also found to exhibit moderate activity against fungi *S. schenckii, T. mentagraphytes*, and *C. parapsilosis*. Other compounds whose MIC was >75 μg/mL are not reported in Table 1 since they were considered to be inactive compounds.

Antibacterial activities of compounds **3**–**15** against various strains of bacteria, for example, *Streptococcus faecalis*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, were carried out according to the broth microdilution technique described by NCCLS and the results are reported in Table 2. The MIC of each compound was determined against test isolates using this technique. The antibacterial activity was compared with those of standard antibacterial drugs, kanamycin, amikacin, tobramycin, and gentamycin, and its MIC value is expressed in μg/mL.

Compound **6a** showed marked antibacterial activity against *K. pneumoniae* and *E. coli* and in vitro showed better results than kanamycin against these two bacteria. Compound **6a** also exhibited better antibacterial activity than amikacin against *S. faecalis* and *S. aureus*, and tobramycin against *S. faecalis* bacteria. Compound **9a** exhibited better antibacterial activity against *S. faecalis* when compared with amikacin and tobramycin and was found to have better antibacterial profile than kanamycin against *K. pneumonia*. Compounds **9b**, **13a**, **13c**, and **15b** also showed better activity against *S. faecalis* when compared with amikacin and tobramycin. However, the compounds referred to in Table 2 did not exhibit better activity than gentamycin.

To the best of our knowledge the reaction of 1,4-naphthoquinones with enantiomerically pure D- or L-α-amino acids/esters has not been described before. The two possible modes of carrying out reaction of 1,4-naphthoquinones **1** with D- or L-α-amino acids/esters are one which involves 1,4-type of Michael addition of nucleophiles to quinone moiety of 1,4-naphthoquinones[18,19], and another the involves the nucleophilic displacement of that readily obtained 2-bromo-1,4-naphthoquinone derivatives **2**.[20] We have explored both the routes for

Table 2. Structures and in vitro antibacterial activities for compounds **3**–**15** (MIC, μg/mL)

Compound	R	R^1	R^2	R^3	MIC (μg/mL)				
					S. faecalis	*K. pneumoniae*	*E. coli*	*P. aeruginosa*	*S. aureus*
3b	H	OH	H	b	50	>50	50	50	50
4a	H	H	CH_3	b	>50	25	>50	>50	>50
6a	H	H	$CH_2CH(CH_3)_2$	b	12.5	6.25	12.5	>50	12.5
9a	H	H	$(CH_2)_2SCH_3$	b	12.5	6.25	>50	>50	>50
9b	H	OH	$(CH_2)_2SCH_3$	b	25	>50	>50	>50	>50
11b	H	OH	b	*i*-Pr	>50	>50	>50	50	>50
13a	H	b	b	b	25	>50	50	>50	50
13c	OH	b	b	b	25	>50	50	>50	50
15b	H	OH	b	b	25	>50	>50	>50	50
Kanamycin[c]					a	32	16	>128	2.0
Amikacin					>64	1.0	1.0	2.0	16.0
Tobramycin					32	1.0	0.5	4.0	0.25
Gentamycin					a	0.39	a	0.78	0.78

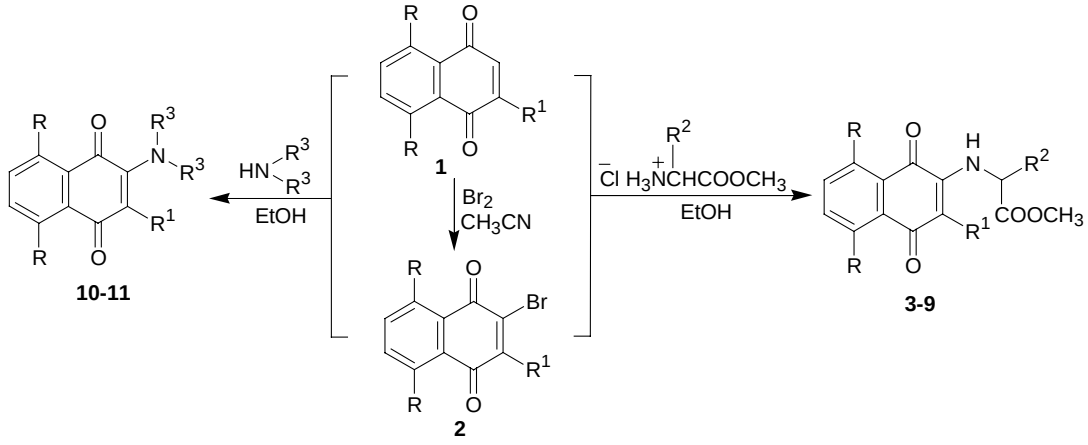
a, activity not reported; b, not required; c, MIC_{90} (MIC, μg/mL).

reaction of enantiomerically pure L-α-amino acids/esters, and other nitrogen and sulfur nucleophiles with 1,4-naphthoquinones **1** and their 2-bromo derivatives **2**.

The reaction of 1,4-naphthoquinones **1** and their bromo derivatives **2** with enantiomerically pure L-α-amino acid methyl ester hydrochlorides to give (*S*)-*N*-(1,4-naphthoquinon-2-yl)-α -amino acid methyl esters **3–9** [21] is shown in Scheme 1. The physical data including $[\alpha]_D$ of **4–9** are shown in Table 3. Since there is no question of inversion of configuration at chiral center, the absolute configuration of L-α-amino acid is reflected in compounds **4–9**. Analogous reaction of 1,4-naphthoquinones **1** and their bromo derivatives **2** with aliphatic secondary amines gave 2-*N*,*N*-dialkylamino-1,4-naphthoquinones **10–11**[21] (Scheme 1). Better yields of **3–11** were obtained by following a second route involving nucleophilic displacement of 2-bromo-1,4-naphthoquinones **2** with the above nitrogen nucleophiles.

In order to study the nucleophilic displacement reaction of 2-bromo-1,4-naphthoquinones **2** with sulfur nucleophiles, 2-mercaptoimidazole and 2-mercaptobenzimidazole seemed to be the reagents of choice.

The reaction of 2-mercaptobenzimidazole and 2-mercaptoimidazole with 2-bromo-1,4-naphthoquinones (**2a** and **2c**) afforded [(2,3-*d*)thiazolo-1′,3′-benzimidazolyl]-1,4-naphthoquinones (**12a** and **12c**) and [(2,3-*d*)thiazolo-1′,3′-imidazolyl]-1,4-naphthoquinones (**13a** and **13c**), respectively. However, the reaction of 2-mercaptobenzimidazole and 2-mercaptoimidazole with 2-hydroxy-3-bromo-1,4-naphthoquinone **2b** afforded 2-hydroxy-3-



(a) R=R^1=H; (b) R=H, R^1=OH; (c) R=OH, R^1=H

(3) R^2=H; (4) R^2=CH$_3$; (5) R^2=(CH$_2$)$_2$COOCH$_3$; (6) R^2=CH$_2$CH(CH$_3$)CH$_3$; (7) R^2=CH$_2$Ph

(8) R^2=CH(CH$_3$)CH$_2$CH$_3$; (9) R^2=(CH$_2$)$_2$SCH$_3$

(10) R^3=Et; (11) R^3=i-Pr

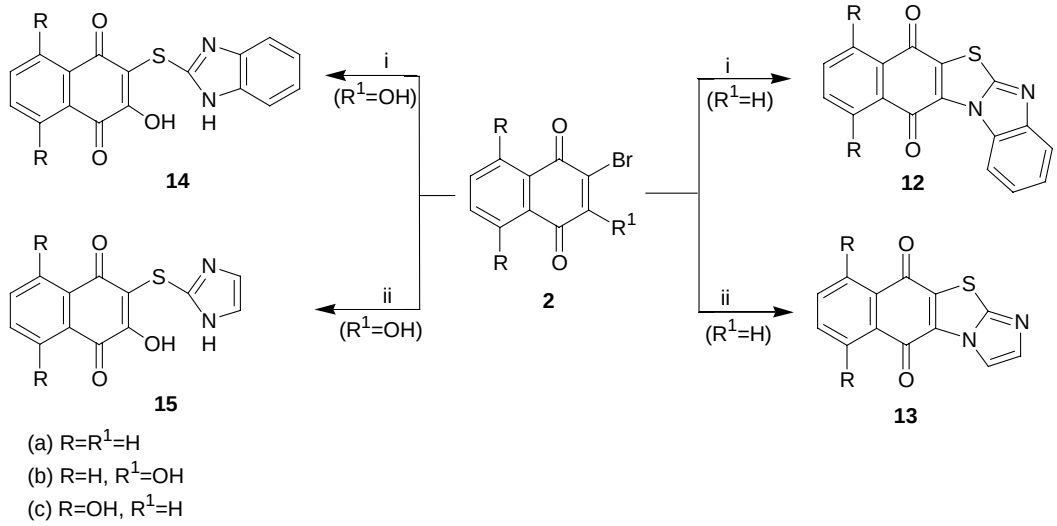
Scheme 1.

Table 3. Structures and optical rotations $[\alpha]_D$ for compounds **4–9** and L-α-amino acid methyl ester hydrochlorides

Compound	R	R^1	R^2	Melting point (°C)	$[\alpha]_D$	Temperature (°C)	Concentration	Solvent
4a	H	H	CH_3	188	+20°	29	0.0009	DMSO
4b	H	OH	CH_3	220	+9°	29	0.0036	DMSO
5a	H	H	$(CH_2)_2COOCH_3$	222	+14°	29	0.0015	DMSO
5b	H	OH	$(CH_2)_2COOCH_3$	230	+14°	29	0.0014	DMSO
6a	H	H	$CH_2CH(CH_3)_2$	122–124	+15°	29	0.0013	DMSO
6b	H	OH	$CH_2CH(CH_3)_2$	185	[b]	—	—	—
7a	H	H	CH_2Ph	152–155	+44°	29	0.0029	DMSO
7b	H	OH	CH_2Ph	150	+45°	29	0.0028	DMSO
8a	H	H	$CH(CH_3)C_2H_5$	>250	+28°	29	0.0008	DMSO
8b	H	OH	$CH(CH_3)C_2H_5$	240	[b]	—	—	—
9a	H	H	$(CH_2)_2SCH_3$	145	+28°	29	0.0007	DMSO
9b	H	OH	$(CH_2)_2SCH_3$	150–153	−5°	29	0.0023	DMSO
$CH_3CH(NH_3Cl)COOCH_3$	[a]	[a]	[a]	107–110	+6.5°	20	10	MeOH
$CH_3OOC(CH_2)_2CH(NH_3Cl)COOCH_3$	[a]	[a]	[a]	89–90	+26°	20	5	H_2O
$(CH_3)_2CHCH_2CH(NH_3Cl)COOCH_3$	[a]	[a]	[a]	150–151	+13°	20	2	H_2O
$PhCH_2CH(NH_3Cl)COOCH_3$	[a]	[a]	[a]	158–161	+37°	20	2	EtOH
$C_2H_5CH(CH_3)CH(NH_3Cl)COOCH_3$	[a]	[a]	[a]	98–100	+27°	20	2	H_2O
$CH_3S(CH_2)_2CH(NH_3Cl)COOCH_3$	[a]	[a]	[a]	151–153	+26°	20	5	H_2O

[a] Not required.

[b] The $[\alpha]_D$ could not be determined due to darkening of solution.



Scheme 2. Reagents and conditions: (i) 2-mercaptobenzimidazole, EtOH, 100 °C; (ii) 2-mercaptoimidazole, EtOH, 100 °C.

(2′-mercaptobenzimidazolyl)-1,4-naphthoquinone **14b** and 2-hydroxy-3-(2′-mercaptoimidazolyl)-1,4-naphthoquinone **15b**, respectively, as exhibited in Scheme 2.[22] The formation of **14b** and **15b** is attributed to the presence of hydroxyl group at 2-position in **2b** which hinders the formation of cyclized product.

In conclusion, we have synthesized a series of (*S*)-*N*-(1,4-naphthoquinon-2-yl)-α-amino acid methyl esters **3**–**9**, 2-*N*,*N*-dialkylamino-1,4-naphthoquinones **10**–**11**, and 2-hydroxy-3-(2′-mercaptobenzimidazolyl)-1,4-naphthoquinones **14**–**15** along with their cyclic analogs **12**–**13**. Amongst the promising compounds **9b** and **13c** have shown in vitro significant antifungal activity against *C. albicans, C. neoformans*, and *S. schenckii*, whereas compound **6a** exhibited marked antibacterial activity in vitro against *S. faecalis*, *K. pneumonia*, *E. coli*, and *S. aureus*. Thus, compounds **9b** and **6a** are lead compounds for antifungal and antibacterial activities, respectively. Further work on compounds **9b** and **6a** is in progress.

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21. General procedure for the synthesis of (*S*)-*N*-(1,4-naphthoquinon-2-yl)-α-amino acid methyl esters **3**–**9** and 2-*N*,*N*-dialkylamino-1,4-naphthoquinones **10**–**11**. Anhydrous K_2CO_3 (1.0 mmol) was added to a stirred reaction mixture of 2-bromo-1,4-naphthoquinone derivatives **2a**–**c** (10 mmol) and L-α-amino acids methyl ester hydrochlorides (10 mmol) in abs EtOH (50 mL). The reaction mixture was refluxed with stirring for 3–5 h at 100 °C. The resulting mixture was filtered and the filtrate was concentrated in vacuo. The residue was subjected to column chromatography on silica gel using EtOAc–hexane (1:10) and the product was crystallized with suitable solvent to give **3**–**9** in 60–85% yield. Compound **9b**; dark yellow crystals after crystallization with $CHCl_3$-hexane, 81% yield; mp 150–153 °C; IR (KBr): 1595 and 1680 (>C=O of quinone), 1745 (>C=O of $COOCH_3$), 3250–3480 (br h, NH and OH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.59 (m, 5H, CH,CH_2,CH_2), 2.68 (s, 3H, SCH_3), 3.93 (s, 3H, $COOCH_3$), 6.20 (s, 1H, NH), 7.74 (m, 2H, C_6-H and C_7-H), 8.10 (m, 2H, C_5-H and C_8-H), 10.5 (br h, 1H, OH). $[\alpha]_D^{29}$ −5° (*c* 0.0023, DMSO). Anal. Calcd for $C_{16}H_{17}O_5NS$ (335): C, 57.31; H, 5.07; N, 4.17; S, 9.55. Found: C, 57.54; H, 5.22; N, 4.33; S, 9.70. Analogous procedure was followed for the synthesis of **10**–**11**. Compound **11a**; light brown crystals after crystallization with abs EtOH; 72% yield; mp > 250 °C; IR (KBr): 1592 and 1680 (>C=O of quinone) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.21 (d, 12H, $CH_3 \times 4$), 3.28 (m, 2H, CH × 2), 5.93 (s, 1H, C_3-H), 7.72 (m, 2H, C_6-H and C_7-H), 8.23 (m, 2H, C_5-H and C_8-H); Anal. Calcd for $C_{16}H_{19}O_2N$ (257): C, 74.70; H, 7.39; N, 5.44. Found: C, 75.04; H, 7.62; N, 5.72.
22. General procedure for the synthesis of 2-hydroxy-3-(2′-mercaptoimidazolyl)-1,4-naphthoquinones and their cyclic analogs **12**–**15**. 2-Mercaptoimidazole (0.846 g; 12 mmol) in abs EtOH (10 mL) was added to a stirred solution of 2-bromo-1,4-naphthoquinone derivatives **2**(**a**–**c**) (10 mmol) in abs EtOH (40 mL) and the reaction mixture was refluxed for 5 h at 100 °C. The solid product obtained on cooling was filtered and crystallized with abs EtOH to yield **13** and **15**. Analogous procedure was followed for synthesis of **12** and **14** by using 2-mercaptobenzimidazole in place of 2-mercaptoimidazole. Compound **13a**; reddish brown crystals; 78% yield; mp 199–200 °C; IR (KBr): 1594 and 1668 (>C=O of quinone) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.98 (s, 1H, imidazolyl CH), 7.26 (s, 1H, imidazolyl CH), 7.77 (m, 2H, C_6-H and C_7-H), 8.09 (m, 2H, C_5-H and C_8-H). Anal. Calcd for $C_{13}H_6O_2N_2S$ (254): C, 61.41; H, 2.36; N, 11.02; S, 12.59. Found: C, 61.66; H, 2.52; N, 11.24; S, 12.72. Compound **15b**; yellowish brown crystals; 85% yield; mp 160 °C; IR (KBr): 1585 and 1642 (>C=O of quinone), 3178 (NH), 3400 (OH) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.08 (br s, 1H, NH), 7.12–8.10 (m, 6H, C_2-H and C_3-H of imidazolyl, C_5-H, C_6-H, C_7-H and C_8-H). Anal. Calcd for $C_{13}H_8O_3N_2S$ (272): C, 57.35; H, 2.94; N, 10.29; S, 11.76. Found: C, 57.66; H, 3.12; N, 10.50; S, 12.02.