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Synthesis of (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazoles and their (1,4)-naphthohydroquinone derivatives as antifungal, antibacterial, and anticancer agents

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Abstract—A series of (1,4)-naphthoquinono [3,2-*c*]-1*H*-pyrazoles and their (1,4)-naphthohydroquinone derivatives **2**–**7** were synthesized and evaluated for antifungal, antibacterial, and anticancer activities. The structure–activity relationship of these compounds was studied and the results show that the compound **2b** exhibited in vitro antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, and also possessed antibacterial profile against *Klebsiella pneumoniae* and *Escherichia coli* whereas **1c** showed anticancer activity against *Walker 256 Carcinosarcoma* in rats.

Heterocyclic 1,4-benzo- and 1,4-naphthoquinones containing nitrogen atom have been reported to possess cytotoxic,[1–5] antibacterial, antiproliferative,[6] antiplatelet, anti-inflammatory, antiallergic,[7,8] and antifungal[9] activities. The structure–activity relationship of heterocyclic quinones has revealed that the cytotoxic activity relies upon the number and position of nitrogen atom in the heterocyclic quinone.[2]

The interesting biological profile of heterocyclic 1,4-benzo- and 1,4-naphthoquinones containing one or more nitrogen atoms and the presence of carboxamido group[7,8] prompted us to synthesize (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazoles and their (1,4)-naphthohydroquinone derivatives **2**–**7** possessing two nitrogen atoms inside the ring and the carboxamido group in the side chain.

The evaluation of antifungal activities of **2**–**7** 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.[10,11] The minimum inhibitory concentration (MIC) of each compound was determined against test isolates using this technique.

The antifungal activity was compared with standard drugs like miconazole, nystatin, fluconazole, and amphotericin B. MIC of standard drugs is 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]-propane sulfonic acid) (Sigma Chemical Co.).

Comparison of the activity of compounds **2**–**7** referred to in Table 1 with that of the antifungal drug miconazole showed that compound **2b** had better activity against fungi *C. albicans* and *C. neoformans*. Compound **2b** also exhibited enhanced activity against fungi *C. albicans* when compared with the antifungal drug nystatin. Compounds **4a** and **4c** had some activity against *C. albicans* when compared with miconazole. Compound **2b** also exhibited moderate activity against fungi *T. mentagraphytes*, *A. fumigatus*, and *C. parapsilosis.* Other compounds whose MIC was >75 μg/mL are not reported in Table 1 as these were considered to be inactive compounds.

Antibacterial activities of compounds **2**–**7** against various strains of the bacteria, for example, *Staphylococcus*

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Table 1. In vitro antifungal activity for compounds **2**–**7**

Compds	MIC (μg/mL)					
	C. albicans	*C. neoformans*	*S. schenckii*	*T. mentagraphytes*	*A. fumigatus*	*C. parapsilosis*
2b	6.25	6.25	50	25	25	12.5
3b	>50	>50	>50	>50	>50	>50
4a	25	25	>50	>50	>50	50
4c	25	>50	>50	>50	>50	>50
6a	>50	>50	>50	>50	>50	>50
6b	>50	>50	>50	>50	>50	>50
7d	>50	>50	>50	>50	>50	50
7f	>50	>50	>50	>50	>50	>50
7g	>50	>50	>50	>50	>50	>50
7h	>50	>50	>50	>50	>50	>50
Miconazole	25	12.5	[a]	<0.78	12.5	[a]
Nystatin	7.8	3.5	13.2	[a]	[a]	[a]
Fluconazole	1.0	1.0	2.0	0.5	2.0	2.0
Amphotericin B	0.39	0.78	[a]	1.56	[a]	[a]

[a] Activity not reported.

Table 2. In vitro antibacterial activity for compounds **2**–**7**

Compds	MIC (μg/mL)				
	S. faecalis	*K. pneumoniae*	*E. coli*	*P. aeruginosa*	*S. aureus*
2b	25	6.25	6.25	>50	25
3b	12.5	12.5	25	>50	25
4a	>50	>50	>50	>50	>50
4c	>50	>50	>50	>50	>50
6a	>50	>50	>50	>50	>50
6b	25	12.5	>50	>50	>50
7d	>50	25	>50	>50	>50
7f	>50	12.5	12.5	>50	>50
7g	>50	25	25	>50	>50
7h	>50	>50	>50	>50	>50
Gentamycin	[a]	0.39	[a]	0.78	0.78
Kanamycin[b]	[a]	32	16	>128	2.0

[a] Activity not reported.
[b] MIC_{90} (μg/mL).

aureus, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* were carried out according to the broth microdilution technique described by NCCLS. The results are reported in Table 2. The MIC of each compound was determined against test isolates using this technique. Gentamycin the standard antibacterial drug was used as a positive control in all the tests and its MIC value is expressed in micrograms per milliliter. The antibacterial activity was also compared with kanamycin[12] (MIC_{90} 75 μg/mL).

Compound **2b** showed marked activity against *K. pneumoniae* and *E. coli* which in vitro showed better results than kanamycin against these two bacteria. Compounds **3b**, **6b**, **7f**, and **7g** exhibited moderate activity against *K. pneumoniae* and *E. coli.* Compounds **2b**, **3b**, and **6b** exhibited moderate activity against *S. faecalis* whereas **2b** and **3b** showed activity against *S. aureus*. However, the compound referred to in Table 2 and discussed above did not exhibit better activity than the standard drug gentamycin.

The anticancer activity was carried out against *Walker 256 Carcinosarcoma* in rats. The anticancer activities of **1c** and **3a** are shown in Table 3. However, none of the compounds tested showed better activity than the marked activity exhibited by naphthazarin **(1c)**.[13]

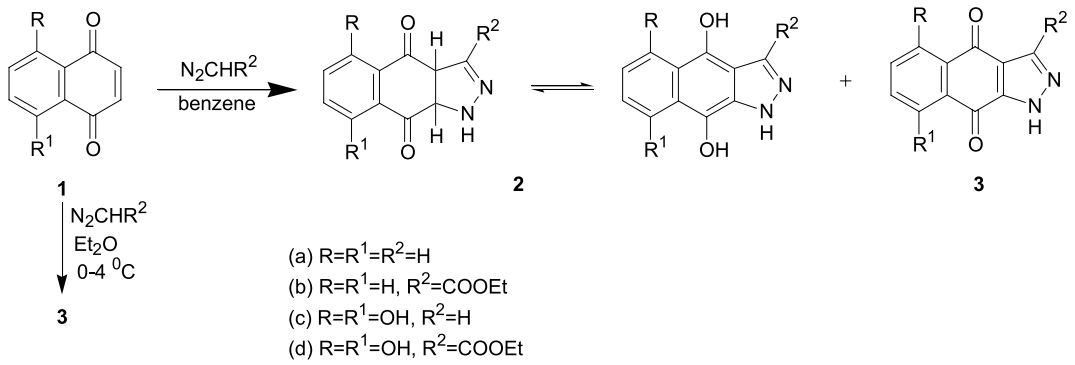
Table 3. Anticancer activity against *Walker 256 Carcinosarcoma* in rats

Compds	Dose (mg/kg)	Survivors	T/C (%)	Remark
1c	10	3/4	37	Active
3a	50	3/3	115	Inactive

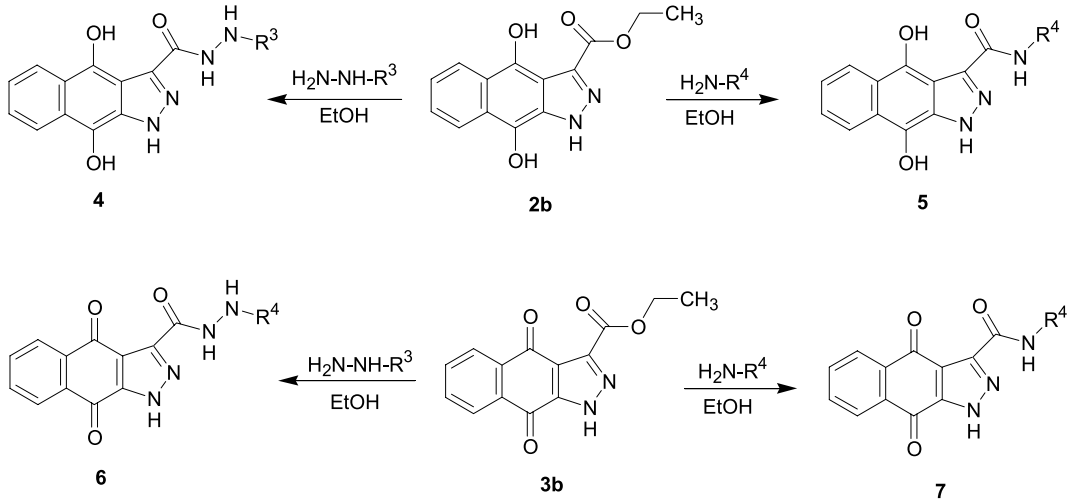
The synthesis of (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazoles and their (1,4)-naphthohydroquinone derivatives **2** and **3** is shown in Scheme 1. Compounds **2** and **3** were synthesized as a mixture of compounds by condensation of 1,4-naphthoquinones **1** with diazomethane and its ethyl ester using benzene as solvent.

Compound **3** was exclusively obtained as a single product by reaction of 1,4-naphthoquinones **1** with diazomethane and its ethyl ester by carrying out reaction in diethyl ether at 0 °C.[14]

Reaction of pyrazoles **2b** and **3b** with substituted hydrazines and primary amines results in the formation of (1,4)-naphthohydroquinono-[3,2-*c*]-1*H*-pyrazole-3-car-



Scheme 1.



Scheme 2.

boxylic acid hydrazides **4a**–**c**, (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazole-3-carboxylic acid hydrazides **6a**–**c**, (1,4)-naphthohydroquinono-[3,2-*c*]-1*H*-pyrazole-3-carboxylic acid amides **5d**–**h**, and (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazole-3-carboxylic acid amides **7d**–**h** as shown in Scheme 2.[15,16]

In conclusion, we have synthesized a series of (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazoles and their (1,4)-naphthohydroquinono derivatives and carried out their biological activities. Among the promising compounds, **2b** has shown in vitro significant antifungal activity against *C. albicans* and *C. neoformans*. Compound **2b** also exhibited marked antibacterial activity against *K. pneumoniae* and *E. coli.* Compounds **3b**, **6b**, **7f**, and **7g** also showed some antibacterial activity. Compound **2b** is the lead compound for both antifungal and antibacterial activities. Among the promising compounds, only **1c** has shown significant activity against *Walker 256 Carcinosarcoma* in rats. Further work on compound **2b** is in progress.

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14. General procedure for the preparation of (1,4)-naphthohydroquinono-[3,2-*c*]-1*H*-pyrazoles **2a**–**d** and (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazoles **3a**–**d**. Ethyldiazoacetate/diazomethane (10 mmol) in benzene (5 mL) was added to a stirred solution of 1,4-naphthoquinone derivatives **1a**–**d** (10 mmol) in benzene (20 mL). The reaction mixture was refluxed with stirring for 1.5 h and allowed to stand overnight at room temperature. To the dark red semi-solid solution was added $CHCl_3$ (20 mL). The solid thus obtained was filtered. The residue consisted of **2a**–**d** whereas filtrate on concentration in vacuo yielded **3a**–**d**. The reaction of ethyldiazoacetate/diazomethane (10 mmol) in diethyl ether (30 mL) with 1,4-naphthoquinones **1a**–**d** (10 mmol) in diethyl ether (50 mL) at 0–4 °C for 48 h resulted in the formation of only **3a**–**d** as the isolated product. Compound **2b**: colorless solid after crystallization with benzene, 48% yield; mp 145 °C; IR (KBr): 3200–3406 (NH), 1725 (>C=O of COOEt) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.54 (t, 3H, CH_3), 4.54 (q, 2H, CH_2), 6.68 (s, 1H, OH), 7.01 (s, 1H, OH), 7.42–7.82 (m, 2H, C_6–H and C_7–H), 8.04–8.14 (m, 2H, C_5–H and C_8–H), 8.87 (s, 1H, NH); MS: M^+ (*m*/*e*) 272. Compound **3b**: Mother liquor on crystallization yielded **3b** as yellow crystals; 47% yield; mp 184–185 °C; IR (KBr): 3181 (NH), 1591 and 1682 (>C=O of quinone), 1747 (>C=O of COOEt) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.50 (t, 3H, CH_3), 1.79 (br h, 1H, NH), 4.56 (q, 2H, CH_2), 7.84 (m, 2H, C_6–H and C_7–H), 8.29 (m, 2H, C_5–H and C_8–H); MS: M^+ (*m*/*e*) 270. Compound **3b** obtained by carrying out reaction in diethyl ether as a solvent had similar spectroscopic data.
15. General procedure for the preparation of (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazole-3-carboxylic acid hydrazides **6a**–**c**. Hydrazine derivatives (20 mmol) were added to a stirred solution of 1,4-naphthoquinone derivative **3b** (2.70 g, 10 mmol) in abs. EtOH (50 mL) at room temperature. The reaction mixture was allowed to stir at rt for 2–5 h. The reddish brown solid thus obtained was filtered and crystallized from CH_3OH. Compound **6b**: 94% yield; mp >280 °C; IR (KBr): 3416 (NH), 1599 and 1686 (>C=O of quinone), 1638 (>C=O of amide) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.55 (s, 1H, NH), 3.48 (br h, 2H, NH × 2), 7.26 (s, 5H, C_6H_5), 7.83 (m, 2H, C_6–H and C_7–H), 8.20 (m, 2H, C_5–H and C_8–H); MS: M^+ (*m*/*e*) 332. Analogous procedure was followed for the synthesis of **4a**–**c**.
16. General procedure for the preparation of (1,4)-naphthoquinono-[3,2-*c*]-1*H*-pyrazole-3-carboxylic acid amides **7d**–**h**. Primary aliphatic amines (100 mmol) were added to a stirred solution of 1,4-naphthoquinone derivative **3b** (2.70 g, 10 mmol) in abs EtOH (50 mL). The reaction mixture was refluxed for 1–5 h. The yellow solid thus obtained was filtered and crystallized from CH_3OH. Compound **7d**: 75% yield; mp >260 °C; IR (KBr): 3433 (NH), 1588 and 1658 (>C=O of quinone), 1634 (>C=O of amide) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.58 (s, 3H, CH_3), 7.66 (s, 1H, NH), 7.81 (m, 2H, C_6–H and C_7–H), 8.24 (m, 2H, C_5–H and C_8–H); MS: M^+ (*m*/*e*) 245. Analogous procedure was followed for the synthesis of **5a**–**c**.