Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 6120–6126

Design, synthesis, and biological evaluation of 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones and related compounds as antifungal and antibacterial agents

Vishnu K. Tandon,[a,*] Dharmendra B. Yadav,[a] Hardesh K. Maurya,[a] Ashok K. Chaturvedi[b] and Praveen K. Shukla[b]

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

Received 28 March 2006; revised 15 April 2006; accepted 17 April 2006
Available online 27 June 2006

Abstract—A series of (*S*)-*N*-(3-chloro-1,4-naphthoquinon-2-yl)-α-amino acid ethyl esters **3** and 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones **6**–**23** were synthesized and evaluated for antifungal and antibacterial activities. The structure–activity relationship of these compounds was studied and the results show that the compounds **3a** and **3b** exhibited in vitro antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Sporothrix schenckii* whereas compounds **12** and **22** showed in vitro antibacterial activity against *Klebsiella pneumoniae* and *Escherichia coli*.

1. Introduction

Heterocyclic quinones, containing nitrogen atom, are known to possess antibacterial,[1,2] antifungal,[3–6] and cytotoxic activities.[7–10] The clinical significance of this class of compounds has stimulated the synthesis of new lead compounds retaining the 'core' quinone chromophore.[11–14]

In the course of a medicinal chemistry program aimed at discovering new heterocyclic quinones endowed with antibacterial and antifungal activities, we have synthesized a series of 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones **6**–**23** possessing two nitrogen atoms in the heterocyclic ring and their precursors *N*-[3-chloro-1,4-naphthoquinon-2-yl]-α-amino acid alkyl esters **3**. The presence of aryl, alkyl group or nitrogen atom was an important factor to affect antifungal and antibacterial activities.

The antifungal screening of compounds **3**–**23** 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 broth microdilution technique described by NCCLS.[15,16] The minimum inhibitory concentration (MIC) of compounds **3**-**23** was determined against test isolates using this technique.

The antifungal activity was compared with those of standard antifungal drugs Miconazole, Nystatin, Fluconazole and Amphotericin-B.[11,12,14] MIC of compounds and standard drugs referred in Table 1 were determined in 96-well tissue culture plates using RPMI 1640 media buffered with MOPS (3-[*N*-morpholino]-propane sulfonic acid, obtained from Sigma Chemical Co.).

Comparison of antifungal activity of compounds **3**–**23** referred in Table 1 with that of antifungal drug Miconazole showed that both compounds **3a** and **3b** had better activity against fungi *C. albicans* and *C. neoformans* and had same antifungal profile against *A. fumigatus*, while compounds **11** and **13** had same activity against *C. neoformans*. Likewise **11** and **20** had similar antifungal profile against *C. albicans* when compared with Miconazole. Compound **20** also had better antifungal activity against *C. albicans* on comparison with Miconazole. On comparison of antifungal activity with that of antifungal drug Nystatin, compounds **3a**

Keywords: Antifungal; Antibacterial; Quinoxaline-5,10-diones; Heterocyclic quinones; 1,4-Naphthoquinone derivatives.
* Corresponding author. Fax: +91 522 2684388; e-mail: vishnutandon@yahoo.co.in

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.04.029

Table 1. Structures and in vitro antifungal activity for compounds **3**–**23** (MIC: μg/mL)

Compound	R	R^1	R^2/Ar	MIC (μg/mL)					
				C. albicans	*C. neoformans*	*S. schenckii*	*T. mentagraphytes*	*A. fumigatus*	*C. parapsilosis*
3a	C_2H_5	H	[b]	12.5	6.25	6.25	6.25	12.5	25
3b	C_2H_5	CH_3	[b]	6.25	6.25	6.25	6.25	12.5	25
6	[b]	H	CH_2CH_2OH	>50	50	>50	50	>50	>50
7	[b]	CH_3	CH_2CH_2OH	>50	>50	50	50	50	>50
11	[b]	CH_3	$CH(CH_3)_2$	25	12.5	25	25	25	50
12	[b]	H	$C(CH_3)_3$	>50	>50	>50	>50	>50	>50
13	[b]	CH_3	$C(CH_3)_3$	50	12.5	>50	25	>50	>50
20	[b]	H	2-OMe–C_6H_4	25	6.25	25	12.5	50	50
22	[b]	H	4-OH–C_6H_4	>50	>50	>50	>50	>50	>50
23	[b]	CH_3	4-OH–C_6H_4	>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]

Mic., Miconazole; Nys., Nystatin; Flu., Fluconazole; and Amp., Amphotericin-B.
[a] Activity not reported.
[b] Not required.

and **3b** showed better antifungal profile against fungi *S. schenckii*. Compound **3b** also exhibited better antifungal activity against *C. albicans* when compared with Nystatin. Other compounds whose MIC was >75 μg/mL are not reported in Table 1. However, none of the compounds referred in Table 1 showed better activity than Fluconazole and Amphotericin-B.

Structure–activity relationship in **3**–**23** showed that cyclic 1-*tert*-butyl-2-hydroxy-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione **12** exhibited better antifungal activity compared to acyclic *N*-(3-chloro-1,4-naphthoquinon-2-yl)glycine ethyl ester **3a** and (*S*)(+)-*N*-(3-chloro-1,4-naphthoquinon-2-yl)alanine ethyl ester **3b**. The replacement of R^2 by $C(CH_3)_3$ in **12** had considerable effect in enhancement of antifungal activity.

The evaluation of antibacterial activity of compounds **3**–**23** against various strains of pathogenic bacteria, for example, *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* was carried out according to the broth microdilution technique described by NCCLS[15,16] and the results are reported in Table 2. The minimum inhibitory concentration (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 micrograms per milliliter.[17]

Comparison of antibacterial activity of compounds **3**–**23** referred in Table 2 with that of antibacterial drug Kanamycin showed that all the compounds except for **7** showed better activity against *K. pneumoniae*. Both compounds **12** and **22** exhibited better activity against *E. coli* when compared with Kanamycin. On comparison of antibacterial activity with those of antibacterial drugs Tobramycin and Amikacin,[18] compounds **3a**,**b**, **11**–**13**, **22**, and **23** were found to exhibit better activity against

Table 2. Structures and in vitro antibacterial activity for compounds **3**–**23** (MIC: μg/mL)

Compound	R	R^1	R^2/Ar	MIC (μg/mL)				
				S. faecalis	*K. pneumoniae*	*E. coli*	*P. aeruginosa*	*S. aureus*
3a	C_2H_5	H	[b]	25	25	25	50	25
3b	C_2H_5	CH_3	[b]	12.5	12.5	25	25	25
6	[b]	H	CH_2CH_2OH	50	12.5	>50	>50	25
7	[b]	CH_3	CH_2CH_2OH	>50	50	50	>50	50
11	[b]	CH_3	$CH(CH_3)_2$	25	12.5	25	25	25
12	[b]	H	$C(CH_3)_3$	25	6.25	12.5	12.5	12.5
13	[b]	CH_3	$C(CH_3)_3$	25	25	25	50	12.5
20	[b]	H	2-OMe–C_6H_4	>50	12.5	>50	50	50
22	[b]	H	4-OH–C_6H_4	25	12.5	12.5	12.5	12.5
23	[b]	CH_3	4-OH–C_6H_4	25	12.5	>50	>50	25
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}: μg/mL.[17]

S. faecalis. However, none of the compounds referred in Table 2 exhibited better activity than Gentamycin.

Structure–activity relationship in **3**–**23** showed that acyclic *N*-(3-chloro-1,4-naphthoquinon-2-yl)-α-amino acid ethyl esters **3** showed better antibacterial activity than cyclic 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones **6**–**23**. In compound **3** replacement of R^1 by CH_3 enhanced antibacterial activity to some extent against *C. albicans*. In tricyclic compounds **6**–**23**, compound **20** exhibited better antibacterial activity compared to other derivatives of the series by replacement of R^1 by 2-methoxyphenyl group.

Comparison of antibacterial activity of compounds **3**–**23** referred in Table 2 with that of antibacterial drug Kanamycin showed that all the compounds except for **7** showed better activity against *K. pneumoniae*. Both compounds **12** and **22** exhibited better activity against *E. coli* when compared with Kanamycin. On comparison of antibacterial activity with those of antibacterial drugs Tobramycin and Amikacin,[18] compounds **3a**,**b**, **11**–**13**, **22**, and **23** were found to exhibit better activity against *S. faecalis*. However, none of the compounds referred in Table 2 exhibited better activity than Gentamycin.

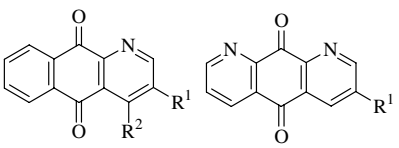
Azaanthroquinones are new class of biologically active 1,4-quinone derivatives and have been found to possess promising biological activities.[18–20] Both mono- and diazaanthroquinones **I** and **II** (Fig. 1) have been synthesized recently and it was found that diazaanthroquinone derivatives were more active than monoazaanthroquinone derivatives.

2. Results and discussion

2.1. Synthetic chemistry

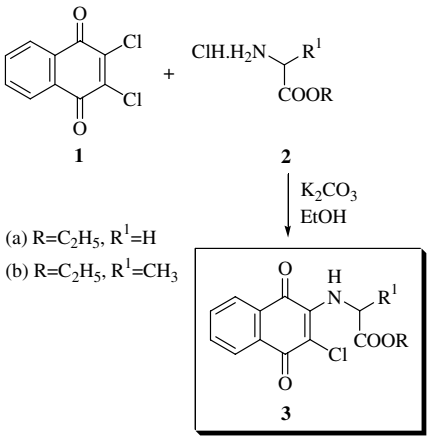
In the program for further extension of our work on the reaction of 2,3-dichloro-1,4-naphthoquinones with chiral enantiomerically pure naturally occurring L-α-amino acid.[14] we have developed a new route to synthesis of 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones **6**–**23** having both nitrogen atoms at 1,4 position in the same ring of diazaanthroquinone nucleus.

The reaction of 2,3-dichloro-1,4-naphthoquinone **1** with enantiomerically pure L-α-amino acid ethyl ester hydrochlorides **2** to give *N*-[3-chloro-1,4-naphthoquinon-2-yl]-α-amino acid ethyl ester **3** is shown in Scheme 1. This reaction involves nucleophilic displacement of Cl atom in 2,3-dichloro-1,4-naphthoquinone **1** with nitrogen



I **II**

Figure 1.



1 **2** **3**

Scheme 1.

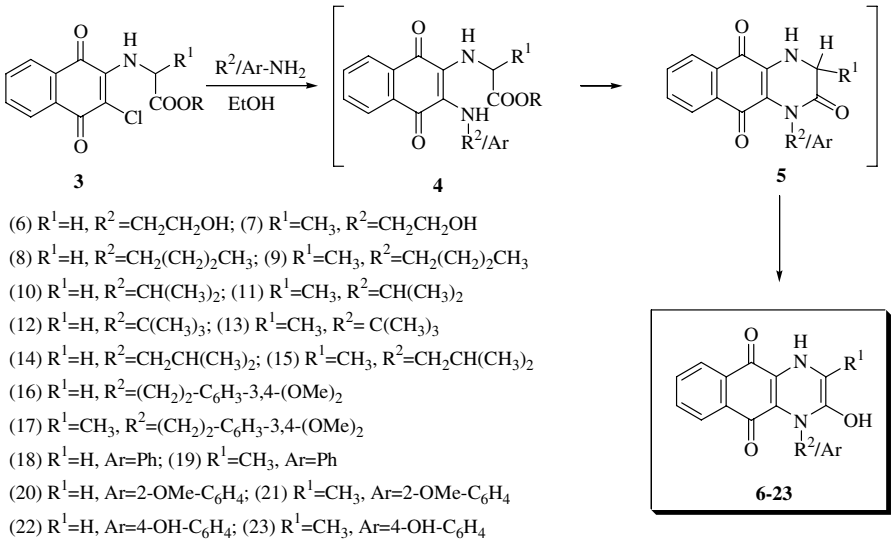
nucleophile. The absolute configuration of L-α-amino acids is reflected in compounds **3** as the question of inversion of configuration at chiral center in **3** does not arise.

Further reaction of **3** with primary aliphatic and aromatic amines resulted in the formation of 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones **6**–**23** as exhibited in Scheme 2. The mechanism of formation of **6**–**23** from **3** is shown in Figure 2 and involves nucleophilic displacement followed by intramolecular nucleophilic addition–elimination and cyclization leading to intermediates **4** and **5**.

Resin-bound solid phase synthesis of 2-amino-3-chloro-5- and 8-nitro-1,4-naphthoquinones has recently been reported by Blackburn.[21] In addition, Kartoflitskaya et al.[22] have used aspartic acid for nucleophilic substitution reaction with 2,3-dichloro-1,4-naphthoquinones. However, none of these two authors have employed α-amino acids used by us for nucleophile substitution reaction with 2,3-dichloro-1,4-naphthquinones.

2.2. In vitro antifungal and antibacterial activity evaluation by MIC assay

The prepared *N*-(3-chloro-1,4-naphthoquinon-2-yl)-α-amino acid ethyl esters **3a**,**b** and 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones **6**–**23** derivatives were evaluated for their in vitro antifungal activity against *C. albicans*, *C. neoformans*, *S. schenckii*, *T. mentagraphytes*, *A. fumigatus* and *C. parapsilosis* (ATCC 22019) and antibacterial activity against *S. faecalis*, *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *S. aureus* at the Division of Fermentation Technology of Central Drug Research Institute, Lucknow, India. In this process, minimum inhibitory concentration of compounds **3**–**23** was tested according to standard microbroth dilution as per NCCLS[15,16] protocol. Briefly, testing was performed in flat-bottomed 96 well tissue culture plates (CELLSTAR® Greiner bio-one GmbH, Germany) in RPMI 1640 medium buffered with MOPS (3-[*N*-morpholino]propane sulfonic acid) (Sigma chem. Co. MO, USA) for fungal strains and in Muller Hinton broth (Titan Biotech Ltd, India) for bacterial strains.



Scheme 2.

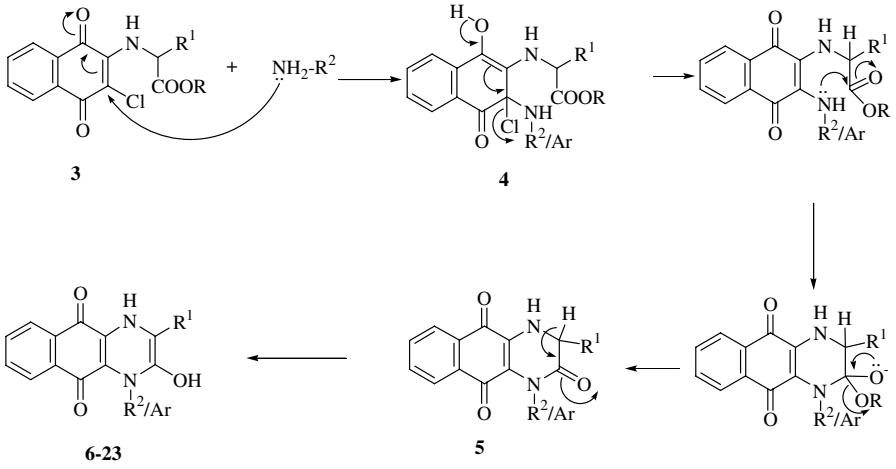


Figure 2.

The concentration range of tested compounds was 50–0.36 μg/mL for standard compounds. Initial inocula of fungal and bacterial strain were maintained at $1–5 \times 10^3$ cells/mL. These plates were incubated in a moist chamber at 35 °C and absorbance at 492 nm was recorded on Versa Max microplate reader (Molecular devices, Sunnyvale, USA) after 48 h for *C. albicans* and *C. parapsilosis*, 72 h for *A. fumigatus*, *S. schenckii*, and *C. neoformans* and 96 h for *T. mentagraphytes* while bacterial strains were incubated for 24 h. MIC was determined as 90% inhibition of growth with respect to the growth control was observed by using SOFTmax Pro 4.3 Software (Molecular Devices, Sunnyvale, USA).

3. Conclusion

In conclusion, we have synthesized a series of *N*-[3-chloro-1,4-naphthoquinon-2-yl]-α-amino acid ethyl esters **3** and 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones **6**–**23**. Amongst the promising compounds **3a** and **3b** have shown in vitro significant antifungal activity against *C. albicans*, *C. neoformans*, and *S. schenckii*. Whereas compound **12** and **22** exhibited marked antibacterial activity in vitro against *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. Thus compounds **3a** and **3b** are lead compounds for antifungal activity and **12** and **22** are the lead compounds for antibacterial activity. Further work on compounds **3a**,**b**, **12**, and **22** is in progress.

4. Experimental

4.1. Materials and methods

The reagents and the solvents used in this study were of analytical grade and were used without further purification. The melting points were determined on an electrically heated Townson Mercer melting point apparatus and are uncorrected. IR spectra were recorded on Beckman Aculab-1G, Perkin-Elmer 881 and FTIR

8201 PC, Schimadzu Spectrophotometers either on KBr discs or in neat. Nuclear magnetic resonance (NMR) spectra were recorded on Perkin-Elmer model R.32 spectrometers using TMS as an internal reference. Microanalyses were carried out on Carlo Erba-1108 instrument and all compounds showed satisfactory elemental analysis for C, H, and N. Progress of reactions and purity of compounds were monitored by thin-layer chromatography (TLC), which was performed on silica gel G and compounds were detected with iodine vapors, where required. Spectra facilities and elemental microanalyses were carried out by SAIF division of Central Drug Research Institute, Lucknow, India. Most reagents were purchased from Lancaster and Merck.

4.2. General procedure of *N*-(3-chloro-1,4-naphthoquinon-2-yl)-α-amino acid ethyl esters (3a,b)

Anhydrous K_2CO_3 (0.001 mol) was added to a stirred reaction mixture of 2,3-dichloro-1,4-naphthoquinone **5** (2.27 g, 0.01 mol) and α-amino acid ethyl ester hydrochlorides (0.01 mol) in abs EtOH (50 mL). The reaction mixture was refluxed for 3–5 h at 110 °C and filtered while hot. The filtrate was concentrated in vacuo until 3/4th of the solvent was distilled off. The resulting liquid was allowed to crystallize at room temperature to give solid, which was further crystallized with a suitable solvent.

4.2.1. *N*-3-(Chloro-1,4-naphthoquinon-2-yl)glycine ethyl ester (3a). The general procedure was followed for 5 h to give scarlet red crystals on crystallization with EtOH; 90% yield; mp: 95 °C; IR (KBr): 578, 679, 714, 785, 844, 884, 953, 1020, 1105, 1160, 1216, 1256, 1279, 1324, 1391, 1437, 1476, 1570, 1594, 1639, 1675, 1735, 3253 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31 (t, 3H, CH_3), 4.28 (q, 2H, OCH_2), 4.61 (s, 2H, CH_2), 6.50 (br h, 1H, NH), 7.72 (m, 2H, C_6–H and C_7–H), 8.11 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{14}H_{12}ClNO_4$): C, 57.25; H, 4.12; N, 4.77 Found: C, 57.05; H, 4.03; N, 4.65. Beilstein test:[23] Cl positive.

4.2.2. (*S*)(+)-*N*-3-(Chloro-1,4-naphthoquinon-2-yl)alanine ethyl ester (3b). The general procedure was followed for 3 h to give scarlet red crystals on crystallization with EtOH; 96% yield; mp: 118–120 °C; IR (KBr): 549, 606, 677, 726, 789, 830, 943, 1028, 1062, 1142, 1181, 1246, 1303, 1341, 1382, 1454, 1521, 1568, 1600, 1684, 1731, 2363, 2986, 3288, 3395 cm^{-1}; ^1H NMR ($CDCI_3$): δ 1.32 (t, 3H, CH_3), 2.72 (dd, 3H, CH_3), 4.13 (q, 2H, OCH_2), 4.24 (q, 1H, CH), 6.50 (br h, 1H, NH), 7.26 (m, 2H, C_6–H and C_7–H) 8.12 (m, 2H, C_5–H and C_8–H), $[\alpha]_D^{29}$ +6.25 (*c* 0.0026, DMSO). Anal. Calcd ($C_{15}H_{14}ClNO_4$): C, 58.55; H, 4.59; N, 4.55. Found: C, 58.66; H, 4.48; N, 4.70. Beilstein test:[23] Cl positive.

4.3. General procedure of 1,2,3-trisubstituted-1,4-dihydrobenzo[*g*]quinoxaline-5,10-diones (6–23)

Primary aliphatic and aromatic amines (0.05 mol) were added to a stirred solution of *N*-3-(chloro-1,4-naphthoquinon-2-yl)-α-amino acid ethyl esters **3** (0.01 mol) in abs EtOH (100 mL). The reaction mixture was refluxed for 05–15 h at 100 °C. The resulting solution was concentrated in vacuo and the oil thus obtained was subjected to column chromatography on silica gel using hexane–EtOAc (10:1) as eluant. Compounds **6**–**23** were obtained as colored solid and were further crystallized with a suitable solvent.

4.3.1. 2-Hydroxy-1-(2-hydroxyethyl)-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (6). The general procedure was followed for 10 h to give red crystals on crystallization with EtOAc–hexane; 67% yield; mp: 145–148 °C; IR (KBr): 531, 563, 595, 641, 677, 731, 785, 817, 881, 976, 1072, 1104, 1127, 1251, 1273, 1309, 1346, 1368, 1432, 1515, 1557, 1595, 1675, 2365, 2834, 2934, 3058, 3344 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25 (s, 1H, OH), 3.37 (q, 2H CH_2), 3.93 (t, 2H, NCH_2), 5.77 (s, 1H, C=CH), 6.22 (br s, 1H, OH/NH), 7.73 (m, 2H, C_6–H and C_7–H), 8.08 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{14}H_{12}N_2O_4$): C, 61.76; H, 4.44; N, 10.29. Found: C, 61.70; H, 4.27; N, 10.34.

4.3.2. 2-Hydroxy-1-(2-hydroxyethyl)-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (7). The general procedure was followed for 15 h to give red crystals on crystallization with EtOAc–hexane; 56% yield; mp: 130–132 °C; IR (KBr): 639, 676, 730, 769, 878, 976, 1074, 1127, 1274, 1349, 1435, 1516, 1597, 1675, 2370, 3344, 3450 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25 (s, 1H, OH), 1.58 (s, 3H, CH_3), 3.36 (q, 2H, CH_2), 3.92 (t, 2H, NCH_2), 5.76 (s, 1H, NH), 7.67 (m, 2H, C_6–H and C_7–H), 8.07 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{15}H_{14}N_2O_4$): C, 62.93; H, 4.93; N, 9.79. Found: C, 62.84; H, 4.89; N, 9.85.

4.3.3. 1-Butyl-2-hydroxy-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (8). The general procedure was followed for 10 h to give red crystals on crystallization with benzene–hexane; 90% yield; mp: 108–110 °C; IR (KBr): 559, 684, 804, 1024, 1103, 1265, 1360, 1446, 1518, 1600, 2370, 2862, 2930, 3314, 3458 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (s, 1H, OH), 0.94 (t, 3H, CH_3), 1.30–1.62 (m, 4H, 2× CH_2), 3.80 (m, 2H, NH_2), 5.78 (s, 1H, C=CH), 6.04 (br s, 1H, NH), 7.66 (m, 2H, C_6–H and C_7–H), 8.02 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{16}H_{16}N_2O_3$): C, 67.59; H, 5.67; N, 9.85. Found: C, 67.51; H, 5.59; N, 9.89.

4.3.4. 1-Butyl-2-hydroxy-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (9). The general procedure was followed for 15 h to give red crystals on crystallization with $CHCl_3$–hexane; 85% yield; mp: 102 °C; IR (KBr): 556, 681, 719, 801, 1023, 1103, 1261, 1355, 1444, 1516, 1599, 1674, 2365, 2863, 2928, 3313, 3450 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80 (s, 1H, OH), 0.90 (t, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.31–1.61 (m, 4H, 2× CH_2), 3.77 (m, 2H, NCH_2), 6.06 (br h, 1H, NH), 7.62 (m, 2H, C_6–H and C_7–H), 8.06 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{17}H_{18}N_2O_3$): C, 68.44; H, 6.08; N, 9.39. Found: C, 68.51; H, 5.59; N, 9.45.

4.3.5. 2-Hydroxy-1-isopropyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (10). The general procedure was followed for 5 h to give red crystals on crystallization with EtOAc–hexane; 70% yield; mp: 110 °C; IR (KBr):

567, 649, 810, 863, 1049, 1097, 1219, 1322, 1407, 1604, 1680, 2370, 3441 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (br h, 1H, OH), 1.34 (d, 6H, 2× CH_3), 4.84 (m, 1H, CH), 5.75 (s, 1H, C=CH), 6.04 (br s, 1H),7.74 (m, 2.H, C_6–H and C_7–H), 8.14 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{15}H_{14}N_2O_3$): C, 66.66; H, 5.22; N, 10.36. Found: C, 66.54; H, 5.40; N, 10.23.

4.3.6. 2-Hydroxy-1-isopropyl-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (11). The general procedure was followed for 8 h to give red crystals on crystallization with EtOAc–hexane; 53% yield; mp: 110–110 °C; IR (KBr): 557, 674, 770, 843, 1023, 1128, 1121, 1295, 1334, 1461, 1516, 1571, 1599, 1676, 2339, 2370, 2928, 3324, 3425 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (br h, 1H, OH), 1.31 (d, 6H, 2× CH_3), 1.59 (s, 3H, CH_3) 4.81 (m, 1H, CH), 5.95 (br h, 1H, NH), 7.70 (m, 2H, C_6–H and C_7–H), 8.09 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{16}H_{16}N_2O_3$): C, 67.59; H, 5.67; N, 9.85. Found: C, 67.85; H, 5.84; N, 10.02.

4.3.7. 4-*tert*-Butyl-3-hydroxy-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (12). The general procedure was followed for 5 h to give red crystals on crystallization with EtOAc–hexane; 85% yield; mp: 100–102 °C; IR (KBr): 481, 527, 555, 640, 678, 719, 788, 816, 860, 915, 941, 1012, 1046, 1131, 1158, 1196, 1226, 1252, 1290, 1328, 1368, 1396, 1459, 1533, 1571, 1599, 1639, 1680, 1855, 1953, 2361, 2975, 3019, 3205, 3300 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (d, 1H, OH), 1.57 (s, 9H, 3× CH_3),5.96 (s, 1H, C=CH), 6.10 (br s, 1H, NH), 7.71 (m, 2H, Cs–H and C_7–H), 8.08 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{16}H_{16}N_2O_3$): C, 67.59; H, 5.67; N, 9.85. Found: C, 67.71; H, 5.60; N, 9.78.

4.3.8. 1-*tert*-Butyl-2-hydroxy-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (13). The general procedure was followed for 8 h to give red crystals on crystallization with EtOAc–hexane; 80% yield; mp: 105–107 °C; IR (KBr): 556, 637, 677, 720, 816, 860, 1012, 1046, 1131, 1196, 1227, 1254, 1291, 1331, 1368, 1460, 1533, 1599, 1680, 2638, 2973, 3302, 3420 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.32 (br s, 1H, OH), 1.57 (s, 9H, 3× CH_3), 1.63 (s, 3H, CH_3), 6.12 (br s, 1H, NH), 7.70 (m, 2H, C_6–H and C_7–H), 8.15 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{17}H_{18}N_2O_3$): C, 68.44; H, 6.08; N, 9.39. Found: C, 68.54; H, 6.29; N, 9.31.

4.3.9. 2-Hydroxy-1-isobutyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (14). The general procedure was followed for 5 h to give red crystals on crystallization with EtOAc–hexane; 81% yield; mp: 110–112 °C; IR (KBr): 520, 564, 723, 820, 1032, 1072, 1128, 1248, 1301, 1365, 1452, 1510, 1600, 1674, 2370, 2962, 3315, 3400 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85 (br s, 1H, OH), 1.01 (d, 6H, 2× CH_3), 1.95 (m, 1H, CH), 3.67 (t, 2H, NCH_2), 6.17 (br s, 1H, NH), 7.69 (m, 2H, C_6–H and C_7–H), 8.09 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{15}H_{14}N_2O_3$): C, 66.66; H, 5.22; N, 10.36. Found: C, 66.73; H, 5.35; N, 10.29.

4.3.10. 2-Hydroxy-1-isobutyl-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (15). The general procedure was followed for 10 h to give red crystals on crystallization with ETOAC–hexane; 78% yield; mp: 115 °C; IR (KBr): 523, 566, 678, 722, 777, 817, 869, 1030, 1070, 1126, 1169, 1247, 1301, 1332, 1361, 1447, 1503, 1574, 1598, 1677, 2340, 2366, 2868, 2955, 3111, 3300 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (br s, 1H, OH), 0.94 (d, 6H, 2× CH_3), 1.21 (s, 1H, CH_3), 1.87 (m, 1H, CH), 3.59 (t, 2H, NCH_2), 6.08 (br h, 1H, NH), 7.58 (m, 2H, C_6–H and C_7–H), 8.01 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{16}H_{16}N_2O_3$): C, 67.59; H, 5.67; N, 9.85. Found: C, 67.42; H, 5.71; N, 9.89.

4.3.11. 1-[2-(3,4-Dimethoxyphenyl)ethyl]-2-hydroxy-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (16). The general procedure was followed for 5 h to give dark red crystals on crystallization with EtOAc–hexane; 75% yield; mp: 135 °C; IR (KBr): 517, 651, 725, 773, 798, 831, 932, 1028, 1151, 1186, 1260, 1305, 1362, 1459, 1512, 1596, 1682, 2367, 2830, 2936, 3067, 3273, 3400 (br h) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25 (s, 1H, OH), 2.92 (t, 2H, C–CH_2), 3.14 (t, 2H, NCH_2), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.79 (s, 1H, C=CH), 6.00 (br s, 1H, NH), 6.79 (m, 3H, Ar-H), 7.67 (m, 2H, C_6–H and C_7–H), 8.07 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{22}H_{20}N_2O_5$): C, 67.34; H, 5.14; N, 7.14. Found: C, 67.48; H, 5.02; N, 7.22.

4.3.12. 1-[2-(3,4-Dimethoxyphenyl)ethyl]-2-hydroxy-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (17). The general procedure was followed for 10 h to give red crystals on crystallization with EtOAc–hexane; 60% yield; mp: 138–139 °C; IR (KBr): 570, 603, 630, 655, 677, 717, 764, 805, 858, 935, 1027, 1066, 1133, 1158, 1260, 1294, 1332, 1441, 1504, 1602, 1674, 2833, 2950, 3004, 3286, 3423 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.56 (s, 3H, CH_3), 2.17 (s, 1H, OH), 2.92 (t, 2H, C–CH_2), 3.87 (s, 6H, 2× OCH_3), 4.09 (t, 2H, NCH_2), 6.11 (br h, 1H, NH), 6.77 (m, 3H, Ar-H), 7.69 (m, 2H, C_6–H and C_7–H), 8.07 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{23}H_{22}N_2O_5$): C, 67.97; H, 5.46; N, 6.89. Found: C, 67.87; H, 5.56; N, 6.98.

4.3.13. 2-Hydroxy-1-phenyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (18). The general procedure was followed for 10 h to give red crystals on crystallization with EtOAc–hexane; 88% yield; mp: 110 °C; IR (KBr): 582, 690, 722, 765, 849, 925, 1020, 1078, 1142, 1243, 1292, 1334, 1385, 1444, 1509, 1602, 1678, 3245, 3450 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.34 (br s, 1H, OH), 6.17 (s, 1H, C=CH), 6.57 (br s, 1H, NH), 7.10–7.67 (m, 5H, Ar-H), 7.75 (m, 2H, C_6–H and C_7–H), 8.16 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{18}H_{12}NO_3$): C, 74.47; H, 4.17; N, 4.83. Found: C, 74.64; H, 4.04; N, 4.98.

4.3.14. 2-Hydroxy-3-methyl-1-phenyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (19). The general procedure was followed for 15 h to give red crystals on crystallization with EtOAc–hexane; 64% yield; mp: 205–207 °C; IR (KBr): 578, 689, 719, 758, 790, 848, 922, 1018, 1046, 1076, 1139, 1242, 1290, 1332, 1383, 1442, 1490, 1509, 1596, 1675, 2940, 2367, 3242, 3460 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.33 (br s, 1H, OH), 1.58 (s, 3H, CH_3), 7.08–7.69 (m, 5H, Ar-H), 7.72–7.80 (m, 2H, C_6–H and C_7–H), 8.16 (m, 2H, C_5–H and C_8–H). Anal. Calcd

($C_{19}H_{14}NO_3$): C, 74.99; H, 4.64; N, 4.60. Found: C, 75.21; H, 4.67; N, 4.68.

4.3.15. 2-Hydroxy-1-(2-methoxyphenyl)-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (20). The general procedure was followed for 10 h to give violet colored oil; 68% yield; IR (neat): 561, 584, 666, 765, 844, 990, 1025, 1045, 1074, 1141, 1182, 1224, 1274, 1342, 1463, 1507, 1616, 1672, 2368, 2838, 2595, 3008, 3199, 3373, 3463 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.30 (br s, 1H, OH), 3.86 (s, 3H, OCH_3), 5.75 (s, 1H, C=CH), 6.90 (m, 5H, Ar-H), 7.71 (m, 2H, C_6–H and C_7–H), 8.15 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{19}H_{14}NO_4$): C, 71.24; H, 4.41; N, 4.37. Found: C, 71.38; H, 4.48; N, 4.28.

4.3.16. 2-Hydroxy-1-(2-methoxyphenyl)-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (21). The general procedure was followed for 15 h to give reddish brown crystals on crystallization with EtOAc–hexane; 62% yield; mp: 130–132 °C; IR (KBr): 466, 498, 529, 568, 595, 633, 689, 719, 753, 789, 817, 852, 903, 932, 1022, 1051, 1114, 1140, 1181, 1226, 1260, 1266, 1325, 1364, 1433, 1480,1513, 1570, 1591,1646, 1667, 2366, 2836, 3067, 3345, 3449 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.33 (br s, 1H, OH), 1.64 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.90–7.68 (m, 5H, Ar-H and NH), 7.75 (m, 2H, C_6–H and C_7–H), 8.15 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{20}H_{16}NO_4$): C, 71.85; H, 4.82; N, 4.19. Found: C, 71.78; H, 4.89; N, 4.26.

4.3.17. 2-Hydroxy-1-(4-hydroxyphenyl)-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (22). The general procedure was followed for 15 h to give violet crystals on crystallization with EtOAc–hexane; 84% yield; mp: 222–224 °C; IR (KBr): 549, 673, 718, 770, 830, 912, 1017, 1048, 1004, 1142, 1238, 1293, 1349, 1439, 1514, 1598, 1678, 2365, 2814, 3297,3369, 3430 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25 (br s, 1H, OH), 4.85 (s, 1H, H), 6.81 (m, 2H, Ar-H), 7.01 (m, 2H, Ar-H), 7.61 (br s, 1H, OH), 7.73 (m, 3H, C_6–C_7–H and NH), 8.14 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{18}H_{12}NO_4$): C, 70.58; H, 3.95; N, 4.57. Found: C, 70.41; H, 3.88; N, 4.64.

4.3.18. 2-Hydroxy-1-(4-hydroxyphenyl)-3-methyl-1,4-dihydrobenzo[*g*]quinoxaline-5,10-dione (23). The general procedure was followed for 8 h to give violet crystals on crystallization with EtOAc–hexane; 80% yield; mp: 210 °C; violet crystals; IR (KBr): 510, 549, 615, 679, 719, 776, 830, 913, 1017, 1047, 1104, 1140, 1163, 1235, 1292, 1338, 1363, 1439, 1512, 1572, 1597, 1670, 2363, 2832, 3296, 3361 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (br s, 1H, OH), 1.59 (s, 3H, CH_3), 6.04 (br s, 1H, NH), 6.80–7.02 (m, 4H, Ar-H), 7.62, (1H, OH), 7.72 (m, 2H, C_6–H and C_7–H), 8.14 (m, 2H, C_5–H and C_8–H). Anal. Calcd ($C_{19}H_{14}NO_4$): C, 71.24; H, 4.41; N, 4.37. Found: C, 71.02; H, 4.65; N, 4.16.

Acknowledgments

We thank Director, SAIF and Division of Fermentation Technology of Central Drug Research Institute, Lucknow, India, for spectral, microanalyses and biological data respectively, reported in the manuscript. One of us, H.K.M., thanks U.G.C., New Delhi, India, for award of J.R.F.

References and notes

1. Tandon, V. K.; Yadav, D. B.; Chaturvedi, A. K.; Shukla, P. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3288.
2. Hung, S. Y.; Chung, K. H.; You, H. J.; Choi, I. H.; Chae, M. J.; Han, J. Y.; Jung, O. J.; Kang, S. J.; Ryu, C. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3563.
3. Ryu, C. K.; Choi, K. U.; Shim, J. Y.; You, H. J.; Choi, I. H.; Chae, M. J. *Bioorg. Med. Chem.* **2003**, *11*, 4003.
4. Ryu, C. K.; Han, J. Y.; Jung, O. J.; Lee, S. K.; Lee, J. Y.; Jeong, S. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 679.
5. Ryu, C. K.; Kang, H. Y.; Yi, Y. J.; Shin, K. H.; Lee, B. H. *Bioorg. Med. Chem.* **2000**, *10*, 1589.
6. (a) Ryu, C. K.; Kim, H. J. *Arch. Pharmacol. Res.* **1994**, *17*, 139; (b) Ryu, C. K.; Sun, Y. J.; Shim, J. Y.; You, H. J.; Choi, K. U.; Lee, H. *Arch. Pharmacol. Res.* **2002**, *25*, 784.
7. Kuo, S. C.; Ibuka, T.; Huang, L. J.; Lien, J. C.; Yean, S. R.; Hung, S. C.; Lednicer, D.; Morris-Natschke, S.; Lee, K. H. *J. Med. Chem.* **1996**, *39*, 1447.
8. Lee, H. J.; Kim, J. S.; Park, S. Y.; Suh, M. E.; Kim, H. J.; Seo, E. K.; Lee, C. O. *Bioorg. Med. Chem.* **2004**, *12*, 1623.
9. Gomez-Monterrey, I. M.; Grieco, P.; Diurno, M. V.; Bolongnese, A.; Colla, P. L; Novellino, E. *Bioorg. Med. Chem.* **2003**, *11*, 3769.
10. Gomez-Monterrey, I. M.; Campiglia, P.; Mazzoni, O.; Novellino, E.; Diurno, M. V. *Tetrahedron Lett.* **2001**, *42*, 5755.
11. Tandon, V. K.; Chhor, R. B.; Singh, R. V.; Rai, S.; Yadav, D. B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1079.
12. Tandon, V. K.; Singh, R. V.; Yadav, D. B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2901.
13. Tandon, V. K.; Yadav, D. B.; Singh, R. V.; Yaish, M.; Chaturvedi, A. K.; Shukla, P. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3463.
14. Tandon, V. K.; Yadav, D. B.; Singh, R. V.; Chaturvedi, A. K.; Shukla, P. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5324.
15. National Committee for Clinical Laboratory Standard. Reference method for broth dilution antifungal susceptibility testing of yeast, approved standard. Document M27-A. National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 1997.
16. National Committee for Clinical Laboratory Standard. 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, 1998.
17. Chambers, H. F.; Sande, M. A. In *The Pharmacological Basis of Therapeutics*; Molinoff, P. B., Ruddon, R. W., Eds., 9th ed.; McGraw-Hill: USA, 1996; p 1108.
18. Krapcho, A. P.; Petry, M. E.; Getahun, E. Z.; Landi, J. J.; Stallman, J.; Plsenberg, J. I.; Gallagher, C. E.; Maresch, M. J.; Hacker, M. P.; Giuliani, F. C.; Geggiolin, G.; Pezzoni, G.; Menta, E.; Manzotti, C.; Oliva, A.; Spinelli, S.; Tognella, S. *J. Med. Chem.* **1994**, *37*, 828.
19. Krapcho, A. P.; Landi, J. J.; Hacker, M. P.; McCormack, J. J. *J. Med. Chem.* **1985**, *28*, 1124.
20. Hazlehurst, L. A.; Krapcho, A. P.; Hacker, M. P. *Biochem. Pharmacol.* **1995**, *50*, 1087.
21. Blackburn, C. *Tetrahedron Lett.* **2005**, *46*, 1405.
22. Kartoflitskaya, A. P.; Kostyukova, S. E.; Marintsova, N. G. *Russ. J. Org. Chem.* **1997**, *33*, 11.
23. Becker, H. *Organicum (Practical Handbook of Organic Chemistry)*; Alddison-Wesley: MA, USA, 1973, p 611.