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Original article

'On water' assisted synthesis and biological evaluation of nitrogen and sulfur containing hetero-1,4-naphthoquinones as potent antifungal and antibacterial agents

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ABSTRACT

2-Chloro-3-(4-methylpiperazin-1-yl)naphthalene-1,4-dione (**3a**), 2-chloro-3-(pyrrolidin-1-yl)naphthalene-1,4-dione (**3b**), 2-chloro-3-(piperidin-1-yl)naphthalene-1,4-dione (**3c**), 2-chloro-3-morpholinonaphthalene-1,4-dione (**3d**), 2-chloro-3-(2-phenylhydrazinyl)naphthalene-1,4-dione (**3e**), 2-(allylamino)-3-chloronaphthalene-1,4-dione (**3f**), 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)acetic acid (**3g**), 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)succinic acid (**3h**), methyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)acetate (**3i**), 2-chloro-3-(2-mercaptoethylthio)naphthalene-1,4-dione (**3j**), 3-hydroxy-4-methyl-4*H*-naphtho[2,3-*b*][1,4]thiazine-5,10-dione (**3k**) and compounds **3l**–**q** have been synthesized by a green methodology approach using water as solvent and evaluated for their antifungal and antibacterial activity. The antifungal profile of **3a**–**n** indicated that compounds **3a**–**d**, **3j**, **3e** and **3k** have potent antifungal activity. Amongst the most promising antifungal compounds, **3a**–**g**, **3j**, **3k** showed better antifungal activity than clinically prevalent antifungal drugs Fluconazole and Amphotericin-B against *Trichophyton mentagraphytes* and compounds **3j** and **3k** have been found to be lead antifungal bicyclic and tricyclic 1,4-naphthoquinones. Compound **3k** also exhibited pronounced antibacterial activity.

1. Introduction

During last one decade, green chemistry has attracted the major scientific discipline due to the application of green chemistry principles which has led to the development of cleaner and more efficient chemical synthesis [1] and water has contributed as an important tool in green chemistry being the most versatile solvent [2]. It has motivated us to develop a green methodology to synthesize medicinally important novel heterocyclic quinone derivatives using water as an economic solvent having environmental safety and societal implications.

The incidence of fungal and bacterial infections still remains an important and challenging problem due to combination of factors including emerging infectious diseases and also due to increase of multi-drug resistant microbial pathogens [3]. The resistance of wide spectrum antifungal and antibacterial agents prompted us to discover and develop new antifungal and antibacterial drugs [4].

The hetero-1,4-naphthoquinones have shown potent biological affinity towards viral [6], molluscidal [7], malarial [8], leishmanial [9], cancer [10,26,31], bacterial and fungal diseases [11–16,27–30], due to their redox potentials [5].

We have earlier reported the synthesis of novel hetero-1,4-naphthoquinoes as potent antiviral, anticancer, antiproliferative, antibacterial and antifungal agent [10–18]. The profound antifungal activity exhibited by compounds **I**–**III** (Fig. 1) [14,16,17] prompted us to envisage 'on water' assisted synthesis of new hetero-1,4-naphthoquinones containing nitrogen and sulfur atoms at 2- and 3-positions of 1,4-naphthoquinone and study their biological activity. We report herein a green methodology concept in quinone chemistry to carryout 'on water' assisted synthesis and biological evaluation of some potent antifungal and antibacterial agents.

2. Results and discussion

2.1. Chemistry

It is well known that the reaction of 2,3-dichloro-1,4-naphthoquinone with nucleophiles proceeds by nucleophilic substitution whereas nucleophilic addition reactions of 1,4-naphthoquinones is augmented by oxidative addition pathway [18]. Based on reactivity and biological activity of 2,3-dichloro-1,4-naphthoquinones and

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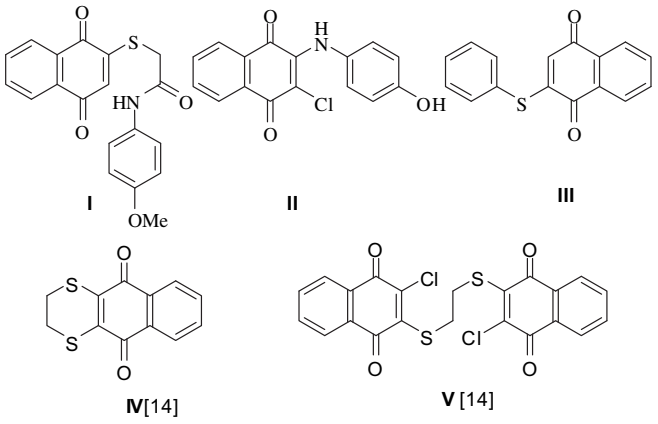
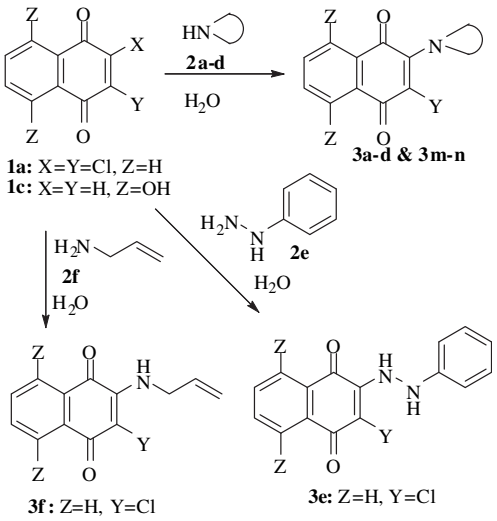


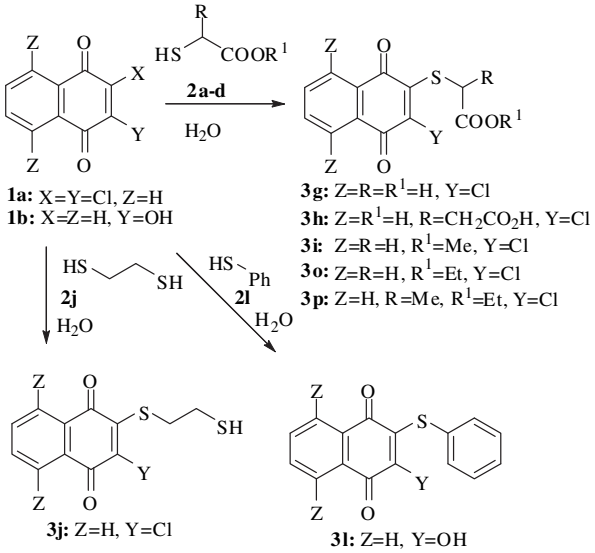
Fig. 1. Lead antifungal agents **I** [14], **II** [17] and **III** [16].

1,4-naphthoquinones [10–18], we studied its reactions with different amines (Scheme 1), thiols (Scheme 2) and intramolecular nucleophilic addition–elimination and cyclization (Scheme 3) **2a–n** in the presence or absence of a base using water as solvent as reported in Table 1 according to our 'on water' assisted synthetic methodology [18] and evaluated their antifungal and antibacterial activity.

When 2,3-dichloro-1,4-naphthoquinone (**1a**) was stirred with different secondary heterocyclic amines (1.1 equivalent) viz. 1-methylpiperazine (**2a**), pyrrolidine (**2b**), piperidine (**2c**) and morpholine (**2d**) [18] at room temperature/50 °C using water as solvent, mono substituted products; 2-chloro-3-(4-methylpiperazin-1-yl)naphthalene-1,4-dione (**3a**), 2-chloro-3-(pyrrolidin-1-yl) naphthalene-1,4-dione (**3b**) [18], 2-chloro-3-(piperidin-1-yl)naphthalene-1,4-dione (**3c**) [18] and 2-chloro-3-morpholinonaphthalene-1,4-dione (**3d**) [18] were obtained respectively in 98–100% yield as reported in Table 1 (Scheme 1). In order to study the structure–activity relationship (SAR) with compounds **II** (Fig. 1) and **3a–d**, 5,8-dihydroxy-2-(4-methylpiperazin-1-yl)naphthalene-1,4-dione (**3m**) and 5,8-dihydroxy-2-(pyrrolidin-1-yl)naphthalene-1,4-dione (**3n**) were synthesized by the reaction of naphthazarine (**1c**) with *sec* heterocyclic amines (**2a**,**b**) respectively as shown in Scheme 1. The reaction of **1a** with phenylhydrazine (**2e**) led to the formation of 2-chloro-3-(2-phenylhydrazinyl)naphthalene-1,4-dione (**3e**) in 96% yield whereas reaction of **1a** with 4-nitrophenylhydrazine and 2,4-dinitrophenylhydrazine do not occur in water due to electron withdrawing inductive effect of nitro group. The reaction of **1a** with prop-2-en-1-amine (**2f**) resulted in the formation of 2-(allylamino)-3-chloronaphthalene-1,4-dione (**3f**) in 100% yield within 15 min in water (Scheme 1). It is pertinent to note that only mono substituted products are formed as coloured solids in water when compared with other organic solvents [18].

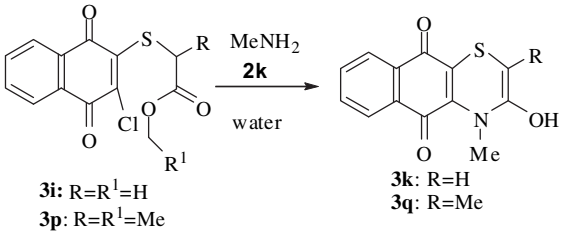


Scheme 1. Reaction of 1,4-naphthoquinones with nitrogen nucleophiles in aqueous medium (For reagents and conditions refer to Table 1).



Scheme 2. Reaction of 1,4-naphthoquinones with sulfur nucleophiles in aqueous medium (For reagents and conditions refer to Table 1).

Compounds **3i** and **3p** undergo further intramolecular nucleophilic addition-elimination and cyclization with methyl amine **2k** in water to yield 3-hydroxy-4-methyl-4*H*-naphtho[2,3-*b*][1,4]



Scheme 3. Intramolecular nucleophilic addition-elimination and cyclization of **3i** and **3p** in aqueous medium (For reagents and conditions refer to Table 1).

dithiine-5,10-dione (**IV**) and 3,3′-(ethane-1,2-diylbis(sulfanediyl)) bis(2-chloronaphthalene-1,4-dione) (**V**) [14] (Fig. 1).

The reaction of **1a** with 2-mercaptoacetic acid (**2g**) and 2-mercaptosuccinic acid (**2h**), have been studied and the products 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)acetic acid (**3g**) and 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)succinic acid (**3h**) respectively were obtained using water as solvent as reported in Scheme 2 (Table 1) whereas the reaction of **1a** with methyl 2-mercaptoacetate (**2i**), ethyl 2-mercaptoacetate (**2m**) and ethyl 2-mercaptopropionate (**2n**) in aqueous medium produced methyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio) acetate (**3i**), ethyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)acetate (**3o**) and ethyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)propanoate (**3p**) in excellent yields (Table 1). The reaction of 2,3-dichloro-1,4-naphthoquinone (**1a**) with ethane-1,2-dithiol (**2j**) in water resulted in the formation of 2-chloro-3-(2-mercaptoethylthio)naphthalene-1,4-dione (**3j**) instead of an expected mixture of 2,3-dihydronaphtho[2,3-*b*][1,4]

Table 1
Reaction conditions of water assisted synthesis of hetero-1,4-naphthoquinone (**3a**–**q**).

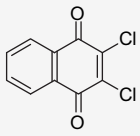
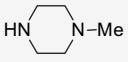
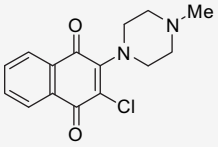
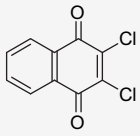
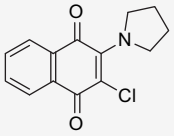
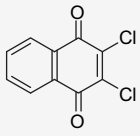
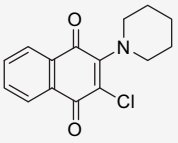
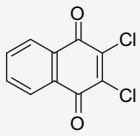
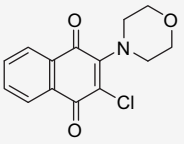
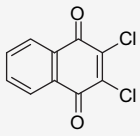
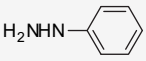
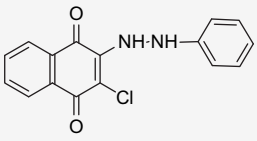
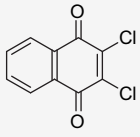
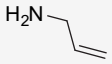
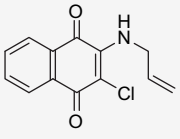
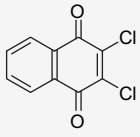
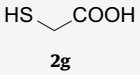
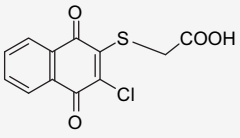
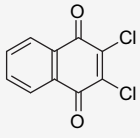
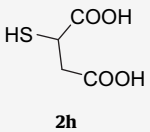
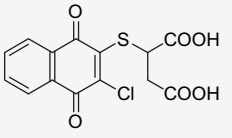
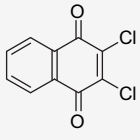
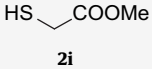
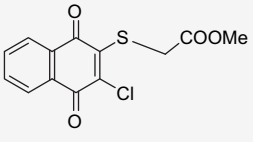
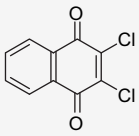
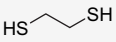
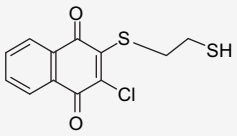
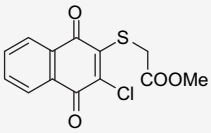
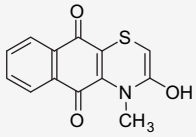
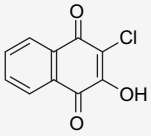
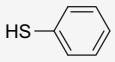
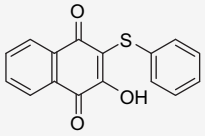
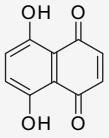
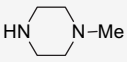
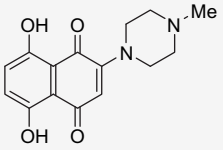
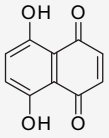
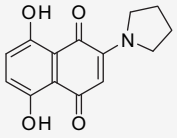
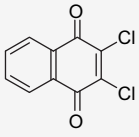
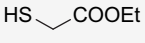
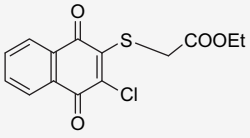
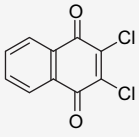
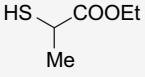
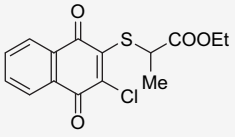
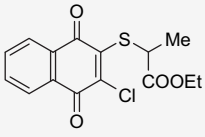
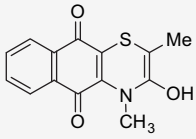
Entry	1	2	Time	b	*T*	Product 3	Mp	Yield%	*c*	W
1	 **1a**	 **2a**	1 h	N	rt	 **3a**	168	98%	100	A
2	 **1a**	 **2b**	10 min	N	rt	 **3b** [18]	80	99%	100	A
3	 **1a**	 **2c**	10 min	N	rt	 **3c** [18]	150	98%	100	A
4	 **1a**	 **2d**	20 min	N	50 °C	 **3d** [18]	110	100%	100	A
5	 **1a**	 **2e**	4 h	N	50 °C	 **3e** [18]	190	96%	95	C
6	 **1a**	 **2f**	30	N	rt	 **3f**	100	100	100	A
7	 **1a**	 **2g**	2h	Et_3N	50 °C	 **3g**	98	89%	91	D
8	 **1a**	 **2h**	2 h	Et_3N	50 °C	 **3h**	>280	89%	90	D
9	 **1a**	 **2i**	1 h	N	rt	 **3i**	115	98%	100	A

Table 1 (*continued*)

Entry	1	2	Time	b	*T*	Product 3	Mp	Yield%	*c*	W
10	 **1a**	 **2j**	15 min	N	rt	 **3j** [18]	196	98%	100	A
11	 **3i**	H_2N-CH_3 **2k**	24 h	Et_3N	70 °C	 **3k**# [18]	170	20%	50	C
12	 **1b**	 **2l**	4 h	N	50 °C	 **3l** [18]	160	98	100	A
13	 **1c**	 **2a**	3 h	N	rt	 **3m**	>260	68%	100	C
14	 **1c**	 **2b**	15 min	N	rt	 **3n** [18]	180	98%	100	A
15	 **1a**	 **2m**	2 h	N	50 °C	 **3o** [18]	96	100%	100	A
16	 **1c**	 **2n**	2h	N	50 °C	 **3p**	83	93%	100	A
17	 **3p**	H_2N-CH_3 **2k**	24 h	K_2CO_3	50 °C	 **3q**# [11]	186	20%	45	C

b: base, N: not required, #other product was not isolated, c: conversion% of **1**, W: workup process, A: product directly filtered, B: product filtered as ppt **3**, C: product filtered and purified by column chromatography, D: water was evaporated or extracted with suitable solvent and purified by column chromatography using silica gel in hexane and ethyl acetate.

thiazine-5,10-dione (**3k**) and 3-hydroxy-2,4-dimethyl-4*H*-naphtho [2,3-*b*][1,4]thiazine-5,10-dione (**3q**) as exhibited in Scheme 1. The structure of **3q** has been confirmed by X-ray crystallography (Fig. 2) [25].

2.2. Antifungal activity

Our laboratory during last five years has been engaged [10–18] to discover new methodologies for the synthesis of potent

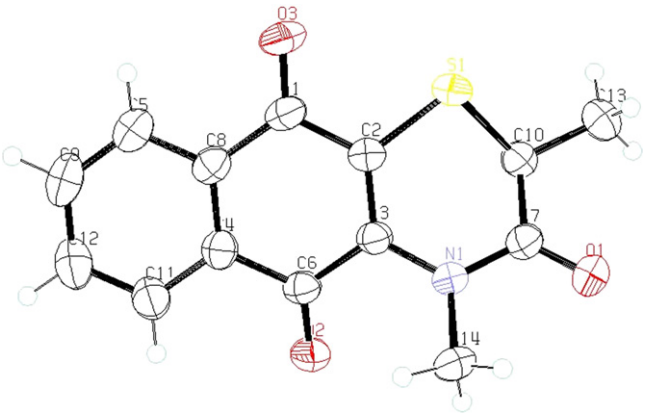


Fig. 2. Crystal structure of 3-Hydroxy-2,4-dimethyl-4*H*-Naphtho[2,3-*b*][1,4]-thiazine-5,10-dione **3q** (No. CCDC765321) [25].

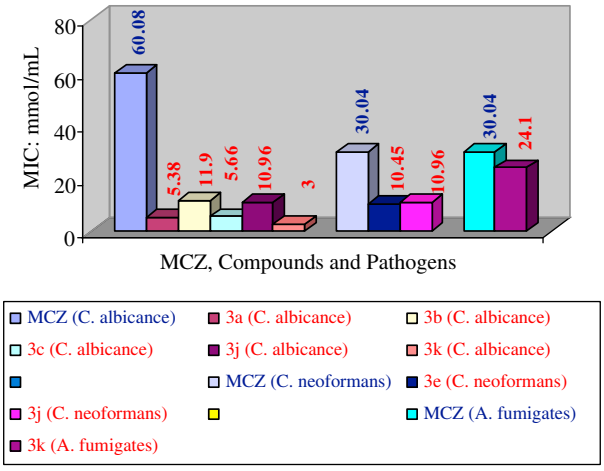


Fig. 3. Comparative antifungal study plot with Miconazole (MCZ), compounds and Pathogens.

antifungal agents containing quinone chromophore [10–18] and in our new endeavors, we have synthesized different heterocyclic naphthoquinones containing compounds **3a**–**n** using water as solvent and evaluated their antifungal activity against a variety of fungi viz. *Candida albicans*, *Cryptococcus neoformans*, *Sporothrix schenckii*, *Trichophyton mentagraphytes*, *Aspergillus fumigatus* and *Candida parapsilosis* by standard micro broth dilution as per NCCLS [23,24] protocol with a view to develop therapeutic agents having broad spectrum of antifungal activity [10–18]. These studies have led to the identification of potent antifungal agents as lead molecules containing quinone chromophore. Subsequently on the basis of structure–activity relationship of antifungal activity of the lead heterocyclic quinone derivatives, we further synthesized and screened antifungal assay of **3a**–**n** as shown in Table 2.

Comparison of antifungal activity of compounds **3a**–**n** with that of antifungal drug Miconazole (MIC_{50} = 60.08 mmol/mL), showed that compound **3k** (MIC_{50} = 3.00 mmol/mL), **3a** (MIC_{50} = 5.38 mmol/mL), **3c** (MIC_{50} = 5.66 mmol/mL), **3e** (MIC_{50} = 10.45 mmol/mL), **3j** (MIC_{50} = 10.96 mmol/mL), **3b** (MIC_{50} = 11.90 mmol/mL), **3d** (MIC_{50} = 45.04 mmol/mL) and **3f** (MIC_{50} = 50.00 mmol/mL) had better antifungal activity against *C. albicans*. Compounds **3e** (MIC_{50} = 10.45 mmol/mL), **3j** (MIC_{50} = 10.96 mmol/mL), **3k** (MIC_{50} = 10.96 mmol/mL) and **3a**–**d** (MIC_{50} = 21.5–22.6 mmol/mL) exhibited better antifungal activity when compared with Miconazole (MIC_{50} = 30.04 mmol/mL) against *C. neoformans*. Compounds **3a**–**c**, **3e**, **3j** and **3k** had shown potent antifungal activity when compared with Miconazole against *T. mentagraphytes*. Compound **3k** (MIC_{50} = 24.10 mmol/mL) had shown promising antifungal profiles on comparison with antifungal drug Miconazole (MIC_{50} = 30.04 mmol/mL) against *A. fumigatus;* (Table 2) as exhibited in Fig. 3.

On comparison of antifungal activity with that of antifungal drug Nystatin (MIC_{50} = 8.42 mmol/mL), compound **3k** (MIC_{50} = 3.00 mmol/mL), **3a** (MIC_{50} = 5.38 mmol/mL), **3c** (MIC_{50} = 5.66 mmol/mL) were found to exhibit better activity against *C. albicans*. Compounds **3j** (MIC_{50} = 2.74 mmol/mL), **3e** (MIC_{50} = 10.45 mmol/mL) and **3a** (MIC_{50} = 10.75 mmol/mL) had better antifungal profile against *S. schenckii* on comparison with Nystatin (MIC_{50} = 14.25 mmol/mL) as exhibited in Fig. 4.

Table 2
Structures and in vitro antifungal activity for compounds **3a**–**q**.

Compounds	MIC: mmol/mL					
	C. albicans	*C. neoformans*	*S. schenckii*	*T. mentagraphytes*	*A. fumigatus*	*C. parapsilosis*
3a	**5.38**[b]	21.54	**10.75**[b]	**1.34**[b,c]	43.09	21.54
3b	11.90[b]	23.85	23.85	**1.49**[b,c]	95.39	23.85
3c	**5.66**[b]	22.69	22.69	**1.42**[b,c]	90.74	11.32[b]
3d	45.04	22.52	22.52	2.81[b,c]	90.09	22.52
3e	10.45[b]	10.45[b]	**10.45**[b]	**1.31**[b,c]	41.88	20.94
3f	50.50	50.50	50.50	**3.15**[b,c]	101.01	50.50
3g	88.50	88.50	88.50	**5.52**[b]	176.99	88.50
3h	>146.74	146.74	73.37	36.68	146.74	146.74
3i	>168.50	168.50	168.50	42.12	168.50	168.50
3j	10.96[b]	10.96[b]	**2.74**[b,c]	**1.37**[b,c]	87.79	43.89
3k	**3.00**[b]	24.10	24.10	**3.00**[b,c]	24.10	6.02[b]
3l	177.11	>177.11	>177.11	177.11	>177.11	>177.11
3m	>173.43	>73.43	173.43	>173.43	173.43	173.43
3n	>192.86	>192.86	>192.86	96.43	>192.86	>192.86
3o [11]	>160.90	40.22	80.45	>160.90	>160.90	>160.90
3p [11]	>153.95	38.49	76.98	>153.95	>153.95	>153.95
3q [11]	22.87	11.42	11.42	5.71	22.87	45.74
Miconazole [13]	60.08	30.04	[a]	<1.87	30.04	[a]
Nystatin [13]	8.42	3.78	14.25	[a]	[a]	[a]
Fluconazole [13]	3.26	1.63	6.53	5.09	6.53	3.26
Amphotericin-B [13]	0.42	0.84	[a]	1.69	[a]	[a]

[a] Activity not reported.
[b] Entries in bold font indicate better activity than reference drugs Miconazole [13] and/ or Nystatin [13].
[c] Entries in bold font indicate better activity than reference drugs Fluconazole [13] and Amphotericin-B [13].

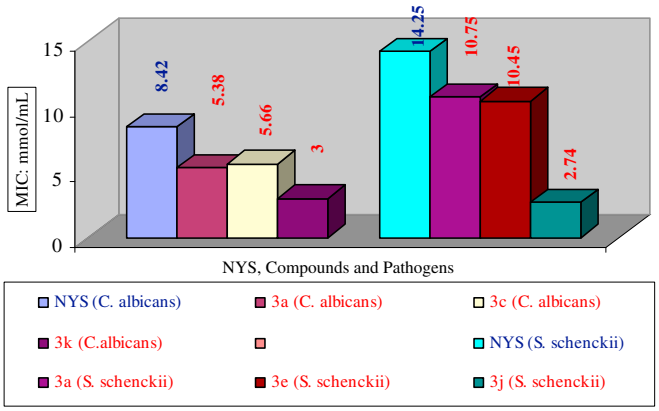


Fig. 4. Comparative antifungal study plot with Nystatin (NYS), compounds and Pathogens.

Compounds **3a**–**f**, **3j**, **3k** (MIC_{50} = 1.32–3.15 mmol/mL) had exhibited extremely potent antifungal activity compared with clinically prevalent antifungal drugs Fluconazole (MIC_{50} = 5.09 mmol/mL) and compounds **3a**–**c**, **3e**, **3j** (MIC_{50} = 1.32–1.49 mmol/mL) compared with Amphotericin-B (MIC_{50} = 1.69 mmol/mL) against *T. mentagraphytes*. Compounds **3k** had also exhibited better activity than clinically prevalent antifungal drug Fluconazole against *C. albicans* and *S. schenckii* respectively (Fig. 5).

Compounds **3k** and **3c** were also shown to posses pronounced antifungal activity MIC_{50} = 6.02 mmol/mL and MIC_{50} = 11.32 mmol/mL against *C. parapsilosis* respectively.

Structure activity relationship of **3a**–**n** revealed that mono substituted secondary heterocyclic amines **3a**–**d**, ethane-1,2-dithiol derivative **3j** and cyclic analogs **3k** of 1,4-naphthoquinone possess potent antifungal activity when compared with aliphatic acids **3g**, **3h**, ester **3i** aryl thiol **3l**. Comparative results have shown that free thiol group in **3j** is an important factor to exhibit potent antifungal activity. The withdrawals of alkylating group in cyclic analog **3q** (Scheme 3 & Table 2) [11] results in slight increase of antifungal activity of compound **3k**.

2.3. Antibacterial activity

Based on mechanism of the antibacterial action of quinones [19–21] and our previous work [10–17], antibacterial activity of

Table 3
Structures and in vitro antibacterial activity for compounds **3a**–**q**.

Compounds	MIC: mmol/mL		
	E. coli	*S. aureus*	*K. pneumoniae*
3a	43.09	43.09	21.54
3b	47.69	95.39	95.39
3c	43.37	90.74	90.74
3d	45.04	180.18	22.52
3e	83.75	83.75	83.75
3f	101.01	101.01	25.25
3g	44.25	44.25	22.12
3h	36.68	146.74	4.58
3i	42.12	42.12	42.12
3j	87.79	43.89	21.95
3k	24.10	3.00	3.00
3l	88.56	177.11	177.11
3m	173.43	173.43	173.43
3n	192.86	192.86	192.86
3o [11]	40.22	40.22	160.90
3p [11]	38.49	38.49	153.95
3q [11]	5.71	11.42	11.42
Kanamycin [13]	33.02	4.13	66.05
Amikacin [13]	1.71	27.32	1.71
Tobramycin [13]	1.07	0.53	2.14
Gentamicin [13]	0.38	1.63	0.82

3a–**n** was elucidated against *Escherichia coli*, *Staphylococcus aureus* (ATCC25923), *Klebsiella Pneumoniae* (ATCC 27736) and *Pseudomonas aeruginosa parapsilosis* by standard micro broth dilution as per NCCLS [23,24] protocol as shown in Table 3.

Comparison of antibacterial activity with that of antibacterial drug Kanamycin (MIC_{50} = 33.02 mmol/mL) showed that compounds **3k** (MIC_{50} = 24.10 mmol/mL) had better activity against *E. coli*. Compound **3k** (MIC_{50} = 3.00 mmol/mL) exhibited better activity when compared with Kanamycin (MIC_{50} = 4.13 mmol/mL) against *S. aureus* (ATCC25923). Compound **3k** (MIC_{50} = 3.00 mmol/mL), **3h** (MIC_{50} = 4.58 mmol/mL), **3a**, **3d**, **3f**, **3g**, **3j** and **3i** also exhibited better activity than Kanamycin against *Klebsiella Pneumoniae* (ATCC 27736).

Compound **3k** (MIC_{50} = 3.00 mmol/mL) had better activity than antibacterial drug Amikacin against *S. aureus* (ATCC25923). Compound **3a**–**n** were also screened against *Pseudomonas aeruginosa* at MIC_{50} = 50.0 μg/mL but did not exhibit significant antibacterial activity (Fig. 6).

Structure activity relationship of **3a**–**n** revealed that cyclic analog **3k** has better antibacterial activity as compared to acyclic mono substituted amino and thiols derivatives of quinone (**3a**–**j**, **3l**)

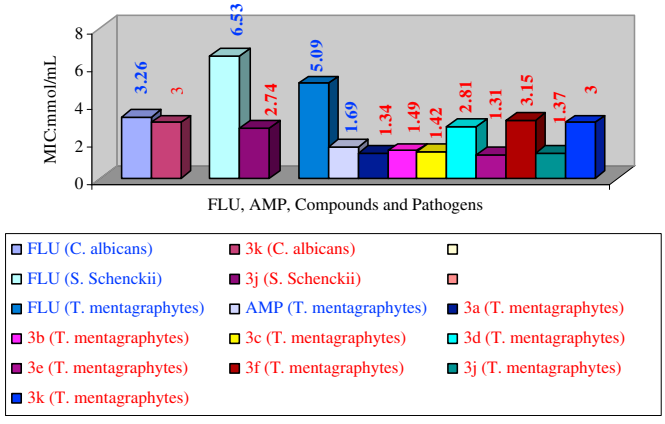


Fig. 5. Comparative antifungal study plot with compounds and Fluconazole (MCZ), Amphotericin-B (AMP), compounds and Pathogens.

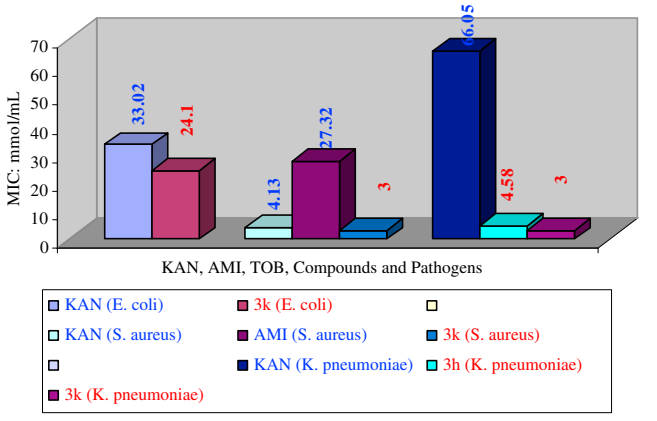


Fig. 6. Comparative antibacterial study plot with Kanamycin (KAN), Amikacin (AMI), Tobramycin (TOB), compounds and pathogens.

3. Conclusion

In conclusion, we are the first to synthesize amino and thiol derivatives of 1,4-naphthoquinone **3a**–**q** by a green methodology approach using water as solvent. The derivatives **3a**–**n** have been evaluated for their antifungal activity. The antifungal profile of **3a**–**n** indicated that compounds **3a**–**d**, **3j**, **3e** and **3k** have potent antifungal activity. Amongst the most promising antifungal compounds, **3a**–**g**, **3j**, **3k** exhibited better antifungal activity than clinically prevalent antifungal drugs, Fluconazole and Amphotericin-B against *T. mentagraphytes*. Compound **3j** is lead compound as potent antifungal agent and compound **3k** exhibited promising antibacterial activity. Compounds **3j** and **3k** are the lead drug candidates and further work is being carryout at Central Drug Research Institute, Lucknow, India concerning its toxicological evaluation. Efforts are paving way to synthesize more potent biologically active derivatives containing quinone chromophore using a green methodology approach.

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 FTIR 8201 PC, Schimadzu Spectrophotometers on KBr discs. Nuclear Magnetic Resonance (NMR) spectra were recorded on Perkin–Elmer model R.32 spectrometers using TMS as an internal reference. All compounds showed satisfactory elemental analysis for C, H, N and S. 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 UV Chamber, where required. Spectra facilities and elemental micro-analyses were carried out by SAIF division of Central Drug Research Institute, Lucknow, India. Most reagents were purchased from Lancaster, Sigma–Aldrich and Merck.

4.2. General procedure for the synthesis of hetero-1,4-naphthoquinones (*3a*–*q*)

Suspension of quinone **1** (1.0 mmol) in water (6 mL) and *sec* heterocyclic amines, aliphatic amines, hydrazines, thioacids or thiols (solid, 1.0 mmol or liquid, 1.1 mmol) **2** was stirred at room temperature or 50–70 °C for 10 min to 24 h. and workup was followed as shown in Table 1. Column chromatography (if required) of the reaction mixture (hexane/EtOAc 50:1 → 15:1) gave **3a**–**q** as orange or red solid (Table 1).

4.2.1. 2-Chloro-3-(4-methylpiperazin-1-yl)naphthalene-1,4-dione (*3a*)

Orange powder; IR (KBr): 1597 and 1675 (>C=O of quinone), ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (t, J = 4.8 Hz, 4H, $C\underline{H}_2$NMe), 2.31 (s, 3H, $NC\underline{H}_3$), 2.85 (t, J = 4.8 Hz, 4H, $NC\underline{H}_2CH_2$NMe), 7.92 (m, 2H, C_5–H and C_8–H), 8.01 (m, 2H, C_6–H and C_7–H); Mass (ESI): 290.10 (M^+, 33%), 291.11 ($M^+ + 1$, 100%); Anal. Calcd for $C_{15}H_{15}ClN_2O_2$: C, 61.97; H, 5.20; N, 9.64. Found: C, 62.09; H, 5.23; N, 9.68; Beilstein test [22]: Cl positive.

4.2.2. 2-Chloro-3-(pyrrolidin-1-yl)naphthalene-1,4-dione (*3b*)

Orange powder; IR (KBr): 1572 and 1670 (>C=O of quinone) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.82 (m, 4H, CH_2), 2.80 (m, 4H, NCH_2), 7.69–7.76 (m, 2H, C_5–H and C_8–H), 8.02 (dd, 1H, J = 8.2 and 1.8 Hz, C_7), 8.10 (dd, 1H, J = 8.2 and 1.8 Hz, C_6); Mass (ESI): 262.09 (M^+, 100%), 263.09 ($M^+ + 1$, 25%), 264.09 ($M^+ + 2$, 33%); Anal. Calcd $C_{14}H_{12}ClNO_2$: C, 64.25; H, 4.62; N, 5.35. Found: C, 64.16; H, 4.56; N, 5.31; Beilstein test [22]: Cl positive.

4.2.3. 2-Chloro-3-(piperidin-1-yl)naphthalene-1,4-dione (*3c*)

Orange powder; IR (KBr): 1585 and 1642 (>C=O of quinone) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 1.59 (m, 6H, $C\underline{H}_2C\underline{H}_2C\underline{H}_2$), 2.96 (m, 4H, $NC\underline{H}_2$), 7.88 (m, 2H, C_5–H and C_8–H), 8.05 (m, 2H, C_6–H and C_7–H); Mass (ESI): 275.01 (M^+); Anal. Calcd $C_{15}H_{14}ClNO_2$: C, 65.34; H, 5.12; N, 5.08. Found: C, 65.22; H, 5.05; N, 5.03; Beilstein test [22]: Cl positive.

4.2.4. 2-Chloro-3-morpholinonaphthalene-1,4-dione (*3d*)

Orange powder; IR (KBr,): 1581 and 1649 (>C=O of quinone) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): 3.62 (t, 4H, J = 4.8 Hz, $NC\underline{H}_2$), 3.82 (t, 4H, J = 4.8 Hz, $OC\underline{H}_2$), 7.68–7.73 (m, 2H, C_5–H and C_8–H), 8.01 (dd, 1H, J = 8.2 and 1.8 Hz, C_7), 8.11 (dd, 1H, J = 8.2 and 1.8 Hz, C_6); Mass (ESI): 277.05 (M^+); Anal. Calcd ($C_{14}H_{12}ClNO_3$): C 60.55, H 4.36, N 5.04. Found: C 60.32, H 4.49, N 5.16; Beilstein test [22]: Cl positive.

4.2.5. 2-Chloro-3-(2-phenylhydrazinyl)naphthalene-1,4-dione (*3e*)

Red powder; IR (KBr): 3442 and 3388 (NH), 1660 and 1585 (>C=O of quinone) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.07 (bs, 1H, NH), 5.00 (bs, 1H, NH), 6.98–7.09 (m, 3H, phenyl), 7.47 (m, 2H, Phenyl), 7.86 (m, 2H, C_5–H and C_8–H), 8.05 (m, 2H, C_6–H and C_7–H); Mass (ESI): 298.05 (M^+); Anal. Calcd for $C_{16}H_{11}ClN_2O_2$: C, 64.33; H, 3.71; N, 9.38. Found: C, 64.18; H, 3.68; N, 9.27; Beilstein test [22]: Cl positive.

4.2.6. 2-(Allylamino)-3-chloronaphthalene-1,4-dione (*3f*)

Red powder; IR (KBr): 3430 (NH), 1589 and 1665 (>C=O of quinone) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (d, J = 4.8 Hz, 2H, $NC\underline{H}_2$), 5.28–5.30 (m, 2H, $C\underline{H}_2CH$), 5.93 (m, 1H, $CH_2C\underline{H}$), 6.06 (bs, 1H, NH); 7.98 (m, 2H, C_5–H and C_8–H), 8.17 (m, 2H, C_6–H and C_7–H); Mass (ESI): 247.04 (M^+); Anal. Calcd for $C_{13}H_{10}ClNO_2$: C, 63.04; H, 4.07; N, 5.66; Found: C, 63.15; H, 4.10; N, 5.68; Beilstein test [22]: Cl positive.

4.2.7. 2-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio) acetic acid (*3g*)

Red powder; IR (KBr): 3421 (OH), 2920 (SCH_2), 2852 (SCH_2), 1736 (>C=O of COOH), 1658 and 1466 (C=O of quinone) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.02 (s, 2H, $SC\underline{H}_2$), 7.98 (m, 2H, C_5–H and C_8–H), 8.17 (m, 2H, C_6–H and C_7–H), 12.78 (bs, 1H, OH); Mass (ESI): 281.98 (M^+); Anal. Calcd for $C_{12}H_7ClO_4S$: C, 50.98; H, 2.50; S, 11.34; Found: C, 51.08; H, 2.53; S, 11.37; Beilstein test [22]: Cl positive.

4.2.8. 2-(3-Chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio) succinic acid (*3h*)

Red powder; IR (KBr): 3441(OH), 3001(SCH_2), 2986(SCH_2), 1785 (>C=O of COOH), 1655 and 1662 (C=O of quinone) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.25 (m, 2H, $C\underline{H}_2CO$); 3.79 (t, 1H, J = 6.3, $SC\underline{H}$), 7.94 (m, 2H, C_5–H and C_8–H), 8.12 (m, 2H, C_6–H and C_7–H), 12.40 (bs, 1H, NH), 12.78 (bs, 1H, NH); Mass (ESI): 339.99 (M^+); Anal. Calcd for $C_{14}H_9ClO_6S$: C, 49.35; H, 2.66; S, 9.41; Found: C, 49.41; H, 2.70; S, 9.45; Beilstein test [22]: Cl positive.

4.2.9. Methyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)acetate (*3i*)

Orange powder; IR (KBr): 1588 and 1670 (>C=O of quinone), 1739 (>C=O of COOMe) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.63 (s, 3H, $OC\underline{H}_3$), 4.00 (s, 2H, $SC\underline{H}_2$), 7.63 (m, 2H, C_5–H and C_8–H), 8.18 (m, 2H, C_6–H and C_7–H); Mass (ESI): 296.00 (M^+); Anal. Calcd $C_{13}H_9ClO_4S$: C, 52.62; H, 3.06; S, 10.81. Found: C, 52.48; H, 2.98; S, 10.74; Beilstein test [22]: Cl positive.

*4.2.10. 2-Chloro-3-(2-mercaptoethylthio)naphthalene-1,4-dione (**3j**)*

Orange powder; IR (KBr): 1582 and 1662 (>C=O of quinone) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.61 (bs, 1H, SH), 3.32 (m, 4H, CH_2CH_2), 7.82 (m, 2H, C_5–H and C_8–H), 8.21 (m, 2H, C_6–H and C_7–H); ^{13}CNMR (300 MHz, $CDCl_3$ + MeOH): δ 26.8, 33.1, 126.7, 127.6, 130.8, 131.4, 133.8, 134.7, 140.7, 143.4, 176.0, 178.6; Mass (ESI): 284.07 (M^+, 100%), 285.07 (M^+ + 1, 25%), 286.07 (M^+ + 2, 33%); Anal. Calcd. for $C_{12}H_9ClO_2S_2$: C, 50.61; H, 3.19; S, 22.52. Found: C, 50.48; H, 3.14; S, 22.49; Beilstein test [22]: Cl positive.

*4.2.11. 3-Hydroxy-4-methyl-4H-naphtho[2,3-b][1,4]thiazine-5,10-dione (**3k**)*

Orange solid; IR (KBr): 1549 and 1658 (>C=O of quinone), 3446 (OH) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.69 (s, 1H, OH), 3.34 (s, 3H, NCH_3), 5.92 (s, 1H, CH), 7.62 (m, 2H, C_5–H and C_8–H), 8.01 (m, 2H, C_6–H and C_7–H); Mass (ESI): 259.03 (M^+); Anal. Calcd for $C_{13}H_9NO_3S$: C, 60.22; H, 3.50; N, 5.40; S, 12.37; Found: C, 60.02; H, 3.46; N, 5.38; S, 12.32.

*4.2.12. 2-Hydroxy-3-(phenylthio)naphthalene-1,4-dione (**3l**)*

Orange solid; IR (KBr): 3440 (O–H), 1584 and 1661 (>C=O of quinone) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.27–757 (m, 5H, phenyl), 7.75 (m, 2H, C_5–H and C_8–H), 8.05 (m, 2H, C_5–H and C_8–H), 10.06 (bs, 1H, OH); Mass (ESI): 283.04 (M^+ + 1); Anal. Calcd for $C_{16}H_{10}O_3S$: C, 68.07; H, 3.57; S, 11.36. Found: C, 67.97; H, 3.58; S, 11.31.

*4.2.13. 5,8-Dihydroxy-2-(4-methylpiperazin-1-yl)naphthalene-1,4-dione (**3m**)*

Red solid, IR (KBr): 1598 and 1679 (>C=O of quinone), 3435 (O–H); ^1H NMR (300 MHz, $CDCl_3$): δ 2.22 (t, J = 4.8 Hz, 4H, CH_2NMe), 2.35 (s, 3H, NCH_3), 2.89 (t, J = 4.8 Hz, 4H, NCH_2), 6.59 (s, 1H, C_3–H), 7.69 (d, 1H, J = 8.2 Hz, C_6–H), 7.71 (d, 1H, J = 8.2 Hz, C_7–H), 12.05 (bh, 2H, OH); Mass (ESI): 289.12 (M^+ + 1); Anal. Calcd for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.59; H, 5.60; N, 9.74.

*4.2.14. 5,8-Dihydroxy-2-(pyrrolidin-1-yl)naphthalene-1,4-dione (**3n**)*

The general procedure was followed to give dark violet crystals after crystallization with methanol; IR (KBr): 3427 (O–H), 1598 and 1649 (>C=O of quinone) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 1.78 (m, 4H, CH_2), 2.69 (m, 4H, NCH_2), 6.56 (s, 1H, C_3–H), 7.60 (m, 2H, C_6–H and C_7–H), 12.20 (bh, 2H, O–H); Mass (ESI): 269.09 (M^+ + 1); Anal. Calcd $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.78; H, 5.00; N, 5.37.

*4.2.15. Ethyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)acetate (**3o**) [11]*

The general procedure was followed to give orange crystals on crystallization with EtOH; IR (KBr): 1737 (>C=O of COOEt), 1669 and 1590 (>C=O of quinone) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.19 (t, 3H, J = 6.3 Hz, CH_3), 3.64 (s, 2H, SCH_2), 4.13 (q, 2H, J = 6.3 Hz, OCH_2), 7.62 (m, 2H, C_5–H and C_8–H), 8.16 (m, 2H, C_6–H and C_7–H); Mass (ESI): 310.01 (M^+); Anal. Calcd for $C_{14}H_{11}ClO_4S$: C, 54.11; H, 3.57; S, 10.32. Found: C, 54.28; H, 3.64; S, 10.48.

*4.2.16. Ethyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio)propanoate (**3p**) [11]*

The general procedure was followed to give orange crystals after crystallization with EtOH; IR (KBr): 1738 (>C=O of COOEt), 1669 and 1591 (>C=O of quinone) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.09 (t, 3H, J = 6.3 Hz, CH_3), 1.57 (d, 3H, J = 7.2 Hz, CH_3), 4.06 (q, 2H, J = 6.3 Hz, OCH_2), 4.68 (q, 1H, J = 7.2 Hz, SCH), 7.72 (m, 2H, C_5–H and C_8–H), 8.05 (m, 2H, C_6–H and C_7–H); Mass (ESI): 324.02 (M^+); Anal. Calcd for $C_{15}H_{13}ClO_4S$: C, 55.47; H, 4.03; S, 9.87. Found: C, 55.36; H, 3.98; S, 9.77.

*4.2.17. 3-Hydroxy-2,4-dimethyl-4H-naphtho[2,3-b][1,4]-thiazine-5,10-dione (**3q**) [11]*

The general procedure was followed to give orange crystals after crystallization with EtOH; IR (KBr): 3443 (OH), 1654 and 1542 (>C=O of quinone) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 1.57 (s, 3H, CH_3), 1.59 (s, 1H, OH), 3.47 (s, 3H, NCH_3), 7.59–7.74 (m, 2H, C_5–H and C_8–H), 8.02–8.16 (m, 2H, C_6–H and C_7–H); ^{13}CNMR ($CDCl_3$): δ 14.50, 35.00, 36.99, 99.98, 126.49, 127.16, 131.20, 131.96, 132.52, 134.03, 134.19, 141.54, 166.07, 180.63; Mass (ESI): 273 (M^+); Anal. Calcd for $C_{14}H_{11}NO_3S$: C, 61.52; H, 4.06; N, 5.12; S, 11.73. Found: C, 61.68; H, 4.14; N, 5.30; S, 11.84.

*4.3. Crystallographic data of 3-hydroxy-2,4-dimethyl-4H-Naphtho [2,3-b][1,4]-thiazine-5,10-dione (**3q**) [11, 25]*

Monoclinic, Space group P 21/n; a = 10.6307(16) Å, b = 8.1826 (12) Å, c = 14.619(2) Å, α = 90, β = 101.380(2), γ = 90; V = 1246.62 Å^3, Z = 4, T = 296 K, λ = 0.71073 Å, F(000) = 544, Crystal size = 0.44 × 0.31 × 0.25 mm^3, Theta range for data collection = 4.72–32.45°,Index ranges = $-23 \le h \le 21$, $-10 \le k \le 10$, $-14 \le l \le 17$, Reflections collected = 11 868, Independent reflections = 2365 [R(int) = 0.0338], Completeness to theta = 25.00°, 99.0%, Absorption correction = Semi-empirical from equivalents, Max. and min. transmission = 1.00000 and 0.84802, Refinement method = Full-matrix least-squares on F^2, Data/restraints/parameters = 2365/0/109, Goodness-of-fit on F^2 = 1.135, Final R indices [I > 2sigma(I)] $R1$ = 0.0615, w$R2$ = 0.2005, R indices (all data), $R1$ = 0.1158, w$R2$ = 0.2349, Largest diff. peak and hole = 0.592 and −0.411 e Å^3 Symmetry: a) x,y,z; b) 1/2−x,1/2 + y,1/2−z; c) −x,−y,−z; d) 1/2 + x,1/2−y,1/2 + z (For detail see Supplementary data).

4.4. In vitro antifungal and antibacterial evaluation by MIC assay

The compounds **3a**–**n** 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 *E. coli*, *S. aureus* (ATCC25923), *Klebsiella pneumoniae* (ATCC 27736) and *P. aeruginosa* at the Division of Fermentation Technology of Central Drug Research Institute, Lucknow, India. In this process minimum inhibitory concentration of compounds **3a**–**n** was tested according to standard micro broth dilution as per NCCLS [23,24] protocol. Briefly, testing was performed in flat bottom 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. Initial inoculums of fungal and bacterial strain were maintained at 1–5 × 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 micro plate 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).

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.02.023.

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[25] Crystallographic data for the structure of Compound 4 have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC765321 Copies of data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0) 1223 33603 or e-mail: deposit@ccdc.cam.ac.uk]. Each request should be accompanied by the complete citation of this publication.
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