European Journal of Medicinal Chemistry 56 (2012) 375–386

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Micelles catalyzed chemo- and regio-selective one pot and one step synthesis of 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-1,4-benzoquinones and 2,5-diaminosubstituted-1,4-benzoquinones "In-Water" and their biological evaluation as antibacterial and antifungal agents

Vishnu K. Tandon [a,*], Sandeep Kumar [a], Nripendra N. Mishra [b], Praveen K. Shukla [b]

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

ARTICLE INFO

Article history:
Received 31 January 2012
Received in revised form
7 July 2012
Accepted 16 July 2012
Available online 1 August 2012

Keywords:
Water
Antifungal
Antibacterial
Chloranil
Micelles
1,4-Benzoquinone

ABSTRACT

Chemo- and regio-selective one pot and one step synthesis of novel 2,3,5,6-tetrakis (substituted thio) cyclohexa-2,5-diene-1,4-diones (**4d**–**14**), 2,5-dichloro-3,6-diaminocyclohexa-2,5-diene-1,4-diones and 2,5-diaminocyclohexa-2,5-diene-1,4-diones (**16**) by economical green methodology approach using LD (Laundry detergent) as a catalyst "In-Water" by nucleophilic addition and substitution reactions of 1,4-benzoquinone and chloranil with sulfur and nitrogen nucleophiles in high yields has been demonstrated. The antifungal profile of **4** and **16** indicates that compounds **4d** and **16f** had better antifungal activity compared to clinically prevalent antifungal drugs Fluconazole, 5-Fluorocytosine and Clotrimazole against *Sporothrix schenckii* and *Trichophyton mentagraphytes*. **16f** had also been found to possess better antibacterial activity compared to Ampicillin *in vitro* against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Compound **16f** did not exhibit any toxicity towards mammalian cells L929.

1. Introduction

In recent years organic reactions performed in water have attracted considerable attention due to unique property of water in promoting reactions and enhancing selectivity [1]. In addition to water being a green solvent and noninflammable, enhancing selectivity effects of organic reactions "In-Water" by hydrophobic effects have been explored by Breslow [2].

The extensive use of water as a medium for organic synthesis has been due to concepts and language by Sharpless et al. [3] who carried out reactions "On-Water" for cases where reactants are insoluble in water.

The surfactant-type catalyst play a dual role both as a catalyst to activate the substrate molecules and as a surfactant to increase the concentration of organic reactants to form micelle particles in water which have been extensively used [4].

In connection with our studies on the reactivity of quinones with nitrogen and sulfur nucleophiles "On-Water" [5] and the utility of surfactants in aqueous medium [4,6–10], we envisaged the reaction of 1,4-benzoquinone and chloranil with sulfur and nitrogen nucleophiles "In-Water" using Laundry detergent (LD, washing powder) as surfactant. Laundry detergent (LD, washing powder) has low cost and is economically viable [11] as compared to other traditional expensive surfactants [4].

The thioether and amino derivatives of 1,4-benzoquinones have exhibited pronounced biological activities due to their redox potentials [12,13]. These derivatives have been found to possess marked antiviral [14], molluscicidal [15], antimalarial [16], anti-leishmanial [17], antiproliferative [18], antibacterial and antifungal activities [18–24]. The profound antifungal activity exhibited by compounds **I**–**III** (Fig. 1) [25–27] prompted us to explore the synthesis of tetraalkyl and tetraarylthio-1,4-benzoquinones as well as 2,5-diaminosubstituted-1,4-benzoquinones in one pot and one step using micelles as catalyst "In-Water". We report herein a green methodology approach in quinone chemistry to carry out "In-Water" micelles catalyzed synthesis and biological evaluation of potent antibacterial and antifungal agents.

* Corresponding author. Tel.: +91 6415066847; fax: +91 522 26848.
E-mail address: vishnutandon@yahoo.co.in (V.K. Tandon).

0223-5234/$ – see front matter
http://dx.doi.org/10.1016/j.ejmech.2012.07.022

2. Results and discussion

2.1. Chemistry

The nucleophilic addition and substitution reactions of 1,4-naphthoquinone derivatives "On-Water" and "In-Water" leading to formation of mono and disubstituted derivatives of 1,4-naphthoquinones have recently been reported by us [5].

The green methodology approach to regioselective and chemoselective nucleophilic substitution reactions of chloranil with alkyl and arylthiols and secondary amines under heterogeneous conditions by the use of K_2CO_3 as a base and water as a solvent to afford various sulfur and nitrogen containing benzoquinone derivatives has been described in detail in this manuscript. The nucleophilic addition reactions of 1,4-benzoquinone with sulfur and nitrogen nucleophiles "On-Water" and "In-Water" have also been studied in detail and compared with nucleophilic substitution reactions of chloranil.

Although the nucleophilic addition reactions of p-benzoquinone with alkanethiols have been extensively studied by Katritzky et al. [28], a mixture of mono and di-substituted products along with their quinol derivatives were isolated in low yields. 2,3,5,6-Tetrakis(alkylsulfanyl)-p-benzoquinones were obtained by Katritzky et al. [28] by further reaction of 2,5-disubstituted derivatives with alkanethiols, the overall yield of 2,3,5,6-tetrakiscyclohexylsulfanyl-p-benzoquinone by the reaction of p-benzoquinone with cyclohexanethiol being 15%.

Ibis et al. [29] have recently reported synthesis of monoalkoxy, tris(thio) and tetrakisthiosubstituted-p-benzoquinones by the reaction of chloranil with various sulfur nucleophiles in alcohol in the presence of sodium carbonate. However, their synthetic procedure led to isolation of mixture of products. A new regioselective, short and high yielding synthetic route for the synthesis of 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones was therefore, desired.

We envisaged a green methodology approach towards synthesis of 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones.

We first explored the nucleophilic addition reactions of benzenethiol (**2a**) on 1,4-benzoquinone (**1a**) in aqueous medium with or without using surfactant as catalyst. In general the mechanism of nucleophilic addition reactions of 1,4-benzoquinone (**1a**) has been outlined in Fig. 2.

Conjugate addition reaction of 1,4-benzoquinone (**1a**) with benzenethiol (**2a**) in 4 mol equivalent in "On-Water" at room temperature for 6 h gave three isolated products viz; hydroquinone, 2,5-bis(phenyl thio)benzene-1,4-diol (**3a**) and 2,5-bis(phenylthio) cyclohexa-2,5-diene-1,4-dione (**3e**) [30] in yields of 45%, 8%, and 12% respectively. The products were purified by silica gel column chromatography and by crystallization from ethyl acetate:hexane (4:1). The basis of the regioselectivity in the formation of 2,5-disubstituted arylthio and alkylthio-1,4-benzoquinones has been unambiguously explained by Katritzky et al. [28] and Becker et al. [30] (Scheme 1).

In order to compare the nucleophilic addition reactions with nucleophilic substitution reactions, we further explored the nucleophilic substitution reactions of chloranil (**1b**) with various sulfur nucleophiles "On-Water". We found that the reaction of chloranil (**1b**) in aqueous medium with benzenethiol (**2a**) at room temperature for 6 h gave the four isolated products viz; 2-hydroxy-3,5,6-tris(phenylthio)cyclohexa-2,5-diene-1,4-dione (**4a**), 2,5-dichloro-3,6-bis(phenylthio)cyclohexa-2,5-diene-1,4-dione (**4b**), 2-chloro-3,5,6-tris(phenylthio)cyclohexa-2,5-diene-1,4-dione (**4c**) and 2,3,5,6-tetrakis(phenylthio)cyclohexa-2,5-diene-1,4-dione (**4d**) in 7%, 10%, 16% and 2% yields respectively (Scheme 2).

Analogous reaction of chloranil (**1b**) with naphthalene-1-thiol (**2b**) and 4-methylbenzenethiol (**2c**) under identical conditions gave mixture of isolated products viz; 2-hydroxy-3,5,6-tris(naphthalen-1-ylthio)cyclohexa-2,5-diene-1,4-dione (**5a**), 2,5-dichloro-3,6-bis-(naphthalen-1-ylthio)cyclohexa-2,5-diene-1,4-dione (**5b**) and 2-chloro-3,5,6-tris(naphthalen-1-ylthio)cyclohexa-2,5-diene-1,4-dione (**5c**) in 4%, 25% and 8% yields respectively and 2-hydroxy-3,5,6-tris(p-tolylthio)cyclohexa-2,5-diene-1,4-dione (**6a**), 2,5-dichloro-3,6-bis(p-tolylthio)cyclohexa-2,5-diene-1,4-dione (**6b**), 2,3,5,6-tetrakis(p-tolylthio)cyclohexa-2,5-diene-1,4-dione (**6d**) in 15%, 5% and 3.5% yields respectively. The products were separated by silica gel column chromatography and by crystallization from ethyl acetate:hexane (4:1) (Scheme 2). It was observed that analogous nucleophilic substitution reactions of chloranil "On H_2O" in absence of LD as surfactant with sulfur nucleophiles (in 4 mol eq.) led to formation of complex mixture of di, tri and tetra substituted products, the yield of 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones being 2–8%.

In order to synthesize 2,3,5,6-tetrakis(substituted-thio)cyclohexa-2,5-diene-1,4-diones, we further carried out the reaction of p-benzoquinone (**1a**) with benzenethiol (**2a**) "In-Water" in presence of 0.5 mol% LD and K_2CO_3 at room temperature for 2 h to yield 2,3,5,6-tetrakis(phenylthio)cyclohexa-2,5-diene-1,4-dione (**4d**) in 51% yield. 2,3,5,6-tetrakis(phenylthio)cyclohexa-2,5-diene-1,4-dione (**4d**) was isolated as red colored solid by filtration from reaction mixture and crystallization of residue from ethyl acetate:hexane (4:1) (Scheme 3).

In order to study the effect of various solvents on the yields, and compare nucleophilic addition and nucleophilic substitution reactions we carried out nucleophilic substitution and addition reactions of chloranil (1b) and benzoquinone (1a) with benzenethiol (2a) in different solvents under identical conditions and found that 2,3,5,6-tetrakis(phenylthio)cyclohexa-2,5-diene-1,4-dione (4d) is best prepared using LD and K_2CO_3 in water at room temperature by reaction of chloranil and thiophenol (Scheme 4).

Using these optimized reaction conditions, we carried out further nucleophilic substitution reactions of chloranil (**1b**) with different sulfur nucleophiles in water using LD as surfactant and K_2CO_3 as base and found that 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones are the only isolable products in high yields. The plausible mechanism of nucleophilic substitution reactions of chloranil has been outlined in Fig. 3.

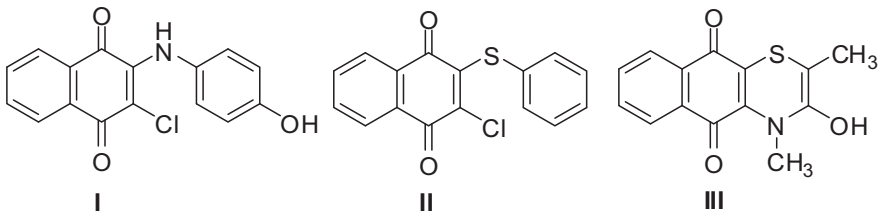


Fig. 1. Lead antifungal agents **I** [25], **II** [26], and **III** [27].

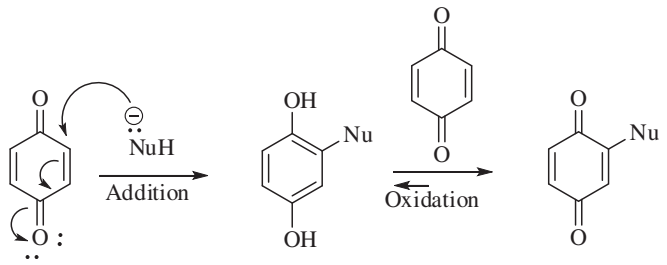
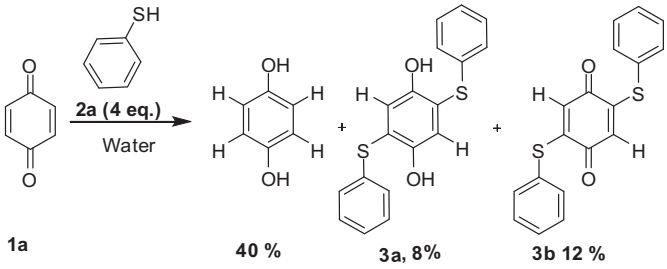


Fig. 2. Addition reactions of 1,4-benzoquinone (**1a**).



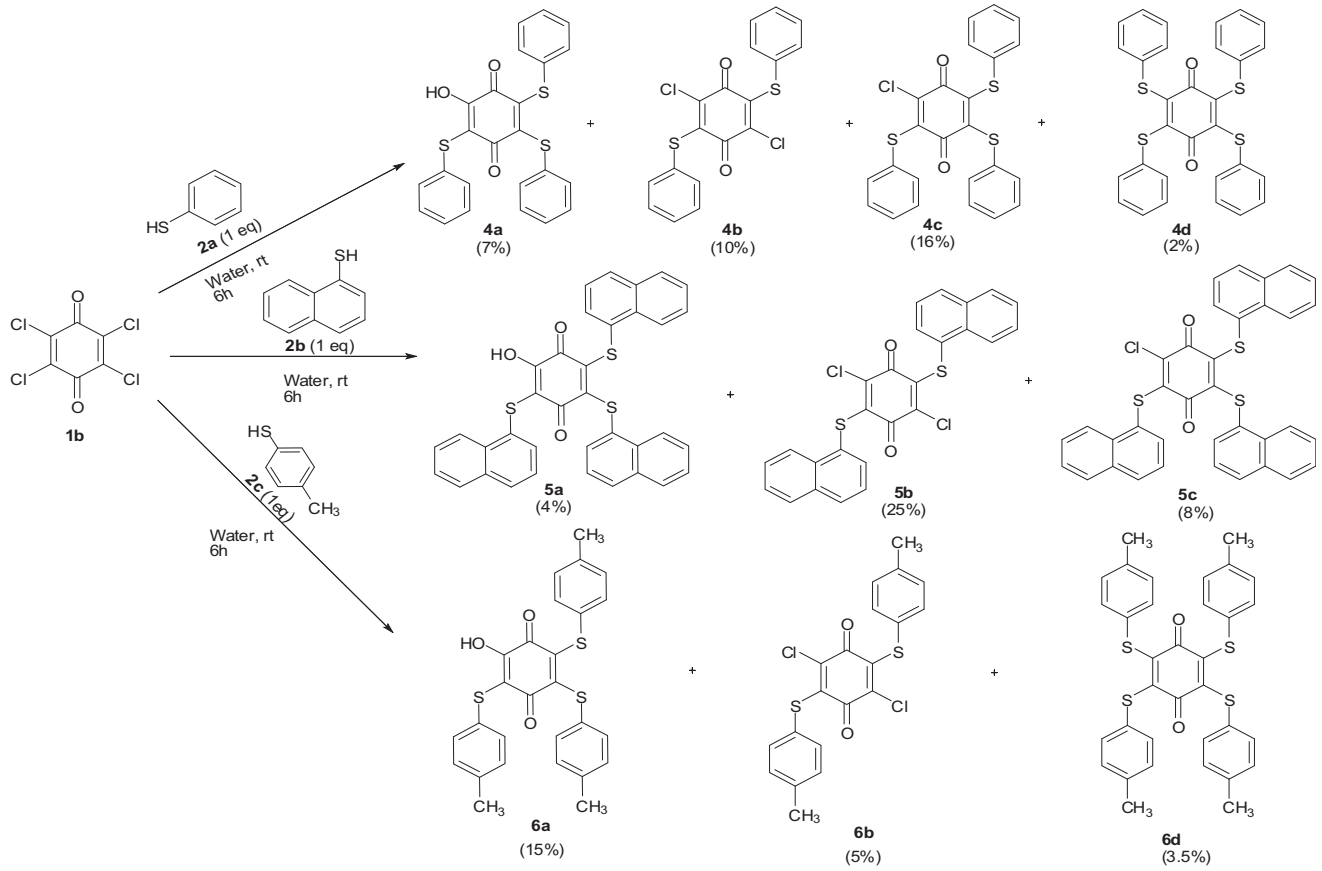
Scheme 1. Nucleophilic addition reaction of 1,4-benzoquinone with benzenethiol (**2a**).

Thus the nucleophilic substitution reaction of chloranil (**1b**) with compounds (**2**), in water in the presence of 0.5 mol % of LD at room temperature for 2–4 h led to isolation of 2,3,5,6-tetrakis(alky and arylsulfanyl)-p-benzoquinones (**4d**–**14**) (Scheme 5). The products were filtered from the reaction mixture and solids thus obtained were crystallized with ethyl acetate:hexane (4:1) leading to the formation of novel 2,3,5,6-tetrakis(alky and arylsulfanyl)-p-benzoquinones (**4d**–**14**), in high yields (Scheme 5).

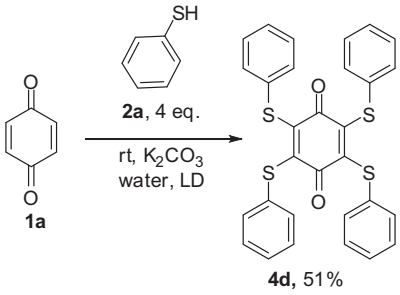
The reaction of naphthalene-1-thiol (**2b**) with chloranil under aforesaid conditions did not afford 2,3,5,6-tetrakis(naphthalen-1-ylthio)cyclohexa-2,5-diene-1,4-dione due to steric bulk of the substituent. The formation of the aforesaid products is explained on the basis of the micelles catalyzed nucleophilic substitution reactions of chloranil (Fig. 4). The mechanism of action of surfactant as catalyst has been extensively studied by Shiri et al. The surfactants play a dual role as a catalyst to activate the substrate molecules as well as increase the concentration of organic reactants to form micelle particles in water.

Analogous nucleophilic substitution and addition reactions were carried out with nitrogen nucleophiles (**15**) to study "In-Water" addition reactions of benzoquinone (**1a**) and substitution reactions of chloranil (**1b**). The disubstituted products were the only products formed in excellent yields using LD and K_2CO_3 in aqueous medium at rt for 6–7 h, with chloranil (**1b**) (Scheme 6).

The formation of regioselective 2,5-diaminosubstituted-p-benzoquinones **16**(**a**–**j**) rather than 2,3,5,6-tetraaminosubstituted-p-benzoquinones analogous to 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones (**4d**–**14**) is due to the fact that the nucleophilic nitrogen atoms in **16** deactivates the adjacent chlorine atoms of 1,4-benzoquinone ring, thereby preventing further the attack of nucleophilic nitrogen on carbon atom of C–Cl bond. On further reaction of Sulfur nucleophiles **2** with compound **16**, the facile nucleophilic substitution took place under the identical



Scheme 2. "On-Water" nucleophilic substitution reactions of chloranil with arylthiols: Reaction mixtures were filtered and crude solids were purified by column chromatography and crystallization.



Scheme 3. Nucleophilic addition of benzenethiol (**2a**) to 1,4-benzoquinone (**1a**) to produce 2,3,5,6-tetrakis(phenylthio)cyclohexa-2,5-diene-1,4-dione.

conditions leading to the formation of 2,5-bis(alkyl and arylthio)-3,6-diaminocyclohexa-2,5-diene-1,4-diones (**17**) (Scheme 6).

It is interesting to note that reactions of nucleophiles with benzoquinones were augmented due to oxidative addition pathway and thus lower yields were obtained for the addition products as compared to substitution products (Figs. 2 and 3).

2.2. Antibacterial activity

The evaluation of antibacterial activities of compounds **3**–**16** against various strains of the bacteria, for example, *Escherichia coli* (ATCC 9637), *Pseudomonas aeruginosa* (ATCC BAA-427), *Staphylococcus aureus* (ATCC 25923) and *Klebsiella pneumoniae* (ATCC 27736) was carried out according to the broth micro-dilution technique described by NCCLS [31,32]. The minimum inhibitory concentration (MIC) of each compound was determined against test isolates using this technique. The MICs of standard antibacterial drugs Ampicillin and Gentamycin were determined in 96-well tissue culture plates using Muller-Hinton broth.

Table 1 shows antibacterial activity of compounds **3**–**16**. The antibacterial activity was compared with Ampicillin and Gentamycin which were used as positive control in all tests with MIC values expressed in μg/mL. Compound **5c** (MIC = 12.5 μg/mL) had

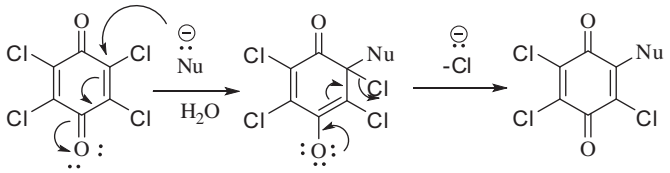


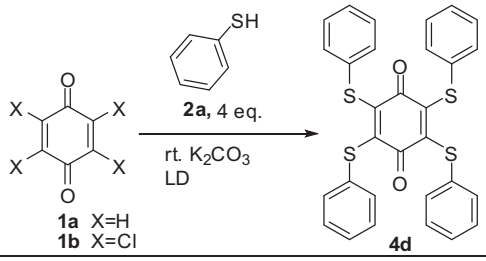
Fig. 3. Nucleophilic substitution reactions of Chloranil (**1b**).

same activity when compared with Ampicillin (MIC = 12.50 μg/mL) and **16f** (MIC = 6.25 μg/mL) showed better antibacterial activity than Ampicillin (MIC = 12.50 μg/mL) against *S. aureus*. Compound **16f** (MIC = 6.25 μg/mL) also exhibited better antibacterial activity than Ampicillin (MIC = 12.50 μg/mL and MIC = >50.00 μg/mL) against *E. coli* and *K. pneumoniae* respectively (Table 1, Fig. 5). On comparison of antibacterial activity of the compounds referred to in Table 1, it was observed that none of the compounds showed better antibacterial activity than Gentamycin.

The study of structure activity relationship in **3**–**16** revealed that 2-chloro3,5,6-tris(arylsulfanyl)-p-benzoquinone derivative **5c** (MIC = 12.50) showed promising antibacterial activity against *S. aureus* referred to in Table 1 than the 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones **4d**, **6d**, **8** and **13**. 2,5-Dichloro-3,6-bis(4-methylpiperazin-1-yl)cyclohexa-2,5-diene-1,4-dione **16f** showed promising antibacterial activity against most of the bacteria when compared with Ampicillin referred to in Table 1 than the analogous morpholino, piperidinyl and pyrolidinyl analogs **16e**, **16i** and **16j**.

2.3. Antifungal activity

The antifungal activity of compounds **3**–**16** against various strains, 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 [31,32]. The Minimum Inhibitory Concentration (MIC) of each compound was determined against test isolates using this



Entry	X	Solvent	base	surfactant	Time[c]	Yield(%)
1	Cl	Ethanol	K_2CO_3	NA	6h	27[a]
2	Cl	THF	K_2CO_3	NA	6h	64[a]
3	Cl	Water	NA	NA	6h	02
4	H	Water	NA	NA	6h	0
5	Cl	Water	K_2CO_3	LD	6h	80[b]

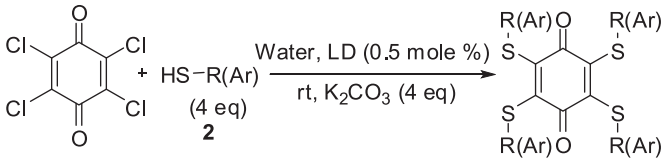
[a]Reaction in water required isolation of product by pouring the reaction mixture in water followed by filtration and recrystallisation

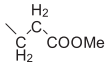
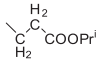
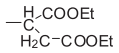
[b]"In-Water" the reaction mixture is directly filtered and crystallized

NA= Not Applicable

[c]There was little or no variation in yields if reactions were carried out with chloranil after 4 h.

Scheme 4. Reactions of 1,4-benzoquinone (**1a**) and chloranil (**1b**) with benzenethiol (**2a**) to yield 2,3,5,6-tetrakis(phenylsulfanyl)-p-benzoquinone in various polar solvents.



Entry	4d-14	-R(Ar)	Optimized time	Yield (%)[a]
1	**4d**		2h	80
2	**6d**		2h	80
3	**7**		3h	85
4	**8**		3h	82
5	**9**		4h	76
6	**10**		3h	83
7	**11**		2.5h	75
8	**12**		3h	76
9	**13**		4h	79
10 [28]	**14**		3h	79

[a]The reaction mixtures were directly filtered and crystallized to get pure products without column chromatography

Scheme 5. "In-Water" substitution reactions of chloranil with aryl and alkyl thiols.

technique. The antifungal activity was compared with those of standard drugs Miconazole, Nystatin, Fluconazole and Amphotericin-B. MIC of standard drugs referred in Table 2 and the compounds **3**–**16** were determined in 96-well tissue culture plates using RPMI 1640 media buffered with MOPS (3-(*N*-morpholino)-propanesulfonic acid) (Sigma Chemical Co.).

Comparison of the activity of compounds **3**–**16** referred to in Table 2 *in vitro* with that of antifungal drug Fluconazole (MIC = 4.00 μg/mL and MIC = 16.00 μg/mL) showed that 2,3,5,6-tetrakis(phenylthio) cyclohexa-2,5-diene-1,4-dione **4d** (MIC = 1.56 μg/mL and MIC = 0.78 μg/mL) had better antifungal activity against fungi *S. schenckii*[a,b,c] and *T. mentagraphytes*[a,b,c] respectively (Table 2). Compound **4d** (MIC = 1.56 μg/mL and MIC = 0.78 μg/mL) also exhibited better antifungal activity compared to antifungal drug 5-Fluorocytosine (MIC = >32.00 μg/mL and MIC = >32.00 μg/mL) and Clotrimazole (MIC = 4.00 μg/mL and MIC = 2.00 μg/mL) against *S. schenckii*[a,b,c] and *T. mentagraphytes*[a,b,c]. Amongst 2,5-diaminosubstituted-p-benzoquinones **16**, only compound 2,5-dichloro-3,6-bis(4-methylpiperazin-1-yl)cyclohexa-2,5-diene-1,4-dione **16f** (MIC = 1.56 μg/mL, MIC = 1.56 μg/mL, MIC = 0.78 μg/mL and MIC = 12.50 μg/mL) showed better antifungal activity against fungi *C. neoformans*, *S. schenckii*, *T. mentagraphytes* and *A. fumigatus* on comparison with antifungal drug Fluconazole (MIC = 2.00 μg/mL, MIC = 4.00 μg/mL, MIC = 16.00 μg/mL and MIC = 32.00 μg/mL) respectively. Compound **16f** (MIC = 1.56 μg/mL, MIC = 0.78 μg/mL and MIC = 12.50 μg/mL) also exhibited better antifungal activity on comparison with 5-Fluorocytosine (MIC = >32.00 μg/mL, MIC >32.00 μg/mL and MIC > 32.00 μg/mL) against *S. schenckii*, *T. mentagraphytes* and *A. fumigatus* respectively. When compared with Clotrimazole (MIC = 4.00 μg/mL and MIC = 2.00 μg/mL), compound **16f** (MIC = 1.56 μg/mL and MIC = 0.78 μg/mL), exhibited better antifungal activity against *S. schenckii* and *T. mentagraphytes* respectively.

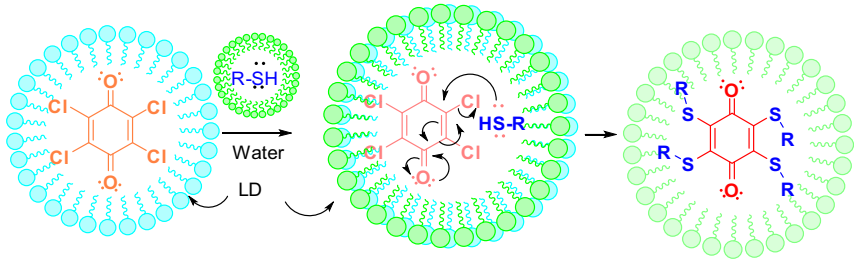
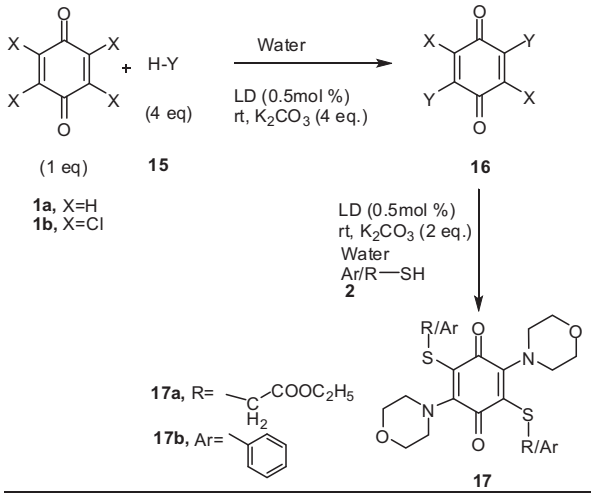
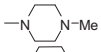
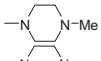
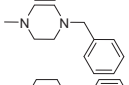
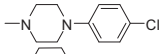


Fig. 4. Effect of aqueous micelle: reaction mechanism of chloranil in water using LD as surfactant.



Entry	16	-Y	X	Optimized Time	Yield[a] (%)
1	**a**		H	6h	64
2	**b**		H	6h	72
3	**c**		H	7h	69
4	**d**		H	6h	76
5	**e**		Cl	6h	78
6	**f**		Cl	7h	86
7	**g**		Cl	6h	82
8	**h**		Cl	6h	80
9	**i**		Cl	7h	88
10	**j**		Cl	6h	81

[a]The reaction mixture were directly filtred and crystallized to get pure products without column chromatography

Scheme 6. "In-Water" addition and substitution reactions of benzoquinone (**1a**) and chloranil (**1b**) with nitrogen nucleophiles.

Table 1
In vitro antibacterial activity of compounds **3**–**16**.

Compound	MIC (μg/mL)			
	E. coli	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*
3e	>50.00	>50.00	>50.00	>50.00
4a	>50.00	>50.00	>50.00	>50.00
4b	>50.00	>50.00	50.00	>50.00
4c	>50.00	>50.00	50.00	>50.00
4d	>50.00	>50.00	>50.00	>50.00
5a	>50.00	>50.00	>50.00	>50.00
5b	>50.00	>50.00	25.00	>50.00
5c	>50.00	>50.00	**12.50**	>50.00
6a	>50.00	>50.00	>50.00	>50.00
6b	>50.00	>50.00	50.00	>50.00
6d	>50.00	>50.00	>50.00	>50.00
8	>50.00	>50.00	50.00	>50.00
13	>50.00	>50.00	>50.00	>50.00
16e	>50.00	>50.00	25.00	>50.00
16f	**6.25**[a]	>50.00	**6.25**[a]	**6.25**[a]
16i	>50.00	>50.00	>50.00	>50.00
16j	>50.00	>50.00	>50.00	>50.00
Ampicillin	12.50	>50.00	12.50	>50.00
Gentamycin	1.56	0.39	1.56	1.56

[a] Entries in bold indicate better antibacterial activity than reference drug Ampicillin.

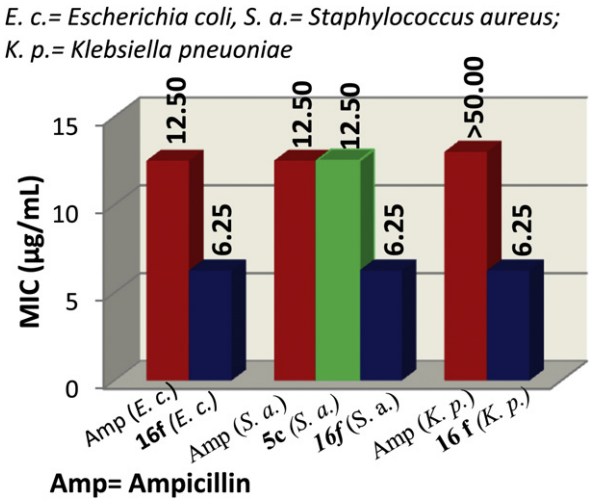


Fig. 5. Comparative antibacterial study plot with Ampicillin (Amp), compounds **16f**, **5c** and pathogens.

Table 2
In vitro antifungal activity of compounds **3**–**16**.

Compounds	MIC (μg/mL)					
	C. albicans	*C. neoformans*	*S. schenckii*	*T. mentagraphytes*	*A. fumigatus*	*C. parapsilosis*
3e	25.00	25.00	50.00	50.00	>50.00	50.00
4a	12.50	12.50	50.00	50.00	>50.00	12.50
4b	25.00	25.00	50.00	50.00	>50.00	25.00
4c	25.00	25.00	50.00	50.00	>50.00	25.00
4d	12.50	12.50	**1.56**[a,b,c]	**0.78**[a,b,c]	50.00	6.25
5a	25.00	25.00	>50.00	50.00	>50.00	25.00
5b	25.00	25.00	50.00	50.00	>50.00	12.50
5c	25.00	25.00	50.00	50.00	>50.00	12.50
6a	12.50	25.00	>50.00	50.00	>50.00	25.00
6b	25.00	25.00	50.00	50.00	>50.00	25.00
6d	25.00	>50.00	50.00	>50.00	>50.00	25.00
8	25.00	50.00	>50.00	50.00	>50.00	25.00
13	25.00	50.00	>50.00	50.00	>50.00	25.00
16e	12.50	12.50	**12.50**[b]	**6.25**[b,c]	>50.00	12.50
16f	6.25	**1.56**[c]	**1.56**[a,b,c]	**0.78**[a,b,c]	**12.50**[b,c]	6.25
16i	25.00	25.00	**25.00**[b]	**12.50**[b]	>50.00	25.00
16j	25.00	25.00	>50.00	50.00	>50.00	50.00
Clotrimazole	0.25	0.25	4.00	2.00	8.00	1.00
5-Fluorocytosine	0.25	0.13	>32.00	>32.00	>32.00	0.13
Fluconazole	1.00	2.00	4.00	16.00	>32.00	0.50

[a] Entries in bold font indicate better activity than reference drug Clotrimazole.
[b] Entries in bold font indicate better activity than reference drug 5-Fluorocytosine.
[c] Entries in bold font indicate better activity than reference drug Fluconazole.

In addition to **16f**, **16e** (MIC = 12.50 μg/mL and MIC = 6.25 μg/mL) and **16i** (MIC = 25.00 μg/mL and MIC = 12.50 μg/mL) exhibited better antifungal activity against *S. schenckii* and *T. mentagraphytes* on comparison with 5-Fluorocytosine (MIC = >32.00 μg/mL and MIC = >32.00 μg/mL) respectively (Table 2, Fig. 6).

2.4. Toxicological studies

The compound **16f** exhibited profound antifungal activity and low MIC values against fungi *C. neoformans*, *S. schenckii*, *T. mentagraphytes* and *A. fumigatus* hence its detailed toxicological studies on mammalian cells L929 was carried out. Morphological anomalies in L929 cells exposed to compound **16f** were evident under phase contrast microscope. L929 cells in control were fairly transparent and attached to the surface of the wells of tissue culture plate (Fig. 7a). The compound did not exhibit any toxicity to L929 cells at MIC and lower concentrations, as was evident from their normal morphology (Fig. 7d and e). However, when exposed to higher concentrations (50 and 6.25 μg/mL), the L929 cells lost their normal morphology (Fig. 7b and c). The MTT assay revealed >90% viability of L929 cells even at higher concentrations.

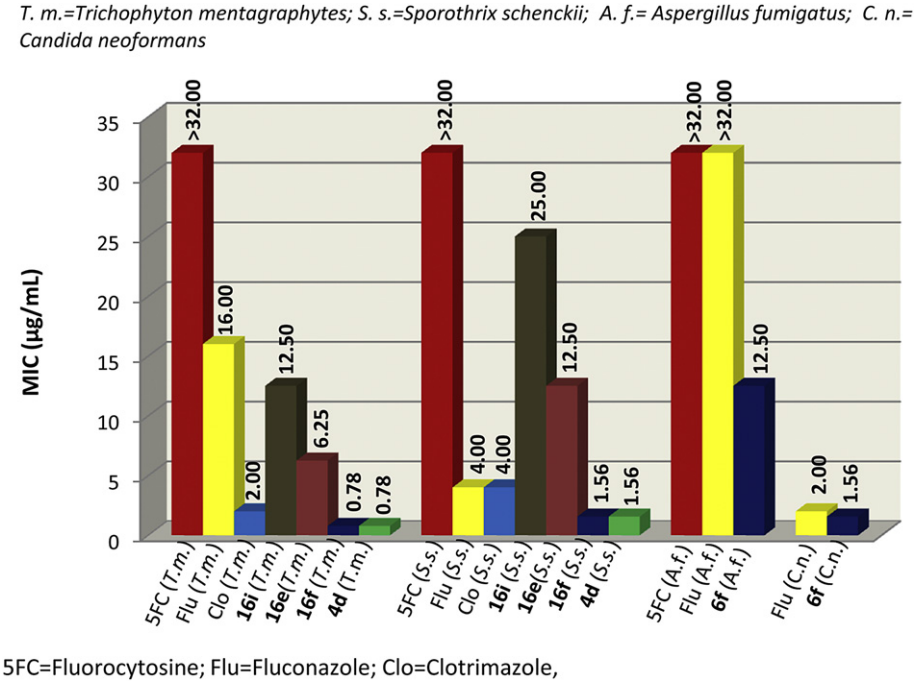


Fig. 6. Comparative antifungal study plot with Miconazole (MIN), Nystatin (NYS), Fluconazole (FLU), Amphotericin-B (AMP), compounds and pathogens.

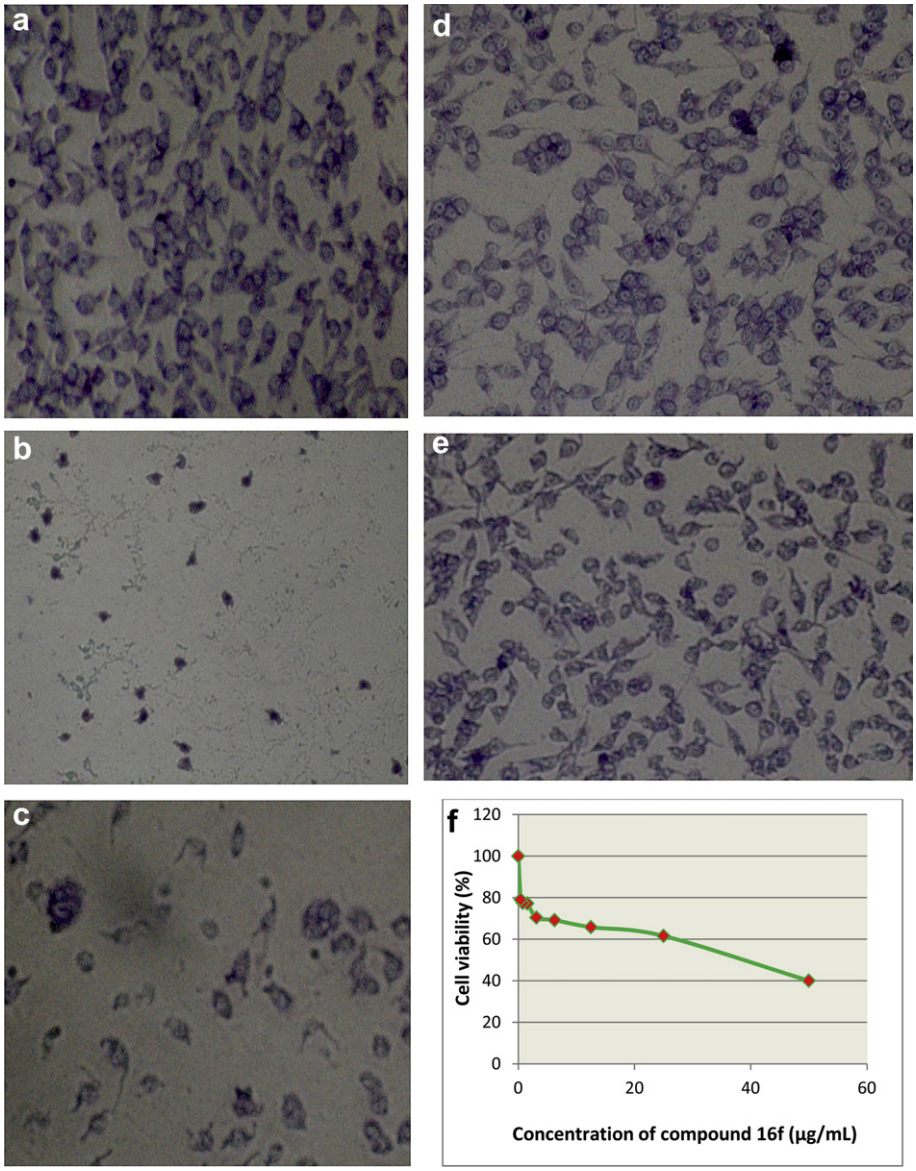


Fig. 7. a. Normal growth of L929 cells. b. Morphological changes in L929 cells at 50 µg/mL of compound **16f**. c. Morphological changes in L929 cells at 6.25 µg/mL of compound **16f**. d. Morphological changes in L929 cells at 1.56 µg/mL of compound 16f. e. Morphological changes in L929 cells at 0.39 µg/mL of compound **16f**. f. Viability (determined by MTT assay) of L929 cells exposed to compound **16f** after 24 h.

3. Conclusion

In conclusion, we have synthesized a series of 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones and 2,5-diaminosubstituted-p-benzoquinones **4**–**16**. Amongst the promising compounds containing sulfur atom in alkyl and arylsulfanyl-p-benzoqinones, compound **4d** showed better antifungal activity than antifungal drugs Fluconazole and Flucytosine *in vitro* against *S. schenckii*, and *T. mentagraphytes*. Amongst 2,5-diaminosubstituted-p-benzoquinones, compound **16f** exhibited better antifungal activity when compared to antifungal drug Fluconazole, against *C. neoformans*, *S. schenckii*, *T. mentagraphytes* and *A. fumigatus*. In addition to profound antifungal effects of **16f** when compared to Fluconazole, **16f** exhibited better antifungal activity against antifungal drugs 5-Fluorocytosine and Clotrimazole against fungi *S. schenckii* and *T. mentagraphytes*. Compound **16f** also showed better antibacterial activity on comparison with antibacterial drug Ampicillin against *E. coli*, *S. aureus* and *K. Pneumonia in vitro*.

Thus compound **16f** is the lead compound for both antifungal and antibacterial activities. The compound **16f** did not exhibit any toxicity towards mammalian cells L929. Further *in vivo* studies on compound **16f** are 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 FTIR 8201 PC, Shimadzu spectrophotometers in KBr discs. Nuclear Magnetic Resonance (NMR) spectra were recorded on Perkin Elmer model R.32 spectrophotometers using TMS as an internal standard. Progress of the reaction and purity of the compound were monitored by thin layer chromatography (TLC),

which was performed on silica gel G and compound were detected with UV chamber, where ever required. Spectral facilities were provided 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 synthesis and spectral data of mono, di, tri and tetra substituted- p-benzoquinones

Thiols (4.2 eq or 1.2 eq) were added to 10 mL distilled water. After stirring for 5 min, was added chloranil(1 eq) or benzoquinone (1 eq) and the resulting reaction mixture stirred at r.t. for 4–6 h and the water layer was decanted and the residue dissolved in chloroform and purified by silica gel column chromatography using ethylacetate and hexane as eluent to give products (**3a**–**6d**).

4.2.1. 2,5-Bis(phenylthio)benzene-1,4-diol (*3a*)

Colorless crystals; mp 110–111 °C; yield 8%; IR (KBr/cm^{-1}): 3427 (OH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.45 (S, 2H), 6.98–7.00 (m, 6H, Ar–H), 7.09–7.19 (m, 6H, Ar–H) ppm; HRMS (ESI): MH$^+$ calcd for $C_{18}H_{15}O_2S_2$ 327.0513, found, 327.0532.

4.2.2. 2,5-Bis(phenylthio)cyclohexa-2,5-diene-1,4-dione (*3b*)

Red crystals; mp 183–184 °C; yield 12%; IR (KBr/cm^{-1}): 1675, 1535 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.77 (s, 2H, quinone H), 7.48–7.52 (m, 10H, Ar–H) ppm; HRMS (ESI): MH$^+$ calcd for $C_{18}H_{13}O_2S_2$, 325.0357, found, 325.0357.

4.2.3. 2-Hydroxy-3,5,6-tris(phenylthio)cyclohexa-2,5-diene-1,4-dione (*4a*)

Colorless crystals; mp 70–71 °C; yield, 7%; IR (KBr/cm^{-1}): 3466, (OH) 1655, 1570 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24–7.35 (m, 9H, Ar–H), 7.51–7.54 (m, 7H, OH and Ar–H), ppm; HRMS (ESI): MH$^+$ calcd for $C_{24}H_{17}O_3S_3$, 449.0339, found 449.0335.

4.2.4. 2,5-Dichloro-3,6-bis(phenylthio)cyclohexa-2,5-diene-1,4-dione (*4b*)

Red crystals; mp: 148–150 °C; yield, 10%; IR (KBr/cm^{-1}): 1638, 1540 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.38 (m, 6H, Ar–H), 7.47–7.50 (m, 4H, Ar–H) ppm; HRMS (ESI): MH$^+$ calcd for $C_{18}H_{11}Cl_2O_2S_2$, 392.9572, found 392.9582.

4.2.5. 2-Chloro-3,5,6-tris(phenylthio)cyclohexa-2,5-diene-1,4-dione (*4c*)

Dark red crystals; mp: 138–140 °C; yield, 16%; IR (KBr/cm^{-1}): 1670, 1518 (>C=O of quinone). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25–7.37 (m, 15H, Ar–H) ppm; HRMS (ESI): MH$^+$ calcd for $C_{24}H_{16}ClO_2S_3$, 467.0001, found 467.0021.

4.2.6. 2,3,5,6-Tetrakis(phenylthio) cyclohexa-2,5-diene-1,4-dione (*4d*)

Red crystals; mp > 250 °C; yield, 2%; IR (KBr/cm^{-1}): 1658, 1573 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18–7.20 (m, 12H, Ar–H), 7.27–7.29 (m, 8H, Ar–H), ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 128.13 (4C), 129.24 (8C), 131.86 (8C), 132.56 (4C), 146.69 (4C), 173.48 (2C) ppm. HRMS (ESI): MH$^+$ calcd for $C_{30}H_{21}O_2S_4$, 541.0424, found, 541.0421.

Alternatively, **4d** was synthesized from chloranil (1 eq), LD (0.5 mol %), K_2CO_3 (4 eq) and **2a** (4 eq) in water at r.t. for 2 h; yield 80%, according to the general procedure 4.2.

4.2.7. 2-Hydroxy-3,5,6-tris(naphthalen-1-ylthio)cyclohexa-2,5-diene-1,4-dione (*5a*)

Colorless crystals; mp 155–156 °C; yield 4%; IR (KBr/cm^{-1}): 3425 (OH), 1622, 1579 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46–7.51 (m, 7H, OH and Ar–H) 7.62–7.66 (m, 3H, Ar–H), 7.74–7.82 (m, 9H, Ar–H), 8.00 (s, 3H, Ar–H), ppm; HRMS (ESI): MH$^+$ calcd for $C_{36}H_{23}O_3S_3$, 599.0809, found 599.0818.

4.2.8. 2,5-Dichloro-3,6-bis(naphthalen-1-ylthio)cyclohexa-2,5-diene-1,4-dione (*5b*)

Red crystals; mp 205–207 °C; yield 25%; IR (KBr/cm^{-1}): 1678, 1538 (>C=O of quinone); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44–7.47 (m, 2H), 7.51–7.55 (m, 4H, Ar–H), 7.79–7.85 (m, 6H, Ar–H), 8.01 (s, 2H, Ar–H); HRMS (ESI): MH$^+$ calcd for $C_{26}H_{15}Cl_2O_2S_2$, 492.9890, found 492.9894.

4.2.9. 2-Chloro-3,5,6-tris(naphthalen-1-ylthio)cyclohexa-2,5-diene-1,4-dione (*5c*)

Red crystals; mp 170–171 °C; yield 8%; IR (KBr/cm^{-1}): 1673, 1581 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05 (s, 2H, Ar–H), 7.3–87.52 (m, 10H, Ar–H), 7.73–7.85 (m, 9H, Ar–H) ppm; HRMS (ESI): MH$^+$ calcd for $C_{36}H_{22}ClO_2S_3$, 617.0470, found 617.0457.

4.2.10. 2-Hydroxy-3,5,6-tris(p-tolylthio)cyclohexa-2,5-diene-1,4-dione(*6a*)

Colorless crystals; mp 40–41 °C; yield 15%; IR (KBr/cm^{-1}): 3455 (OH), 1593, 1488 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.30 (s, 9H, 3× CH_3), 7.07 (d, J = 8.1 Hz, 6H, Ar–H), 7.35 (d, J = 8.1 Hz, 6H, Ar–H) ppm; HRMS (ESI): MH$^+$ calcd for $C_{27}H_{23}O_3S_3$, 491.0809, found, 491.0818.

4.2.11. 2,5-Dichloro-3,6-bis(p-tolylthio)cyclohexa-2,5-diene-1,4-dione (*6b*)

Red needles; mp 136–138 °C; yield, 5%; IR (KBr/cm^{-1}): 1675, 1529 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 6H, 2× CH_3), 7.14 (d, J = 8.1 Hz, 4H, Ar–H), 7.35 (d, J = 8.1 Hz, 4H, Ar–H), ppm; HRMS (ESI): MH$^+$ calcd for $C_{20}H_{15}Cl_2O_2S_2$, 420.9890, found, 420.9874.

4.2.12. 2,3,5,6-Tetrakis(p-tolylthio) cyclohexa-2,5-diene-1,4-dione (*6d*)

Dark red crystals; mp 205–207 °C, yield 80%; IR (KBr/cm^{-1}): 1659, 1489 (>C=O of quinone). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 12H, 4× CH_3), 6.94 (d, J = 8.0 Hz, 8H, Ar–H), 7.14 (d, J = 8.0 Hz, 8H, Ar–H), ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 21.46 (4C), 129.23 (4C), 129.96 (8C), 132.13 (8C), 138.06 (4C), 146.58 (4C), 173.63 (2C) ppm; HRMS (ESI): MH$^+$ calcd for $C_{34}H_{29}O_2S_4$, 597.1050, found 597.1049.

Alternatively, **6d** was synthesized from chloranil (1 eq), LD (0.5 mol %), K_2CO_3 (4 eq) and **2c** (4 eq) in water at r.t. for 2 h; yield 80%.

4.3. General procedure for synthesis and spectral data of 2,3,5,6-tetrakis(alkyl and arylsulfanyl)-p-benzoquinones(*7*–*14*)

Thiols (**2**) (4 eq) were added to an aqueous suspension of surfactant (0.5 mol % LD). After stirring for 5 min chloranil (**1b**) (1 eq) was added followed by addition of 4 eq of K_2CO_3 and the reaction mixtures were stirred at r.t. for 2–4 h. The reaction mixture was filtered and the solids thus obtained were crystallized from ethyl acetate:hexane (4:1) to get products (**7**–**14**)

4.3.1. Tetramethyl-2,2′,2″,2‴-(3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrayl)tetrakis(sulfanediyl)tetraacetate (*7*)

Dark red crystals; mp 195–198 °C; yield, 85%; IR (KBr/cm^{-1}): 1725 (>C=O, of ester), 1665, 1514 (C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 12H, 4× CH_3), 3.82 (s, 8H, 4× CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 34.97 (4C), 52.99 (4C), 144.90 (4C), 169.49 (4C), 173.59 (2C) ppm; HRMS (ESI): MH$^+$ calcd for $C_{18}H_{21}O_{10}S_4$, 525.0017, found 525.0012.

*4.3.2. Tetraethyl 2,2′,2″,2‴-(3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrayl)tetrakis(sulfanediyl) tetraacetate (**8**)*

Dark red crystals; mp 62–63 °C; yield, 82%; IR (KBr/cm^{-1}): 1727 (>C=O of ester), 1659, 1497 (>C=O of quinone). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.27 (t, J = 6.0 Hz, 12H, 4× CH_3), 3.83 (s, 8H, 4× CH_2), 4.18 (q, J = 6.0 Hz, 8H, 4× CH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 14.08 (4C), 35.00 (4C), 61.90 (4C), 144.58 (4C), 168.80 (4C), 173.45 (2C) ppm; HRMS (ESI): MH^+ calcd for $C_{22}H_{29}O_{10}S_4$ 581.0643, found 581.0653.

*4.3.3. Isopropyl-2,2′,2″,2‴-(3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrayl)tetrakis(sulfanediyl)tetraacetate (**9**)*

Red colored solid; mp 59–60 °C; yield, 76%; IR (KBr/cm^{-1}): 1720 (>C=O of Ester), 1665, 1482 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.22 (d, J = 6.0 Hz, 24H, 8× CH_3), 3.76 (s, 8H, 4× CH_2), 4.99 (sep, J = 6.0 Hz, 4H, 4× CH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 21.88 (8C), 35.46 (4C), 69.79 (4C), 144.67 (4C), 168.50 (4C), 173.59 (2C) ppm; HRMS (ESI): MH^+ calcd for $C_{26}H_{37}O_{10}S_4$, 637.1269, found, 637.1267.

*4.3.4. Tetramethyl-3,3′,3″,3‴-(3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrayl)tetrakis(sulfanediyl)tetrapropanoate (**10**)*

Red solid; mp 235–238 °C; yield, 83%; IR (KBr/cm^{-1}): 1738 (>C=O of ester), 1665, 1473 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70 (t, J = 6.9 Hz, 8H, 4× CH_2), 3.33 (t, J = 6.9 Hz, 8H, 4× CH_2), 3.69 (s, 12H, 4× CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 29.36 (4C), 35.40 (4C), 52.10 (4C), 146.10 (4C), 171.97 (4C), 174.27 (2C) ppm. HRMS (ESI): MH^+ calcd for $C_{22}H_{29}O_{10}S_4$, 581.0643, found, 581.0641.

*4.3.5. Tetraethyl 3,3′,3″,3‴-(3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrayl)tetrakis(sulfanediyl)tetrapropanoate (**11**)*

Brown oil; yield, 75%; IR (KBr/cm^{-1}): 1729 (>C=O of Ester), 1653, 1489 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, J = 6.0 Hz, 12H, 4× CH_3), 2.68 (t, J = 5.4 Hz, 8H, 4× CH_2), 3.32 (t, J = 6.0 Hz, 8H, 4× CH_2), 4.14 (q, J = 6.0 Hz, 8H, 4× CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 14.10 (4C), 29.17 (4C), 35.38 (4C), 60.78 (4C), 145.88 (4C), 171.28 (4C), 174.11 (2C) ppm; HRMS (ESI): MH^+ calcd for $C_{26}H_{36}O_{10}S_4$, 636.1191, found, 636.1190.

*4.3.6. Isopropyl 3,3′,3″,3‴-(3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrayl)tetrakis(sulfanediyl)tetrapropanoate (**12**)*

Red colored solid; mp > 250 °C; yield, 76%; IR (KBr/cm^{-1}): 1722 (>C=O of Ester), 1668, 1485 (>C=O of quinone); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.67 (d, J = 6.0 Hz, 24H, 8× CH_3), 2.64 (t, J = 6.9 Hz, 8H 4× CH_2), 3.21 (t, J = 6.9 Hz, 8H, 4× CH_2), 4.88 (m, 4H, 4× CH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6): 21.50 (8C), 29.04 (4C), 35.11 (4C), 67.60 (4C), 145.49 (4C), 170.71 (4C), 173.52 (2C) ppm; HRMS (ESI): MH^+ calcd for $C_{30}H_{45}O_{10}S_4$, 693.1895, found, 693.1894.

*4.3.7. Octaethyl-2,2′,2″,2‴-(3,6-dioxocyclohexa-1,4-diene-1,2,4,5-tetrayl)tetrakis(sulfanediyl)tetrasuccinate (**13**)*

Brown oil; yield, 79%; IR (KBr/cm^{-1}): 1734 (>C=O of ester) 1664, 1467 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.21–1.41 (m, 24H), 2.79–3.1 (m, 8H), 4.16–4.33 (m, 20H) ppm. HRMS (ESI): calcd for $C_{38}H_{53}O_{18}S_4$, 924.2036, found 924.2048.

*4.4. General procedure for synthesis and spectral data of diaminocyclohexa-2,5-diene-1,4-diones (**16**)*

Amines (**15**) (4 eq) were added to an aqueous suspension of surfactant (0.5 mol % LD). After stirring for 2 min chloranil (**1b**) (1 eq) or benzoquinone (**1a**) (1 eq) and K_2CO_3 were added and the reaction mixtures were stirred at r.t. for 6–7 h and the solid thus obtained was crystallized from ethyl acetate:hexane to give crystalline products (**16**)

*4.4.1. 2,5-Dimorpholinocyclohexa-2,5-diene-1,4-dione (**16a**)*

Green solid; mp 230–231 °C; yield, 64%; IR (KBr/cm^{-1}): 1632, 1562 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.56 (bs, 8H, 4× CH_2), 3.82 (bs, 8H, 4× CH_2), 5.5 (s, 2H, quinone-H), ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 49.23 (4C), 66.69 (4C), 107.19 (2C), 152.50 (2C), 182.93 (2C), ppm. HRMS (ESI): MH^+ calcd for $C_{14}H_{19}N_2O_4$, 279.1344, found, 279.1329.

*4.4.2. 2,5-Bis(4-methylpiperazin-1-yl)cyclohexa-2,5-diene-1,4-dione (**16b**)*

Green powder; mp 210–211 °C; yield, 72%; IR (KBr/cm^{-1}): 1628, 1567 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 6H, 2× CH_2), 2.51 (t, J = 4.8 Hz, 8H, 4× CH_2), 3.57 (t, J = 4.8 Hz, 8H, 4× CH_2), 5.54 (s, 2H, quinone-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 46.09, (2C), 48.83 (4C), 54.81 (4C), 106.98 (2C), 152.69 (2C), 182.83 (2C) ppm; HRMS (ESI): calcd for $C_{16}H_{25}N_4O_2$, 305.1977, found, 305.1977.

*4.4.3. 2,5-Di(piperidin-1-yl)cyclohexa-2,5-diene-1,4-dione (**16c**)*

Green powder; mp: >250 °C; yield; 69%; IR (KBr/cm^{-1}): 1630, 1571 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67 (bs, 12H, 6× CH_2), 3.53 (bs, 8H, 4× CH_2), 5.53 (s, 2H, quinone-H), ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 24.53 (2C), 26.05 (4C), 50.54 (4C), 105.68 (2C), 153.40 (2C), 182.69 (2C), ppm. HRMS (ESI): MH^+ calcd for $C_{16}H_{23}N_2O_2$, 275.1759, found, 275.1748.

*4.4.4. 2,5-Di(pyrrolidin-1-yl)cyclohexa-2,5-diene-1,4-dione (**16d**)*

Green powder; mp > 300 °C; yield, 76%; IR (KBr/cm^{-1}): 1620, 1557 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.90 (bs, 8H, 4× CH_2), 3.27 (bs, 4H, 2× CH_2), 3.96 (bs, 4H, 2× CH_2), 3.53 (bs, 8H, 4× CH_2), 5.27 (s, 2H, quinone-H), ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 23.83 (2C), 26.83 (2C), 50.74 (2C), 51.76 (2C), 99.96 (2C), 149.98 (2C), 180.61 (2C), ppm. HRMS (ESI): MH^+ calcd for $C_{14}H_{19}N_2O_2$. 247.1446, found 247.1445.

*4.4.5. 2,5-Dichloro-3,6-dimorpholinocyclohexa-2,5-diene-1,4-dione (**16e**)*

Green powder; mp 205–207 °C; yield, 78%; IR (KBr/cm^{-1}): 1653, 1581 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.60 (t, J = 6.0 Hz, 8H, 4× CH_2), 3.83 (t, J = 6.0 Hz, 8H, 4× CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 52.08 (4C), 67.49 (4C), 116.33 (2C), 147.99 (2C), 175.97 (2C). HRMS (ESI): MH^+ calcd for $C_{14}H_{17}Cl_2N_2O_4$, 346.0565, found 347.0573.

*4.4.6. 2,5-Dichloro-3,6-bis(4-methylpiperazin-1-yl)cyclohexa-2,5-diene-1,4-dione (**16f**)*

Green powder; mp 199–201 °C; yield, 86%; IR (KBr/cm^{-1}): 1652, 1579 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 6H, 2× CH_3), 2.55 (bs, 8H, 4× CH_2), 3.60 (bs, 8H, 4× CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 46.25 (2C), 51.78 (4C), 55.78 (4C), 116.18 (2C), 148.53 (2C), 176.16 (2C) ppm. HRMS (ESI): MH^+ calcd for $C_{16}H_{23}Cl_2N_4O_2$, 373.1198, found 373.1175.

*4.4.7. 2,5-Bis(4-benzylpiperazin-1-yl)-3,6-dichlorocyclohexa-2,5-diene-1,4-dione (**16g**)*

Green powder; mp 280–281 °C; yield, 82%; IR (KBr/cm^{-1}): 1653, 1580 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45 (bs, 8H, 4× CH_2), 3.56 (s, 4H, 2× CH_2), 3.60 (bs, 8H, 4× CH_2), 7.26–7.33 (m, 10H, Ar–H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 51.79 (4C), 53.82 (4C), 63.07 (2C), 116.03 (2C), 127.56 (2C), 128.54 (4C), 129.43 (4C), 137.43 (2C), 148.55 (2C), 176.14 (2C) ppm. HRMS (ESI): MH^+ calcd for $C_{28}H_{31}Cl_2N_4O_2$, 525.1824, found, 525.1858.

*4.4.8. 2,5-Dichloro-3,6-bis(4-(4-chlorophenyl)piperazin-1-yl) cyclohexa-2,5-diene-1,4-dione (**16h**)*

Green powder; mp 200–201 °C; yield, 81%; IR (KBr/cm^{-1}): 1646, 1549 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.30 (bs, 8H 4× CH_2), 3.75 (bs, 8H 4× CH_2), 6.88 (d, J = 5.4 Hz, 4H, Ar–H), 7.25 (d, J = 5.4 Hz, 4H, Ar–H) ppm; HRMS (ESI): MH^+ calcd for $C_{16}H_{25}Cl_4N_4O_2$, 565.0731, found, 565.0729.

*4.4.9. 2,5-Dichloro-3,6-di(piperidin-1-yl)cyclohexa-2,5-diene-1,4-dione (**16i**)*

Green powder; mp 143–145 °C; yield, 81%; IR (KBr/cm^{-1}): 1646, 1549 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70 (bs, 12H, 6× CH_2), 3.48 (bs, 8H, 4× CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 24.24 (2C), 27.08 (4C), 53.44 (4C), 115.52 (2C), 149.52 (2C), 176.20 (2C) ppm; HRMS (ESI): MH^+ calcd for $C_{16}H_{21}Cl_2N_2O_2$, 343.0980, found, 343.0954.

*4.4.10. 2,5-Dichloro-3,6-di(pyrrolidin-1-yl)cyclohexa-2,5-diene-1,4-dione (**16j**)*

Green powder; mp 170–172 °C; yield, 88%; IR (KBr/cm^{-1}): 1631, 1532 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.95 (bs, 8H, 4× CH_2), 1.90 (bs, 8H, 4× CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): 25.69 (4C), 54.63 (4C), 101.65 (2C), 150.12 (2C), 175.32 (2C) ppm.; HRMS (ESI): MH^+ calcd for $C_{14}H_{17}Cl_2N_2O_4$, 315.0667, found, 315.0678.

*4.5. General procedure for synthesis and spectral data of diaminodithialkylcyclohexa-2,5-diene-1,4-diones (**17**)*

Thiols (**2**) (2 eq) were added to an aqueous suspension of surfactant (0.5 mol % LD). After stirring for 5 min compound (**16**) (1 eq) was added followed by addition of K_2CO_3 and the reaction mixtures were stirred at r.t. for 2–4 h. The reaction mixture was filtered and the solids thus obtained were crystallized from chloroform to give diaminodithialkylcyclohexa-2,5-diene-1,4-diones (**17**).

*4.5.1. Diethyl 2,2′-(2,5-dimorpholino-3,6-dioxocyclohexa-1,4-diene-1,4-diyl)bis(sulfanediyl)diacetate (**17a**)*

Red powder; mp 180–181 °C; yield, 95%; IR (KBr/cm^{-1}): 1735 (>C=O of ester), 1652, 1578 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.24 (t, J = 6.9 Hz, 6H, 2× CH_3), 3.48 (s, 4H, 2× CH_2), 3.62 (bs, 8H, 4× CH_2), 3.82 (bs, 8H, 4× CH_2), 4.13 (q, J = 6.9 Hz, 4H, 2× CH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 14.15 (2C), 35.16 (2C), 52.91 (4C), 61.28 (2C), 67.38 (4C), 112.97 (2C), 155.28 (2C), 169.28 (2C), 179.72 (2C), ppm; HRMS (ESI): MH^+ calcd for $C_{22}H_{31}N_2O_8S_2$, 515.1521, found, 515.1521.

*4.5.2. 2,5-Dimorpholino-3,6-bis(phenylthio)cyclohexa-2,5-diene-1,4-dione (**17b**)*

Red powder; mp 204–205 °C; yield, 96%; IR (KBr/cm^{-1}): 1661, 1574 (>C=O of quinone); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.52 (t, J = 4.5 Hz, 8H, 4× CH_2), 3.68 (t, J = 4.5 Hz, 8H, 4× CH_2), 7.15–7.32 (m, 6H, Ar–H), 7.49 (d, J = 7.5 Hz, 4H, Ar–H), ppm; ^{13}C NMR (75 MHz, $CDCl_3$): 52.21 (4C), 67.46 (4C), 110.05 (2C), 127.02 (2C), 127.44 (4C), 129.01 (4C), 136.45 (2C), 155.52 (2C), 182.83 (2C) ppm; HRMS (ESI): MH^+ calcd for $C_{26}H_{27}N_2O_4S_2$, 495.1412, found, 495.1401.

4.6. In vitro antifungal and antibacterial evaluation by MIC assay and toxicological evaluation

The compound **4**–**16** were evaluated for their *in vitro* antifungal activity against *C. albicans*, *C. neoformans*, *S. schenckii*, *T. mentagraphytes*, *A. fumigates* and *C. parapsilosis* (ATCC 22019) and antibacterial activity against *E. coli*, *S. aureus*, (ATCC 25923), *K. Pneumoniae* (ATCC 27736) and *P. aeruginosa* at the Division of Fermentation Technology of central Drug Research Institute, Lucknow, India. In this process Minimum Inhibitory Concentrations of compounds **4**–**16** according to the broth microdilution technique as per NCCLS [31,32] protocol. Briefly testing was performed in 96-well tissue culture plates (CELLSTAR® Greiner bio-one Gmbh, Germany) in RPMI 1640 media buffered with MOPS (3-(*N*-morpholino)-propanesulfonic acid) (Sigma Chemical Co. MO, USA) for fungal strains and in Muller Hinton Broth (Titan Biotech Ltd, India) for bacterial strains. Initial inoculums of fugal and bacterial strains 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 *C. neoformans*, *S. schenckii*, *A. fumigates* 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).

To test the toxicity of lead compound **16f** against mammalian cells, mouse fibroblast cell line L929 was used. Stock solutions (1 mg/mL) of the test compounds were prepared in DMSO. The cell line L929 was grown in RPMI 1640 medium supplemented with 10% FBS and 1× antimycotic and antibacterial solution (sigma, USA) at 37 °C in humidified atmosphere having 5% CO_2. One hundred μL (1 × 10^3 cells μL in RPMI) of the confluent fibroblast stock suspension (1 × 10^5 cells/mL) was dispensed in 96 well tissue culture plates. The original medium from the wells was replaced with 100 μL serum free RPMI when the cells reached 80% confluence after incubation in a CO_2 incubator at 37 °C. Various concentrations of the test compounds (25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09 μg/mL) were added to the growing cells along with control (with no compounds) and incubated for 24 h. 200 μL of MTT solution (0.5 mg of MTT in RPMI 1640 medium) was added to each well after removing the media completely and incubated for 4 h at 37 °C to allow MTT metabolism. An aliquot of 100 μL of DMSO solvent was added to each well and the plate was incubated for 30 min at room temperature. Response of L929 cells to the test compounds was determined spectrophotometrically at 570 and 630 nm. The difference between absorbance at 570 and 630 nm was used as an index of the cell viability.

$$\left[\frac{(A570-A630)\text{sample}}{(A570-A630)\text{control}}\right]\times 100\%$$

The morphology of the cells was observed using Giemsa stain under Phase contrast microscope. After fixation of the cells in the wells of 96 well tissue culture plates, Giemsa stain was added to each well and incubated for 30 min at 37 °C. The excess stain was removed by thorough washing with phosphate buffer saline and the culture plates were air dried and observed under a phase contrast microscope.

Acknowledgments

Sandeep Kumar thanks DST, New Delhi, India for award of INSPIRE Fellowship [IF10379]. We thank Director, SAIF of Central Drug Research Institute, Lucknow India for spectral analysis data reported in the manuscript.

References

[1] C.I. Herrerias, Y. Xiaoquan, Z. Li, C.-J. Li, Chem. Rev. 107 (2007) 2546–2562.
[2] R. Breslow, Acc. Chem. Res. 37 (2004) 471–478.

[3] S. Narayan, J. Muldoon, M.G. Finn, V.V. Fokin, H.C. Kolb, K.B. Sharpless, Angew. Chem. Int. Ed. 44 (2005) 3275–3279.
[4] M. Shiri, M.A. Zolfigol, Tetrahedron 65 (2009) 587–598.
[5] (a) V.K. Tandon, H.K. Maurya, Tetrahedron Lett. 50 (2009) 5896–5902;
(b) V.K. Tandon, H.K. Maurya, M.K. Verma, R. Kumar, P.K. Shukla, Eur. J. Med. Chem. 45 (2010) 2418–2426;
(c) V.K. Tandon, H.K. Maurya, Tetrahedron Lett. 51 (2010) 3843–3847.
[6] P. Gogoi, P. Hazarika, D. Konwar, J. Org. Chem. 70 (2005) 1934–1936.
[7] B. Xin, Y. Zhang, K. Cheng, Synthesis (2007) 1970–1978.
[8] S. Shirakawa, S. Kobayashi, Org. Lett. 9 (2007) 311–314.
[9] K. Manabe, S. Kobayashi, Org. Lett. 1 (1999) 1965–1967.
[10] S. Bhattacharya, A. Srivastava, S. Sengupta, Tetrahedron Lett. 46 (2005) 3557–3560.
[11] Laundry detergent (LD, washing powder) used as surfactant is Ariel, detergent product made in India by Procter and Gamble home products Ltd. Mumbai 400099 (Tel.: +91 22 24942113, e-mail: intouch.lm@pg.com) No.: 4902430214933, MRP rupees 2.00, packed 03/2009, net weight: 14g while the cost of SDS is Rs. 1123.00 for 25 g in the catalog of sigma-Aldrich (436143-25G)-2008. In place of Ariel, other Laundry detergent (washing powder) can also be used. LD, the common Laundry detergent contains alkylbenzenesulfonates [33].
[12] P.I. Gutierrez, Free Radic. Biol. Med. 6 (1989) 405–445.
[13] G. Tudor, P. Gutierrez, A. Aguilera-Gutierrez, E.A.S. Ville, Biol. Chem. Pharmacol. 65 (2003) 1061–1075.
[14] V.K. Tandon, D.B. Yadav, R.V. Singh, A.K. Chaturvedi, P.K. Shukla, Bioorg. Med. Chem. Lett. 15 (2005) 5324–5328.
[15] T.M.S. Silva, C.S. Camara, T.P. Barbosa, A.Z. Soares, L.C. da Cunha, A.C. Pinto, M.D. Vargas, Bioorg. Med. Chem. 13 (2005) 193–196.
[16] C. Biot, H. Bauer, R.H. Schirmer, E. Davioud-Charret, J. Med. Chem. 47 (2004) 5972–5983.
[17] A. Mantyla, J.T. GarnierRautio, T. Nevalanien, J. Vepsalainen, A. Koskinen, S.I. Croft, T. Jarvinen, J. Med. Chem. 47 (2004) 188–195.
[18] V.K. Tandon, D.B. Yadav, H.K. Maurya, A.K. Chaturvedi, P.K. Shukla, Bioorg. Med. Chem. 14 (2006) 6120–6126.
[19] (a) C.K. Ryu, K.U. Choi, J.Y. Shim, H.J. You, I.H. Choi, M.J. Chai, Bioorg. Med. Chem. 11 (2003) 4003–4008;
(b) C.K. Ryu, J.Y. Han, O.J. Jung, S.K. Lee, J.Y. Lee, S.H. Jeong, Bioorg. Med. Chem. Lett. 15 (2005) 679–682.
[20] C.K. Ryu, H.J. Kim, Arch. Pharmacol. Res. 17 (1994) 139–144.
[21] V.K. Tandon, D.B. Yadav, R.V. Singh, M. Vaish, A.K. Chaturvedi, P.K. Shukla, Bioorg. Med. Chem. Lett. 15 (2005) 3463–3466.
[22] V.K. Tandon, R.B. Chhor, R.V. Singh, S. Rai, D.B. Yadav, Bioorg. Med. Chem. Lett. 14 (2004) 1079–1083.
[23] V.K. Tandon, R.B. Singh, D.B. Yadav, Bioorg. Med. Chem. Lett. 14 (2004) 2901–2904.
[24] V.K. Tandon, H.K. Maurya, Heterocycles 76 (2008) 1007–1010.
[25] V.K. Tandon, H.K. Maurya, N.N. Mishra, P.K. Shukla, Eur. J. Med. Chem. 44 (2009) 3130–3137.
[26] V.K. Tandon, H.K. Maurya, A. Tripathi, G.B. ShivaKeshava, P.K. Shukla, P. Srivastava, D. Panda, Eur. J. Med. Chem. 44 (2009) 1086–1092.
[27] V.K. Tandon, H.K. Maurya, D.B. Yadav, A. Tripathi, M. Kumar, P.K. Shukla, Bioorg. Med. Chem. Lett. 16 (2006) 5883–5887.
[28] A.R. Katritzky, D. Fedoseyenko, P.P. Mohapatra, P.J. Steel, Synthesis (2008) 777–787.
[29] C. Ibis, M. Yildiz, C. Sayil, Bull. Korean Chem. Soc. 30 (2009) 2381–2386.
[30] J.Y. Becker, J. Bernstein, S. Bittner, E. Harlev, J.A.R.P. Sarma, S.S. Shaik, New J. Chem. 12 (1988) 875–880.
[31] 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, 1977.
[32] 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 Standards Wayne, PA, USA, 1998.
[33] For detail refer to http://en.wikipidia.org/wiki/Laundrydetergent.