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Design, synthesis and evaluation of novel 1,4-naphthoquinone derivatives as antifungal and anticancer agents

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Abstract—A series of 1,4-naphthoquinone derivatives were synthesized and tested for antifungal and antitumor activity against a number of fungal disease causative species and Walker 256 carcinoma cell lines. The results show that the compounds **8a**,**e** and **11b** possess pronounced antifungal proifile where as **7b** and **f** were found to be active against Walker 256 carcinoma cell lines. Moreover **7c** and **11a** showed inhibitory effect against reverse transcriptase enzyme from *Rauscher Murine Leukemia* Virus.

1. Introduction

Fungal diseases in humans are of two types, superficial and systemic. The types of species relies upon the affected area of body. Systemic fungal infections are one of the serious causes of high degree of mortality in severely affected cases with impaired immune systems brought about by use of cytotoxic drugs, immunosuppressives and HIV infections. Nearly all the antifungal drugs usually suppress the growth of the fungal reservoirs and in turn the fungal strains get an opportunity to develop resistance. Use of antifungal agents in the management of related infections among AIDS and cancer patients, led to an emergence which is a cause of serious concern. Hence, multidrug resitance is becoming a significant problem, therefore, the efficacious antimycotic agents with a broad spectrum of antifungal activity and non-toxic profile are warranted.

1,4-Naphtoquinone pharmacophore is known to impart anticancer activity in a number of drugs for example, streptonigrin,[1] actinomycins,[2] mitomycins,[3] etc.

Scant reports are available concerning antifungal profile of 1,4-naphthoquinones.[4,5]

Naturally occuring 2-methoxy-1,4-naphthoquinone, **1** (Fig. 1) was found to be highly fungistatic against several plant pathogenic fungi.[6] The substitution of a sulfur atom for oxygen in **1** led to the synthesis of 2-methylsulfanyl-[1,4]naphthoquinone **2** (Fig. 1) and its fungistatic activity measured which results in a marked increase in its activity profile. While the compounds **3** (Fig. 1) were found as antifungal agents as they possess in vitro activity against several fungal cultures capable of causing disease in vertibrates. A different class of

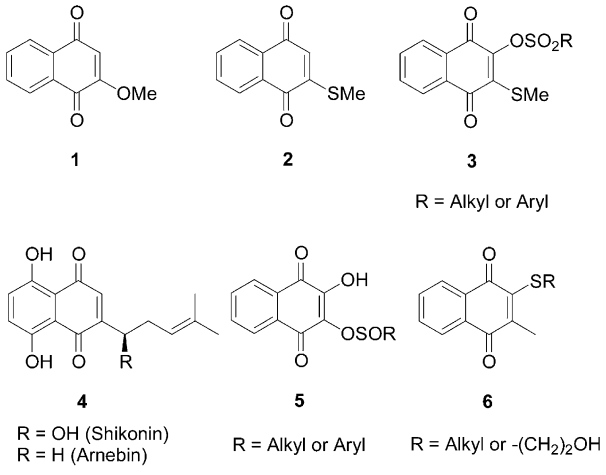

Figure 1. Existing antifungal activity bearing quinones.

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naphthoquinone derivatives **4** shikonin and arnebin (Fig. 1) exhibited pronounced antidermatophytic properties against a number of fungal strains.[7]

Compounds **5**, (Fig. 1) have shown to possess anticancer properties[8] while compounds **6** (Fig. 1) were found to show cell growth inhibitory properties.[9] The antifungal and antitumor profile of compounds **2**, **3**, **5** and **6** (Fig. 1), prompted us to synthesize 1,4-naphthoquinones possessing sulfur atom in them.

The mode of action of naphthoquinones for antifungal behaviour is not yet known. However, a number of strides have been made in order to ascertain the molecular mechanism of action for their antibiotic, phytotoxic[10] and anticancer properties.[11]

The effect of naphthoquinones on microbial cells reflect their ability to suppress oxidative phosphorylation which could be the cause of their antibiotic properties.[12] There are few naphthoquinone candidates for example, gunacin and 2-hydroxyjuglone isolated from *Ustilago sp*. having high degree of activity against gram positive bacteria, various strains of fungus and mycoplasm.[13]

The mechanism of cytotoxic action is related to the interference of naphthoquinones in the DNA synthesis.[14] 2-Hydroxyjuglones were found to inhibit the respiratory function of fungal spheroblasts and suppress the biosynthesis of RNA. It blocks the protein, lipids and glucose transports by affecting basic biochemical processes of membrane systems in the cells.[15] The broad spectrum activity of the thio analogues of 1,4-naphthoquinones outlined in this paper could be the consequence of the aforementioned mechanism(s).

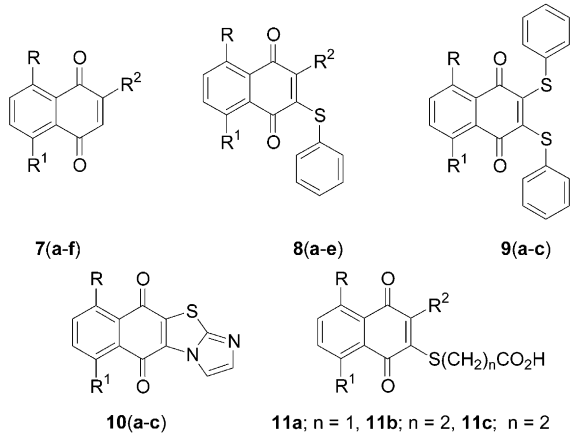


Figure 2. Structures of quinones synthesized for the antifungal and anticancer evaluation.

2. Chemistry

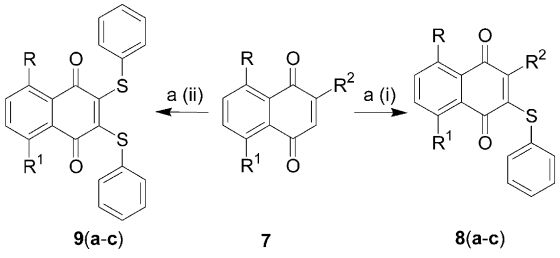
Figure 2 shows the series of the compounds used in this study. Compounds **8**–**11** were synthesized by the reaction of 1,4-naphthoquinones (**7**) with thiophenol, 2-mercaptoimidazole and mercapto alkanoic acids according to known protocols as previously published.[16]

Compounds **8** and **9** were prepared by treating (**7**) with thiophenol in absolute ethanol and further recrystallization from methanol afforded the products while compound **10** was obtained by treating (**7**) with 2-mercaptoimidazole in absolute ethanol and crystallization after completion of the reaction.

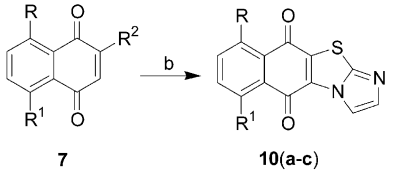
The compounds bearing carboxylic acid functionality, **11**, have been synthesized by the reaction of thioglycolic acid and **7** in absolute ethanol. The reaction mixture was stirred for 2 h and extracted with saturated $NaHCO_3$ solution and the aquous phase acidified with dilute HCl which after crystallization afforded the products (Schemes 1–3).

3. Biology

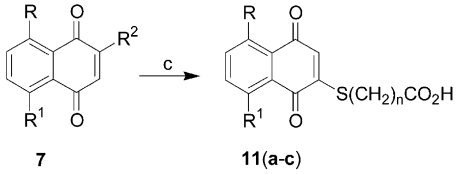
The evaluation of antifungal properties of compounds were conducted against various strains of pathogenic fungi, for example, *C. albicans*, *C. neoformans*, *S. schenckii*, *T. mentagraphytes, M. cannis* and *A. fumigatus*.



Scheme 1. Reagents: (a) (i) 1 equiv C_6H_5SH, EtOH, 100 °C; (ii) 2 equiv C_6H_5SH, EtOH, 100 °C.



Scheme 2. Reagents: (b) 2-mercaptoimidazole, EtOH, 100 °C.



Scheme 3. Reagents: (c) $HS(CH_2)_nCO_2H$, EtOH, 100 °C.

Table 1. Antifungal activity of the compounds **2**–**5** (MIC: μg/mL)

Compounds	*C. albicans*	*C. neoformans*	*S. scenckii*	*T. mentagraphytes*	*A. fumigatus*	*M. cannis*
8a	12.5	1.56	[b]	<0.78	25	<0.98
8e	25	1.56	[b]	1.56	<0.78	1.56
10a	50	12.5	25	25	25	25
10b	25	<12.5	<12.5	25	b	25
10c	25	6.25	[b]	6.25	3.1	6.25
11b	3.125	1.56	[b]	3.125	b	3.125
Miconazole[a]	25	12.5	[c]	≤0.78	12.5	≤0.78
Nystatin	7.8–7.9	3.5–3.9	13.2	[c]	[c]	[c]
Am. B[d]	0.39	0.78	[c]	1.56	[c]	1.56

[a] Miconazole.
[b] No activity.
[c] Activity not reported.
[d] Amphotericin B.

Table 2. Anticancer activity against Walker 256 carcinosarcoma [dose (mg/kg)]

Compounds	Dose	Survivors	T/C%	Remark
7b	10	3/4	37	Active
7f	2.5	4/4	23	Active

The anticancer screening of **7**–**11** was done by a standard procedure[17] in which the tumour containing Walker 256 carcinoma cell was implanted in the right thigh muscle of the rat while inhibitory effect of the compounds **7**–**11** to RNA-dependent DNA polymerase enzyme of *Rauscher Murine Leukemia* Virus was assayed by the known method.[18]

The compounds having minimum inhibitory concentration (MIC) of 50 μg/mL or less were considered active and compared with standard antifungal drugs as summarized in Table 1 while anticancer activity is shown in Table 2.

Comparison of activity of the compounds with the miconazole is effectively presented in Figure 3 which reveals that compounds **8a** and **11b** are more effective than miconazole against *C. albicans* while **10b**, **10c**, **8a**,**e** and **11b** have better activity against *C. neoformans*.

Surprisingly **8a** was found to possess the same activity profile as miconazole against *T. mentagraphytes*. Against *A. fumigatus* **8e** and **10c** were found to show better activity than MCZ.

Figure 4 presents a comparative study of compounds with nystatin in which **11b** showed more than two-fold activity against *C. albicans*. Against *C. neoformans*, nystatin holds MIC value more than double compared to **8a**,**e** and **11b** separately.

Compound **10b** was found slightly better than nystatin while used against *S. schenckii*.

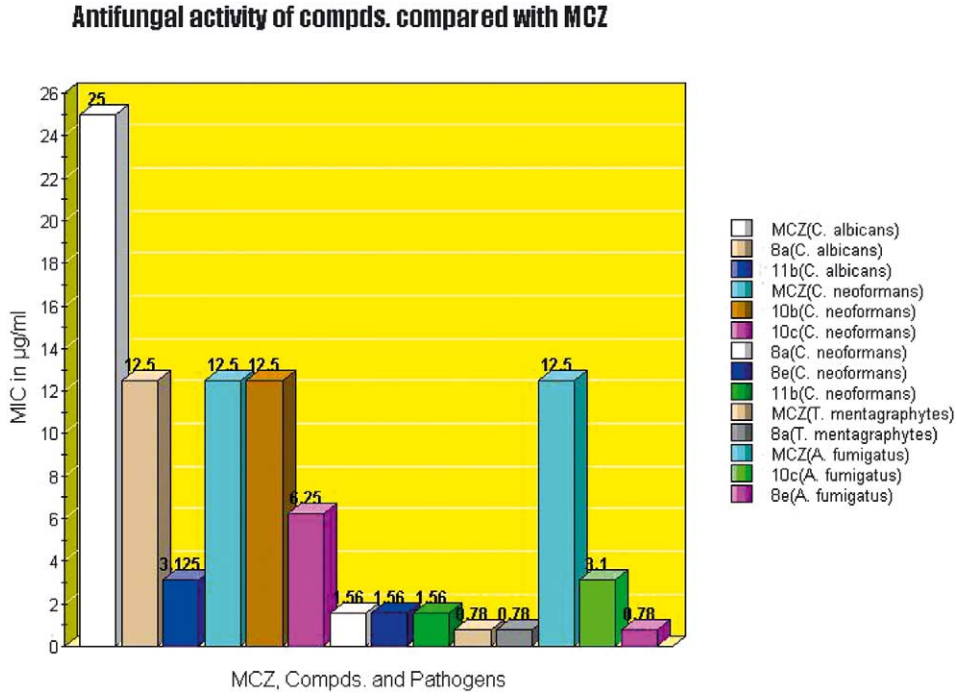


Figure 3. Comparative antifungal study plot with compounds and miconazole.

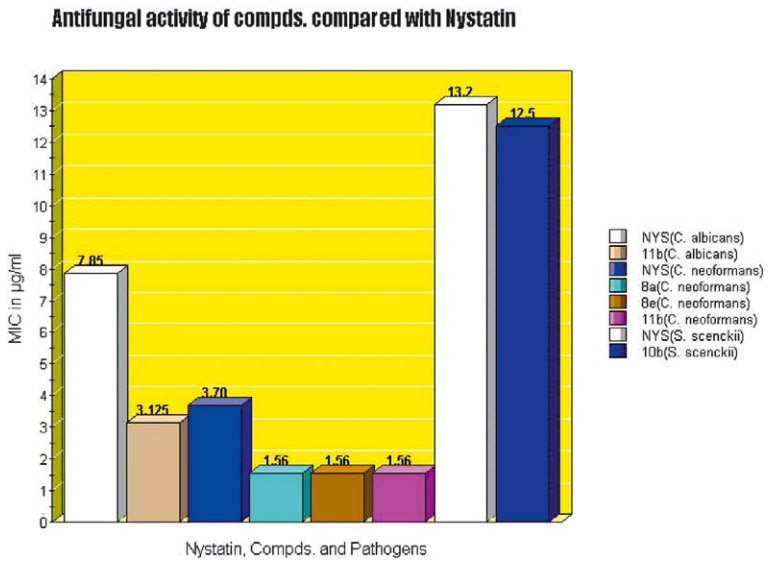


Figure 4. Comparative antifungal study plot with compounds and nystatin.

Amphotericin B is one of the most promising antifungal drugs and its inhibitory effects have been compared with synthesized compounds, some of them have either found the same or better activity against few pathogenic fungal strains.

As shown in Figure 5, **8a** and **e** held the same potential as Amphotericin B against *T. mentagraphytes* and *M. cannis*, respectively, whereas **8a** was found to be far better against *M. cannis*.

4. Results and Discussion

MIC values were used to determine growth inhibition in the presence of synthesized compounds. MIC of **8a** was 1.56 µg/mL, against *C. neoformans* and <0.78 µg/mL against *T. mentagraphytes* whereas **8e** was found effective enough to inhibit the growth of the *C. neoformans* at 1.56 µg/mL, *T. mentagraphytes* at 1.56 µg/mL and *A. fumigatus* at <0.78 µg/mL which are comparable with the antifungal drugs amphotericin B and miconazole.

Compounds containing an imidazole ring also showed antifungal activity against a large number of pathogens. The MIC value of **10b** was <12.5 µg/mL against *C. neoformans* while **10c** was found to have 6.25 µg/mL MIC against both *C. neoformans* and *T. mentagraphytes* separately and 3.1 µg/mL against *A. fumigatus*. Compound **10c** also showed moderate inhibition against *S. faecalis*, *K. pneumoniae*, *E. coli*, *P. aerginosa*, *S. aureus* and *C. albicans*.

The promising inhibitory effect of 1,4-naphthoquinones containing a sulfur atom attached to carboxylic group, **11b** was pronounced against a number of fungi. MIC

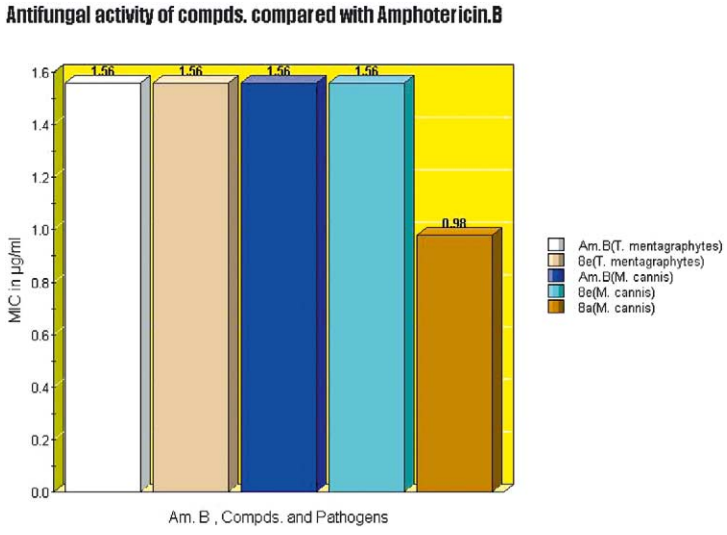


Figure 5. Comparative antifungal study plot with compounds and Amphotericin B.

value of this compound was 3.12 μg/mL against *C. albicans, T. mentagraphytes* and *M. cannis* whereas it holds 1.56 μg/mL MIC value against *C. neoformans*. This compound is active against all the fungi against which both amphotericin B and miconazole are known to act.

Compound **7b** and **f** were found active having T/C% of 37 and 23, respectively, against Walker 256 carcinosarcoma. Compound **7c** showed 97% activity at concentration of 56 μM against RNA dependent DNA polymerase enzyme from *Rauscher murine Leukemia* virus while **11a** showed >98% activity at 56 μM concentration. Compound **7c** also showed anticancer activity at higher dose in KB system tissue culture.

5. Conclusion

Here we describe a series of sulfur containing 1,4-naphthoquinone derivatives that inhibit fungal growth in a MIC range of well established drugs. Several are particularly promising as their MIC values are less than the subjected drugs for study. There are less antifungal reports on such type of quinones that to possess remarkable broad spectrum antifungal properties, therefore, it is worthwhile to explore these synthetic compounds as novel antifungal agents and further studies are being carried out in our laboratory.

Acknowledgements

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