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Short Communication

Synthesis and evaluation of antifungal activity of naphthoquinone derivatives

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Abstract

3-Arylamino-2-phenylsulfinylnaphthoquinones, 2,3-diarylthio-naphthoquinones and 2-phenylsulfinyl-3-arylthio-1,4-dihydronaphtalenes are synthesized and tested against five fungi. The activities of these products were better than amphotericine B against all the strains except for *Candida albicans*.

Keywords: Naphthoquinone derivative; Amino-sulfinylnaphthoquinone; 2-Phenylsulfinyl-3-arylthio-1,4-dihydronaphtalenes; Antifungal activity

1. Introduction

Systemic fungal infections are one of serious causes of mortality in HIV infections and the emergence of multiresistance strains is a significant problem. It is known that some sulfide-, sulfoxide- and sulfone-quinones have antifungal activities. 2-Phenylthio- and 2-alkylthionaphthoquinones **1** and the corresponding sulfoxides **2** showed a high level of activity against *Bothrytis* [1]. Arylamino-dioxobenzothiazoles **3** were tested in vitro against pathogen fungi such as *Candida* species and *Aspergillus niger* [2] (Scheme 1). Some of them have antifungal activities without cytotoxicity in mammalian cells [3,4]. A NADPH-quinone oxido-reductase inhibition can explain this activity: some compounds have a cytotoxicity against human solid cancer cell lines and lethal effect with KB [3–5]. Recently Ryu et al. [6] have shown that bis(arylthio)juglone derivatives have good activities against *Candida albicans* and *Candida tropicalis*.

Thus, in our plan to study biological activity of quinones we decided to synthesize sulfidesulfoxide-quinones **4,** disulfide-quinones **5** and amino-sulfoxide-quinones **6** (Scheme 2).

In this work we described an efficient synthesis of compounds **5** and we studied the activity of compounds such as **4, 5** and **6** against different fungi strains.

2. Chemistry

2,3-Bis(arylthio)-1,4-naphthoquinones were soon obtained by Clark [1], Ryu et al. [6], Fieser and Brown [7] and Tjepkema [8] by substitution reaction with 2,3-dichloronaphthoquinone derivatives and the appropriate arylthiol in ethanol by heating to reflux. Ropmanyuk synthesized **5a** (X = H) from 2,3-bis(benzotriazol-1-yl)-1,4-naphthoquinone with 2 equivalents of benzenethiol [9]. Recently, Tandon et al. [10] obtained **5a** (X = H) by heating naphthoquinone in ethanol with 2 equivalents of benzenethiol but no yield was reported.

Different arylthiols were added, and **8a–d** were obtained with good yields (Table 3).

In our hands, stirring of an excess of benzenethiol and naphthoquinone at room temperature did not give disulfide; the addition at room temperature of an excess of benzenethiol

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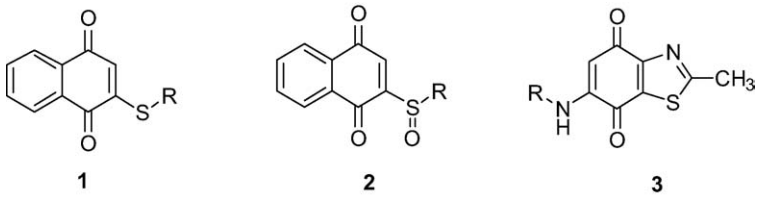
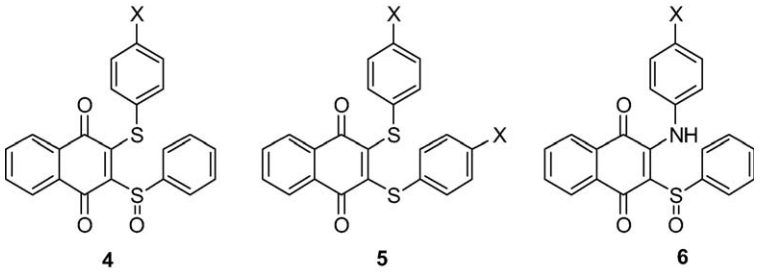
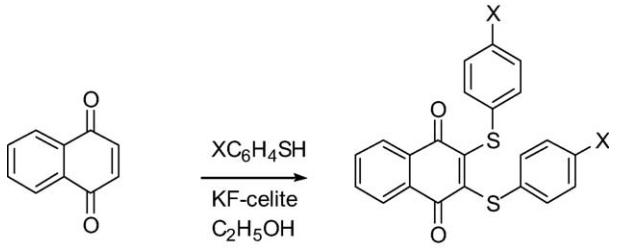


Scheme 1.



Scheme 2.



Scheme 3.

to 2-phenylthio-naphthoquinone **1** (R = C_6H_5) led to a complex mixture and no product could be isolated.

We observed that a mixture of naphthoquinone and an excess of benzenethiol (3 equivalents) in ethanol stirred at room temperature for 2 hours in the presence of potassium fluoride adsorbed onto celite (KF/celite = 1:1 in weight, KF-celite/naphthoquinone = 0.50 g per 1 mmol) lead to bis-sulfide **5a** (R = H) under mild conditions. Thus, different compounds **5a–c** were obtained by addition of corresponding arylthiols with naphthoquinone under these conditions. (Table 1 and Scheme 3).

Sulfoxide-arylamino-quinones **6a–e** were obtained by addition of arylamines to sulfoxide naphthoquinone **2** (R = C_6H_5). These new compounds were obtained with moderate yields (Table 2).

In this reaction the amino-hydroquinones **7,** obtained by [1, 4]-addition were re-oxidized in the reactional mixture into quinone **6** with quinone **2.** Thus, this fact can explain moderate yields observed with these reactions (Scheme 4).

To obtain arylthio-sulfoxide-naphtoquinone **4a** (X = H) we added benzenethiol to sulfoxide **2** (1 equivalent) in ethanol at room temperature. After two hours white crystals were isolated. These compounds were not the attempted phenylthio-sulfoxide **4a** (X = H) but sulfidesulfoxide-phenol **8a.** We never succeeded in oxidize **8** into quinone **4** (Scheme 5).

Table 1
Addition of arylthiols to naphtoquinone in the presence of KF-celite

X	Product	%
H	**5a**	55
CH_3	**5b**	49
OCH_3	**5c**	48

Table 2
Addition of arylamines to sulfoxide-naphtoquinone **2**

X	Products	%
H	**6a**	54
Cl	**6b**	50
Br	**6c**	51
OCH_3	**6d**	46
NO_2	**6e**	54

Table 3
Addition of arylthiols to sulfoxide-naphtoquinone

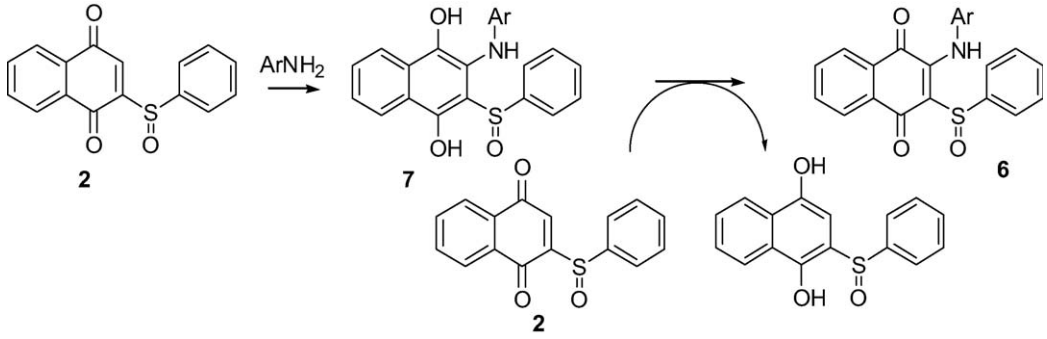
X	Compound	%
H	**8a**	95
Br	**8b**	62
NO_2	**8c**	42
CH_3	**8d**	61

All the analytical data of the different compounds were consistent with their structures **(6a–d** and **8a–d)** or with the bibliographic data **(5a–c).**

3. Antifungal activity

Thirteen compounds were performed in vitro screening for their growth inhibitory activity against five fungi strains: C*andida albicans* CIP 884.65 and *C. tropicalis* CIP 1275.81 (Collection of Pasteur Institut, Paris, France) *Aspergillus niger* ATCC 16404 (American Type Culture Collection, 10801, University Boulevard Manassas (VA) 20110-2209 USA), *Fusarum*

oxysporum LMFAB 8 (Laboratory of Fundamental Mycology Applied in Biotechnologies, Lyon, France) and *Trychophyton tonsurans* LMFAB 12 (Laboratory of Fundamental Mycology Applied in Biotechnologies, Lyon, France). Amphotericin B was used as a reference in the different tests. The growth-inhibition induce by the different compounds are been deter-



Scheme 4.

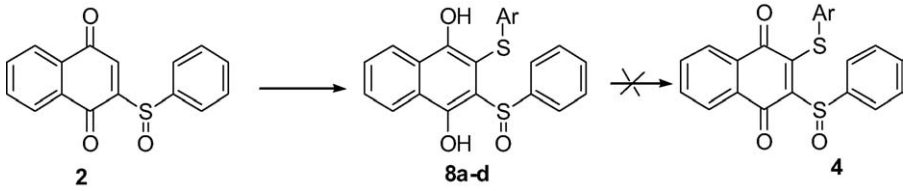


Table 4
Growing-inhibition of different strains of fungi at the 0.4 M ml^{-1} concentration

Product	*C. albicans*	*C. tropicalis*	*A. niger*	*F. oxysporum*	*T. tonsurans*
Ampho B	79	29	75	69	70
5a	42	52	58	58	82
5b	38	54	60	61	78
5c	43	59	63	63	77
6a	48	53	82	82	98
6b	41	62	85	81	88
6c	44	56	96	96	95
6d	39	40	47	48	85
8a	41	59	38	38	83
8b	39	54	54	54	90
8c	42	56	57	57	82
8d	44	56	61	61	87

Ampho B = amphotericin B.

Table 5
Determination of MIC_{50} of synthesized new molecules

	C. albicans *MIC_{50}	*C. tropicalis* MIC_{50}	*A. niger* MIC_{50}	*F. oxysporum* MIC_{50}	*T. tonsurans* MIC_{50}
Ampho B	0.067–0.13	> 3	< 0.2	< 0.2	< 0.2
5a	2–3	< 0.2	< 0.2	< 0.2	< 0.2
5b	> 3	0.2–0.3	< 0.2	< 0.2	< 0.2
5c	> 3	< 0.2	< 0.2	< 0.2	< 0.2
6a	> 3	< 0.2	< 0.2	< 0.2	< 0.2
6b	0.53–1	< 0.2	< 0.2	< 0.2	< 0.2
6c	> 3	< 0.2	< 0.2	< 0.2	< 0.2
6d	0.53–1	< 0.2	< 0.2	< 0.2	< 0.2
6e	> 3	0.2–0.3	< 0.2	1–2	< 0.2
8a	> 3	< 0.2	< 0.2	0.2–0.3	< 0.2
8b	2–3	< 0.2	< 0.2	< 0.2	< 0.2
8c	> 3	< 0.2	< 0.2	< 0.2	< 0.2
8d	> 3	< 0.2	< 0.2	< 0.2	< 0.2

*MIC_{50} is expressed as μg ml^{-1}. Ampho B = amphotericin B.

mined (Table 4). The minimum inhibitory concentration (MIC) values were determined by comparison with amphotericin B as a standard agent. The data are summarized in Table 5. Most of these compounds generally showed potent antifungal activities. Except for *Candida albicans*, amphotericin B was more active than all the synthesized compounds, three products (i.e., **6b**, **6c**, **6d**) were very active against the other strains (*Candida tropicalis, Aspergillus niger*, *Fusarum oxysporum* and *Trichophyton tonsurans*).

MIC_{50} of the synthesized molecules against *C. albicans* was important in comparison to amphotericin B. MIC_{50} were lower than 0.2 μg ml^{-1} except to **6e** which had a MIC_{50} at 0.3 μg ml^{-1} for *C. tropicalis*. For *A. niger, T. tonsurans* and *F. oxysporum,* all MIC_{50} were lower than 0.2 μg ml^{-1} except the MIC_{50} of **8a** and **6e** which are 0.3 μg ml^{-1} and 2 μg ml^{-1}, respectively.

No evident structure-activity relationship seems exist with these different series.

In this work, all synthesized compounds were more active than amphotericin B against these strains. The inhibition ratio product: amphotericin B was better than 2:1 for all compounds except for *C. albicans. C. tropicalis* is often present in opportunistic infections such as in HIV, and is known to be a resistant-amphotericin B strain. thus sulfoxide- and sulfide-quinones can be chosen as candidates to a more extensively in vivo study. Further investigation is needed to clarify the mechanism of the antifungical activity.

4. Experimental part

4.1. antifungal activity tests

The stock solution from each molecule was prepared in DMSO and the final concentration was made in RPMI 1640 medium.

MICs (minimal inhibitory concentrations) of amphotericin B and the synthesized molecules were calculated by the NCCLS broth microdilution method [11] for *C. albicans* and *C. tropicalis* and M38-P [12] for *A. niger*, *F. oxysporum* and *T. tonsurans.* The final concentrations of the antifungal agents ranged from 0–3 μg ml^{-1}. The inoculi were adjusted to a concentration of $4–6 \times 10^5$ CFU/ml^{-1} in RPMI 1640 medium and an aliquot of 0.1 ml was added to each well of the microdilution plate which were incubated at 35 °C for *C. albicans* and *C. tropicalis* and at 37 °C for *A. niger*, *F. oxysporum* and *T. tonsurans.* The plates were read after 48 h with a microplate spectrophotometer set at 405 nm. MICs were calculated based on the density of the growth control and were the lowest drug concentration that resulted in a 50% reduction in growth compared with that of the drug-free growth control. The data are summarized in Table 5.

4.2. Chemistry

4.2.1. Materials

Melting points were determined on a Kofler hot-plate melting point apparatus and were not corrected. Infra-red spectra were obtained on a PERKING-ELMER FT/IR-1600 instrument. Absorption bands are expressed in cm^{-1} and only noteworthy absorptions are listed. ^1H and ^{13}C NMR spectra were recorded on a BRUKER BZH 200/52 instrument, working at 200 MHz (^1H NMR) and 70 MHz (^{13}C NMR). Chemical shifts are reported in ppm downfield from tetramethylsilane. The electronic ionization mass spectrometry experiments were performed on a FINNIGAN MAT 95 XL instrument. Compounds **1** and **2** were synthesized with known procedures [7,13].

4.2.2. General procedure for the synthesis of disulfide naphthoquinones ***5a–c***

To a solution of naphthoquinone (0.250 mmol) in ethanol (15 ml) and 0.500 g of celite-KF (celite/KF: 1:1 in weight) was added a solution of arylsulfide (0.750 mmol). The suspension was stirred for 2 hours. After filtration the solvent was evaporated and the crude product was crystallized into ethanol (Table 6).

Table 6
Physico-chemical data of sulfides **5a–d**

Compound	X	Physico-chemical data
5a	H	MP (EtOH): 136 °C IR (KBr): 3435, 1667, 1593, 1498, 1267 cm^{-1}. ^1H NMR ($DMSOd_6$): 7.90 (2H) m; 7.15 (2H) m; 7.10 (2H) m. ^{13}C NMR ($DMSOd_6$): 178.1, 147.7, 134.0, 133.8, 132.5, 129.9, 129.1, 127.2, 126.6. MS: *m/z* 374 ($M^{+\cdot}$) HRMS: calculated for: $C_{22}H_{14}S_2O_2$: 374.0435; found: 374.0432.
5b	CH_3	MP (EtOH): 169 °C IR (KBr): 2920, 1664, 1649, 1589, 1505, 1491, 1260. ^1H NMR ($CDCl_3$): 8.0 (2H) m; 7.6 (2H) m; 7.3 (4H) m; 7.1 (4H) m; 7.5 (6H) s. ^{13}C NMR ($CDCl_3$): 178.8, 148.3, 138.0, 133.6, 132.9, 131.5, 130.2, 129.9, 127.1, 21.2. MS: *m/z* 402 (M^+). HRMS: calculated for: $C_{24}H_{18}O_2S_2$ 402.0748, found: 402.0742
5c	OCH_3	MP (EtOH): 108 °C IR (KBr): 2942, 1663, 1589, 1491, 1244. ^1H NMR ($CDCl_3$): 8.0 (2H) m; 7.6 (2H) m; 7.4 (4H) d ($J = 10$ Hz); 6.7 (4H) d ($J = 10$ Hz); 3.8 (6H) s. ^{13}C NMR: 178.9, 159.7, 148.1, 133.8, 133.6, 132.9, 127.0, 124.1, 114.7, 55.3. MS: *m/z* 435 ($MH^{+\cdot}$) HRMS: calculated for $C_{24}H_{18}O_4S_2H^+$: 4350725, found: 435.0726.

Table 7
Physico-chemical data of amino-sulfoxides **6a–e**

Compound	X	Physico-chemical data
6a	H	MP (EtOH): 155 °C IR (KBr): 3400, 1685, 991 cm^{-1} ^1H NMR ($DMSOd_6$) : 11.1 (1H) s; 8.1 (1H) d ($J = 8$ Hz); 8.0–7.7 (4H) 7.5–7.2 (4H) m; 7.10 (1H) d, ($J = 8$ Hz). ^{13}C NMR (DMSOd6): 180.2, 149.7, 143.5, 139.6, 134.8, 133.0, 131.8, 131.6, 131.2; 129.4; 129.1; 126.9; 126.3; 126.0; 124.8; 124.2; 116.8. MS: *m/z* 373 ($M^{+\cdot}$) HRMS: calculated for: $C_{22}H_{15}NO_3S$: 373.0773, found: 373.0768
6b	Cl	MP (EtOH): 167 °C IR (KBr): 3400, 1680, 990 cm^{-1} ^1H NMR ($DMSOd_6$): 11.0 (1H) s; 8.0 (1H) d, ($J = 8$ Hz); 7.0 (3H) d, ($J = 8$ Hz); 7.9–7.2 (7H) m. ^{13}C NMR ($DMSOd_6$): 180.1, 179.8, 149.4, 143.3, 138.2, 134.9, 133.2, 131.8, 131.7, 131.5, 131.3, 129.5, 129.2, 127.0, 126.0, 125.4, 124.7, 117.6. MS: *m/z* 407 ($M^{+\cdot}$) HRMS: calculated for $C_{22}H_{14}NO_3SCl$: 407.0382, found: 407.0372
6c	Br	MP (EtOH): 165 °C IR (KBr): 3500, 1683, 994 cm^{-1}. ^1H NMR ($DMSOd_6$): 11.0 (1H) s; 8.0 (1H) d; ($J = 8$ Hz); 7.9–7.0 (5H) m; 6.9 (1H) d, ($J = 8$ Hz). ^{13}C NMR (DMSOd6): 180.0, 179.8, 149.3, 143.3, 138.7, 134.9, 133.2, 132.2, 131.7, 131.5, 131.3, 127.0, 126.1, 125.7, 124.7, 119.5, 117.7. MS: 451 ($M^{+\cdot}$) HRMS: calculated for: $C_{22}H_{14}NO_3SBr$: 450.9878, found: 450.9873
6d	NO_2	MP (EtOH): 192 °C IR (KBr): 3492, 1672, 1592, 1573, 1300 cm^{-1}. ^1H NMR ($DMSOd_6$): ^{13}C NMR ($DMSOd_6$) : MS: *m/z* 419 ($MH^{+\cdot}$) HRMS: calculated for: $C_{22}H_{14}N_2O_5SH^+$: 419.0702, found: 419.0703.

Table 8
Physico-chemical data of sulfoxide-sulfides **8a–d**

Compound	X	Physico-chemical data
8a		MP (EtOH): 179 °C IR (KBr): 3377, 3048, 1578, 1442, 1280, 1167 cm^{-1}. ^1H NMR ($DMSOd_6$): 12.1 (1H) s; 10.0 (1H) s; 8.2 (2H) m; 7.8 (4H) m; 7.4 (3H) m; 7.2 (3H) m; 7.0 (2H) m. ^{13}C NMR ($DMSOd_6$): 151.0, 149.6, 143.6, 135.8, 131.9, 129.7, 129.1, 128.5, 126.4, 128.0, 127.1, 125.6, 123.6, 121.9, 116.1, 103.6. MS: *m/z* 392 ($M^{+\cdot}$) HRMS: calculated for $C_{22}H_{16}O_3S_2$: 392.0541, found: 392.0539.
8b	Br	MP (EtOH): 186 °C IR (KBr): 3370, 3073, 1565, 1473, 1304, 1167 cm^{-1}. ^1H NMR ($DMSOd_6$): 12.3 (1H) s; 10.0 (1H) s; 8.2 (2H) m; 7.9 (5H) m; 7.8 (4H) m; 6.9 (2H) m. ^{13}C NMR ($DMSOd_6$): 151.0, 149.7, 143.5, 135.4, 131.9, 131.8, 129.7, 128.6, 128.1, 127.8, 127.5, 127.1, 126.5, 123.3, 122.0, 118.4; 115.8; 103.2. MS: *m/z* 471 (MH^+, 100%), 473 (80%). HRMS: calculated for: $C_{22}H_{15}O_3S_2BrH^+$: 470.9724, found: 470.9724.
8c	NO_2	MP (EtOH): 190 °C IR (KBr): 3375, 3064, 2922, 1596, 1579, 1340, 1167 cm^{-1}. ^1H NMR ($DMSOd_6$): 12.0 (1H) s; 8.5 (1H) d ($J = 10$ Hz); 8.3 (1H) d ($J = 10$ Hz); 8.0 (1H) d ($J = 10$ Hz); 7.8 (H) m; 7.3 (4H) m; 7.0 (2H) m; 6.5 (1H) m. ^{13}C NMR ($DMSOd_6$): 152.9, 149.0, 143.9, 132.2, 129.6, 128.9, 128.8, 127.1, 124.3, 123.4, 122.9. MS: *m/z* 438 (MH^+). HRMS: calculated for $C_{22}H_{15}O_5NS_2H^+$: 438.0472, found: 438.0472.
8d	CH_3	MP (EtOH): 145 °C IR (KBr): 3358, 3059, 2916, 1564, 1303, 1167 cm^{-1}. ^1H NMR (DMSOd6): 12.2 (1H) s; 9.9 (1H) s; 8.21 (2H); 7.9 (1H) m; 7.7 (2H) m; 7.5 (3H) m; 7.1 (2H) d ($J = 8$ Hz); 6.9 (2H) d ($J = 8$ Hz); 2.1 (1H) m. ^{13}C NMR ($DMSOd_6$): 150.9, 149.4, 143.6, 135.2, 132.2, 131.9, 129.7, 128.5, 127.9, 127.5, 127.1, 126.3, 121.9, 116.1, 104.3, 20.4. MS: 406 ($MH^{+\cdot}$). HRMS: calculated for $C_{23}H_{18}O_3S_2$: 406.0697, found: 406.0681

4.2.3. General procedure for the synthesis of 3-arylamino-2-phenylsulfinylnaphthoquinones ***6a–e***

To a solution of sulfinylnaphthoquinone 2 (0.71 mmol) in ethanol (15 ml) was added a solution of arylamine (1.42 mmol). The solution was stirred for 2 hours then the solvent was evaporated and the crude product was crystallized from ethanol (Table 7).

4.2.4. General procedure for the synthesis 2-arylsulfide-3-phenylsulfinylnaphtalene ***8a–d***

To a solution of 2-sulfinylnaphthoquinone (1.80 mmol) in dichloromethane (2 ml) solution was stirred overnight, then the solvent was evaporated and the crude product was crystallized from ethanol (Table 8).

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