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Original article

Synthesis and biological evaluation of novel nitrogen- and sulfur-containing hetero-1,4-naphthoquinones as potent antifungal and antibacterial agents

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ABSTRACT

1,4-Naphthoquinones are unique reagents in organic synthesis and have been employed in several well known and recently developed areas of application. Furthermore, these 1,4-naphthoquinones have demonstrated high reactivity in nucleophilic vinylic substitutions, in the preparation of sulfurated, (hetero)cyclic and several other transformations. This study describes the synthesis and biological evaluation of derivatives of monosulfurated naphthalene-1,4-dione (**3**), 3-chloro-2-ethoxy-naphthalene-1,4-dione (**4**), disulfurated naphthalene-1,4-dione (**5**), and symmetrical bis-1,4-naphthoquinones (**7**, **9**) were obtained from the reaction of 2,3-dichloro-naphthaquinone (**1**) with S-, O-substituted mono-, di-, and tetrathiols, respectively. The structures of the novel products were characterized by spectroscopic methods.

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1. Introduction

(Hetero)cyclic quinones constitute an important group of substrates. The structure of these substrates is often found in naturally occurring compounds and is incorporated into synthetic biologically active compounds [1]. Structure–activity relationship studies from quinonoid compounds showed that the position and number of nitrogen atoms were considerably important factors to affect the biological activities [2,3]. Generally, increasing the number of substituent nitrogen atoms enhances the activities. There is a report that bis(arylthio)-quinoline-5,8-diones and 6-arylamino-quinoline-5,8-diones exhibited antifungal activity against pathogenic fungi [4,5]. The presence of amino, thio, or chloro moiety on the quinines was considerably important factor to effect antifungal activity [6].

Systemic fungal infections are seriously causes of mortality in HIV infections and the emergence of multi-resistance strains is a significant problem. It is already known that some sulfide-, sulfoxide-quinones have antifungal activities [7]. There are numerous reports on biological evaluation of substituted 1,4-naphthoquinones that show the biological relevance of this system, in particular when they contain thio substituents [8].

The incidence of fungal and bacterial infections still remains an important and challenging problem because of the combination of factors including emerging infectious diseases and also because of increasing of multi-drug resistant microbial pathogens [9]. The resistance of spectrum antifungal and antibacterial agents has prompted us to discover and develop new antifungal and antibacterial drugs [10].

As part of our research program on the synthesis of biologically active quinones, we became interested in the synthesis and biological evaluation of 1,4-naphthoquinones containing nitrogen and sulfur atom, having a range of similar redox potentials. The profound antifungal and antibacterial activity exhibited by compounds [11] has prompted us to synthesize 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 methodology concept in quinone chemistry to carry out biological evaluation of some potent antifungal and antibacterial agents.

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2. Results and discussion

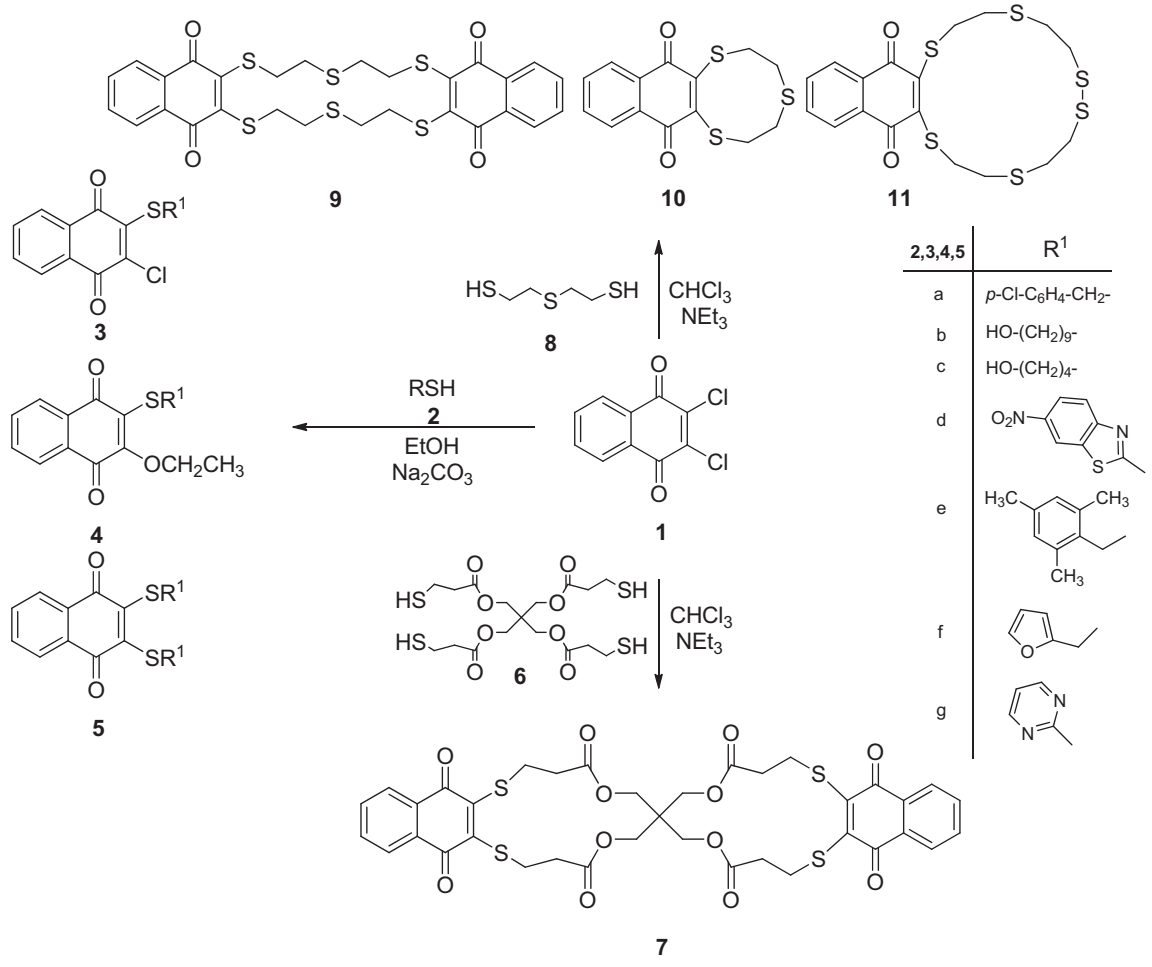
2.1. Chemistry

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 [12]. Based on reactivity and biological activity of 2,3-dichloro-1,4-naphthoquinone derivatives [13], we have studied its reactions with different mono-, di-, and tetrathiols (Scheme 1) in the presence or absence of a base as reported and have evaluated their antifungal and antibacterial activity.

Our investigation was to synthesize both di(thio)-substituted products and mono(thio)-substituted products containing chlorine atom. As intended, the di(thio)- and mono(thio)-substituted compounds were obtained. In some cases, mono(thio)-substituted compounds containing chlorine atom derivatives were not observed potentially due to the decreased thiol amount in the medium of the reaction, while the ethoxy derivatives of mono(thio)-substituted compounds were obtained successfully.

The reaction of 2,3-dichloro-1,4-naphthoquinone (**1**) with different alkyl-, arylthiols (1.1 equivalent) in ethanol in the presence of Na_2CO_3 gave **3a**–**b**, **4c**, **4e**, and **5a**–**g** compounds (Scheme 1). While compound **3a**–**b** are mono(thio)-substituted naphthoquinone, the compound **4c** and **4e** is mono(thio)-substituted ethoxy derivatives. In the mass spectrum of the compounds **4c** and **4e** the accurate mass measurement of the molecular ion peak are noticed at *m/z* 306 $(M)^+$ and 389 $(M)^+$, respectively. In the ^1H NMR spectra of **4c** and **4e**, protons in methylene group (-O–CH_2–) situated in ethoxy group and which are adjacent to the oxygen atom are observed as multiplet at 4.4–4.8 ppm. However, these peaks are not observed in the spectra of **5a**–**g** since these compounds are mono(thio)-substituted naphthoquinone. In the mass spectrum of compounds **5a**–**g**, the accurate mass measurements of the molecular ion peaks were noticed at *m/z* 471 $(M)^+$, 506 $(M)^+$, 366 $(M)^+$, 578 $(M)^+$, 509 $(M + Na)^+$, 382 $(M)^+$, and 401 $(M + Na)^+$, respectively. The ^{13}C NMR shifts of the methylene carbon atoms of compound **4c** and **4e** -adjacent to the oxygen atoms (-O–CH_2–)- have showed their resonances in the downfield at 69 ppm. The ^{13}C NMR shifts of the carbon atoms of compounds **5a**–**g** have appeared at around 150 ppm as one peak only, while the carbon atoms of compounds **3a**–**b**, **4c**, and **4e** have showed their resonances at around 141 and 148 ppm as two peaks. The spectra of compound **3a**–**b**, **4c**, and **4e**, carbon atoms of carbonyl groups are observed at around 175 and 180 ppm as two peaks while the carbon atom signals of carbonyl groups of **5a**–**g** have showed their resonances at around 179 ppm as one peak only. For compounds **3a**–**b**, **4c**, and **4e**, this is due to a carbonyl being beta to oxygen or chlorine atoms and the other carbonyl being beta to sulfur atoms.

The reaction of 2,3-dichloro-1,4-naphthoquinone (**1**) with 2,2′-thiodiethanethiol (**8**) resulted in the formation of intramolecular



Scheme 1. Reaction of 1,4-naphthoquinones with mono-, di-, and tetrathiols.

cyclization to yield heterocyclic diquinone 7,8,10,11,20,21,23,24-octahydrodinaphtho[2,3-*b*:2′,3′-*k*] [1,4,7,10,13,16]hexathiacy-clooctadecine-5,13,18,26-tetraone (**9**), condensed heterocyclic 2,3,5,6-tetrahydronaphtho[2,3-*b*] [1,4,7]trithionine-8,13-dione (**10**), and heterocyclic containing disulfide group 2,3,5,6,9,10,12,13-octahydronaphtho[2,3-*i*] [1,2,5,8,11,14]hexathiacyclohexadecine-15,20-dione (**11**) as exhibited in Scheme 1. In the mass spectrum of compounds **9**, **10**, and **11**, the accurate mass measurements of the molecular ion peaks were noticed at *m/z* 639 $(M + Na)^+$, 308 $(M)^+$, and 460 $(M)^+$, respectively.

It is pertinent to note that symmetrical bis-1,4-naphthoquinones (**7**) were prepared through the addition of tetrathiol nucleophile 2,2-bis(((3-mercaptopropanoyl)oxy)methyl)propane-1,3-diyl bis(3-mercaptopropanoate) (**6**) to naphthoquinone (**1**) representing a common synthetic route to many fused heterocyclic rings which have been used as synthetic intermediates in medicinal chemistry and for dyestuffs.

Monosulfanyl-1,4-naphthoquinones (**3**) are (depending on the character of thiol) positive by Beilstein's test [14], and therefore, contained chlorine, but and bissubstituted (sulfanyl- and ethoxy sulfanyl) and cyclic 1,4-naphthoquinones (**4**, **5**, **7**, **9**, **10**, **11**) are negative by the same test because all chlorines have been substituted by the thiolate (Scheme 1).

2.2. Biological activities as antibacterial and antifungal

In our new endeavors, we have synthesized different (hetero) cyclic naphthoquinones and evaluated their antifungal activity against fungi *Candida tenuis VKM Y-70* and *Aspergillus niger F-1119* by diffusion method [15] and serial dilution method [16] with a view to develop therapeutic agents having broad spectrum of antifungal activity. Antibacterial activity of synthesized compounds was elucidated against *Escherichia coli B-906*, *Staphylococcus aureus 209-P*, and *Mycobacterium luteum b-917* by diffusion method in Table 1 and serial dilution method as shown in Tables 2 and 3. Their activities were compared with those of the known antibacterial agent Vancomicine and the antifungal agent Nystatin. Earlier studies have led to the identification to potent antibacterial and antifungal agents as lead molecules containing quinone chromophore. Afterwards, on the basis of structure–activity relationship of antifungal activity of the (hetero)cyclic quinone derivatives, we have further synthesized and screened antibacterial and antifungal assay of **3a**–**b**, **4c**, **4e**, **5a**–**g**, **7**, and **9** by diffusion method as shown Table 1.

Data presented in Tables 1, 2, and 3 shows that there are substances with antibacterial and fungicidal action among the study compounds. The test-culture *E. coli* appeared not to be sensitive to all compounds. The *S. aureus* and *M. luteum* was not sensitive and low sensitive to compound **3a**–**b**, **4e**, **5a**–**b**,**d**–**f**, **7**, and **9** by diffusion method. The *S. aureus* strain was sensitive to compound **4c** at a concentration of 0.5% (diameter of the inhibition zone was 19.4 mm), while the compound **5c** at a concentration of 0.5% had diameter of the inhibition zone 26 mm.

Compounds **4c** and **5c** showed high activity against *M. luteum* at 0.5% (diameter of the inhibition zone–25.4 mm and 31 mm respectively). Antifungal activity against *C. tenuis* was observed for **5d** at concentration of 0.5% (d = 24 mm). Compound **3a** has low activity against *C. tenuis* (d = 14 mm at 0.5% concentration). Compounds **3b**, **4e**, **5a**, **5e**, **7**, and **9** have no antifungal activity against *A. niger* at 0.5% and 0.1% evaluated concentrations by diffusion method.

Compound **3a** and **4c** (at 0.5% concentration) were found to exhibit low antifungal activity against *C. tenuis* on comparison with antifungal drug Nystatin evaluated by diffusion method.

Table 1
Antibacterial and antifungal activities of compounds by diffusion method.

Compounds	Concentration (%)	Inhibition diameter of microorganism growth, mm				
		Antibacterial activity			Antifungal activity	
		E. coli	*S. aureus*	*M. luteum*	*C. tenuis*	*A. niger*
3a	0.5	0	7.0	0	14.0	7.0
	0.1	0	0	0	10.0	0
3b	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
4c	0.5	0	19.4	25.4	14.7	10
	0.1	0	12.0	14.7	8.4	0
4e	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
5a	0.5	0	0	0	7.4	0
	0.1	0	0	0	0	0
5b	0.5	0	0	0	0	6.0
	0.1	0	0	0	0	0
5c	0.5	0	26.0	31.0	8.0	6.0
	0.1	0	18.0	22.0	0	0
5d	0.5	0	8.0	11.7	24.0	15.0
	0.1	0	0	0	15.0	8.0
5e	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
5f	0.5	0	7.0	10.4	0	10.0
	0.1	0	0	7.7	0	8.0
5g	0.5	0	9.0	11.7	7.0	9.0
	0.1	0	6.0	6.7	6.0	7.0
7	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
9	0.5	0	0	0	0	0
	0.1	0	0	0	0	0
C[a]	0.1	14.0	15.0	18.0	19.0	20.0

[a] Vancomicine was used as a control in the tests of antibacterial activity of the synthesized compounds, and Nystatin was used in the tests of antifungal activity of the synthesized compounds.

Compound **5d** (at 0.5% concentration) had better antifungal activity against *C. tenuis* on comparison with Nystatin (Fig. 1).

Comparison of antibacterial activity with antibacterial drug Vancomicine (at 0.1% concentration) showed that **4c** (at 0.5% concentration) and **5c** (at 0.5 and 0.1% concentration) had better activity against *S. aureus* and *M. luteum* (Fig. 2).

Evaluation of antibacterial activity of synthesized compounds showed that **3a** and **5b** have MIC = 500 μg/mL, **5a** and **5d** have MIC = 125 μg/mL, and **4c** has MIC = 31.2 μg/mL for *M. luteum*. **4c** and **5b** have MIC = 250 μg/mL, **5g** has MIC = 62.5 μg/mL for

Table 2
Antibacterial activities of compounds by serial dilution method.

Compounds	MIC (μg/mL)		
	E. coli	*S. aureus*	*M. luteum*
3a	+	+	500.0
3b	+	+	+
4c	+	250.0	31.2
4e	+	+	+
5a	+	+	125.0
5b	+	250.0	+
5c	+	15.6	15.6
5d	+	+	125.0
5e	+	+	+
5f	+	+	*
5g	500.0	62.5	500.0
7	+	+	+
9	+	+	+

+: Growth of microorganisms.
*In the investigated concentrations the indexes of biocidic effect were not determined.

Table 3
Antifungal activities of compounds by serial dilution method.

Compounds	MIC (μg/mL)	
	C. tenuis	*A. niger*
3a	15.6	15.6
3b	31.2	+
4c	15.6	31.2
4e	62.5	+
5a	15.6	31.2
5b	+	+
5c	7.8	31.2
5d	15.6	31.2
5e	125.0	+
5f	15.6	62.5
5g	125.0	500.0
7	15.6	+
9	31.2	+

+: Growth of microorganisms.
In the investigated concentrations the indexes of biocidic effect were not determined.

S. aureus, and **5c** has MIC = 15.6 μg/mL for *S. aureus* and *M. luteum* (Table 2 and Table 3).

Evaluation of antifungal activity of compounds **3a**–**b**, **4c**, **4e**, **5a**, **5c**, **5g**, **7**, and **9** showed their activity in concentrations 7.8–125 μg/mL against test-culture *C. tenuis*. MIC of **3a**, **4c**, **5a**, **5c**, **5d**, **5f**, and **5g** was observed at 15.6–500 μg/mL against test-culture *A. niger*.

3. Conclusion

A convenient synthesis route of 2- and 2,3-substituted naphthalene-1,4-diones from 2,3-dichloro-1,4-naphthoquinone has been reported. Among the synthesized compounds with antimicrobial activity at low concentrations against *S. aureus*, *M. luteum* bacteria and *C. tenuis* and *A. niger* fungi in comparison with control were identified. 2-chloro-3-((4-chlorobenzyl)thio)naphthalene-1,4-dione (**3a**), 2-ethoxy-3-((4-hydroxybutyl)thio)naphthalene-1,4-dione (**4c**), 2,3-Bis((4-chlorobenzyl)thio)naphthalene-1,4-dione (**5a**), 2,3-Bis((4-hydroxybutyl)thio)naphthalene-1,4-dione (**5c**), 2,3-bis((6-nitrobenzo[d]thiazol-2-yl)thio)naphthalene-1,4-dione (**5d**), 2,3-bis((furan-2-ylmethyl)thio)naphthalene-1,4-dione (**5f**), and 2,3-Bis(pyrimidin-2-ylthio)naphthalene-1,4-dione (**5g**) are promising as biologically active compounds.

The latter approach mostly could rely on the coordination of transition-metal cations to monosite (**9**, **10**, **11**) and/or multisite (**7**) ligands. Investigations concerning the biological activity in science of the compounds have presented herein.

4. Experimental

4.1. Materials and methods

Melting points were measured using a Buchi B-540 melting point apparatus and are uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. Infrared (IR) spectra were recorded in KBr pellets in Nujol mulls on a Perkin Elmer Precisely Spectrum One FTIR spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$, DMSO on a Varian Unity INOVA spectrometer. Mass spectra were obtained on a Thermo Finnigan LCQ Advantage MAX LC/MS/MS spectrometer using the ESI or APCI technique. Products were isolated by column chromatography on silica gel (Fluka silica gel 60, particle size 63–200 μm). Thin-layer chromatography (TLC) was performed on Merck silica gel plates ($60F_{254}$), and detection was carried out with ultraviolet light (254 nm). All chemicals were reagent grade and used without further purification. Moisture was excluded from the glass apparatus using $CaCl_2$ drying tubes.

4.2. General procedures for the synthesis of 1,4-naphthoquinones

4.2.1. General procedure 1: for the synthesis of mono-, disubstituted thio-substituted-1,4-naphthoquinones, and ethoxy-substituted thio-substituted-1,4-naphthoquinones

Sodium carbonate (1.52 g) was dissolved in ethanol as reaction media (65 mL). 2,3-dichloro-1,4-naphthoquinone (**1**) and thiol (**2a**–**g**) were added to the solution, respectively. Without heating, the mixture was stirred for 6–8 h. The color of the solution changed quickly, and the extent of the reaction was monitored by TLC. The reaction mixture was extracted in a Soxhlet extractor with dichloromethane. After recovery of the solvent, the crude product was purified by column chromatography.

*4.2.1.1. 2-Chloro-3-((4-chlorobenzyl)thio)naphthalene-1,4-dione (**3a**).* Orange powder; yield 1.2 g (78%); m.p. 120.5–122.5 °C; R_f: 0.82 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3299, 3038 cm^{-1} (C–$H_{aromatic}$), 1589, 1496 (C=C), 1672, 1660 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.52 (s, 2H, S–CH_2), 7.17–7.23 (m, 4H, $CH_{aromatic}$), 7.65–7.67 (m, 2H, $CH_{aromatic}$), 7.98–8.05 (m, 2H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 38.05 (S–CH_2), 127.53, 129.17, 130.77, 131.39, 132.71, 133.92, 134.18, 134.44, 135.45 ($C_{aromatic}$), 148.13, 141.02 (=C–S), 180.11, 175.29 (C=O); MS (ESI): 349 $(M)^+$; Anal. Calcd. for $C_{17}H_{10}Cl_2O_2S$: C, 58.47; H, 2.89; S, 9.18. Found C, 58.40; H, 2.97; S, 8.55.; Beilstein test [14]: Cl positive.

*4.2.1.2. 2-Chloro-3-((9-hydroxynonyl)thio)naphthalene-1,4-dione (**3b**).* Red powder; yield 0.53 g (30%); m. p. 62.3–62.5 °C; R_f: 0.7 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3583 (O–H), 2926 (C–$H_{aromatic}$), 2851

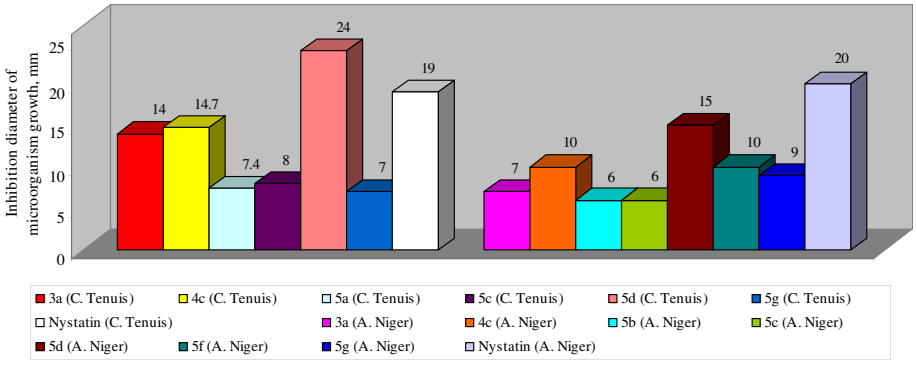


Fig. 1. Comparative antifungal study plot with Nystatin and compounds.

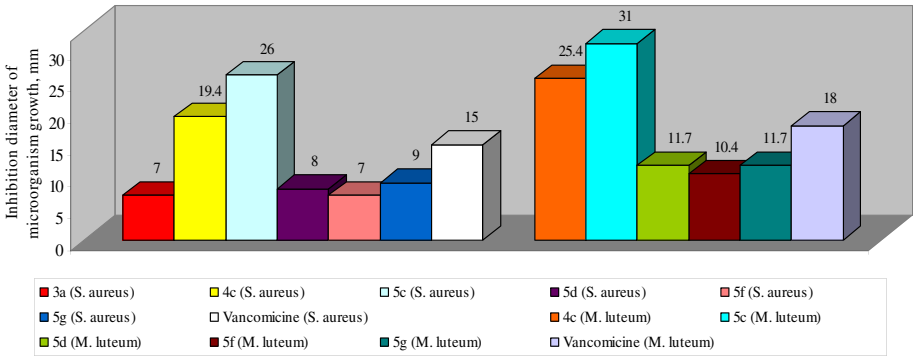


Fig. 2. Comparative antibacterial study plot with Vancomicine and compounds.

($C-H_{aliphatic}$), 1665 (C=O), 1590 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 1.2–1.8 (m, 14H, CH_2), 3.3 (t, J = 7.32 Hz, 2H, -S–CH_2), 3.5 (t, J = 6.83 Hz, 2H,-O–CH_2), 7.2–8.2 (m, 4H, $H_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.75, 29.40, 28.34, 28.27, 27.97, 27.58, 24.68 (–CH_2), 33.32 (–S–CH_2), 62.00 (HO–CH_2), 133.09, 132.77, 131.62, 130.28, 126.86, 126.19 ($C_{aromatic}$, $CH_{aromatic}$), 138.75 (=C–S), 148.41 (=C–Cl), 178.89 (C=O); MS (ESI): 366 $(M)^+$; Anal. Calcd. for $C_{19}H_{23}ClO_3S$: C, 62.20; H, 6.32; S, 8.74. Found C, 61.15; H, 5.20; S, 8.54; Beilstein test [14]: Cl positive.

*4.2.1.3. 2-Ethoxy-3-((4-hydroxybutyl)thio)naphthalene-1,4-dione (**4c**).* Red powder, yield 0.53 g (% 36); m. p. 64.6–64.8 °C; R_f: 0.4 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3411 (O–H), 2979 (C–$H_{aromatic}$), 2936 (C–$H_{aliphatic}$), 1660 (C=O), 1591 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 1.3–1.4 (q, 3H, CH_3), 1.5–1.7 (m, 4H, CH_2), 3.2 (t, 2H, J = 6.83 Hz, S–CH_2), 3.6 (t, 2H, J = 6.34 Hz –CH_2–OH), 4.8 (q, 2H, O–CH_2), 7.5–8.0 (m, 4H, $H_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.91 (CH_3), 25.59–30.61 (CH_2), 31.78 (S–CH_2), 61.11(CH_2–OH), 69.01 (O–CH_2), 125.38, 125.56, 130.42, 131.34, 132.56, 32.62 ($C_{aromatic}$, $CH_{aromatic}$), 133.26 (=C–S), 156.94 (=C–O), 177.86, 181.87 (C=O); MS (ESI): 306 $(M)^+$; Anal. Calcd. for $C_{16}H_{18}O_4S$: C, 62.72; H, 5.92; S, 10.47. Found C, 61.65; H, 5.76; S, 10.01; Beilstein test [14]: Cl negative.

*4.2.1.4. 2-Ethoxy-3-((2,4,6-trimethylbenzyl)thio)naphthalene-1,4-dione (**4e**).* Orange powder; yield 0.51 g (32%); m. p. 125.1–126.4 °C; R_f : 0.68 [PET/CH_2Cl_2(1:1)]; IR (KBr): ν (cm^{-1}) 3067 (C–$H_{aromatic}$), 2987, 2944, 2959, 2909, 2857 (C–$H_{aliphatic}$), 1671 (C=O), 1590, 1556 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 1.36 (t, J = 7.32 Hz, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.36 (s, 6H, CH_3), 4.39–4.43 (m, 4H, S–CH_2, O–CH_2), 6.79 (s, 2H, $CH_{aromatic}$), 7.62–7.64 (m, 2H, $CH_{aromatic}$), 7.98–8.00 ppm (m, 2H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.9, 18.6, 18.6, 19.9 (CH_3), 31.8 (S–CH_2), 69.1 (O–CH_2), 125.4, 125.6, 128.1, 128.8, 130.5, 131.5, 132.5, 132.6, 134.6, 136.3, 136.7 ($CH_{aromatic}$, $C_{aromatic}$), 156.6 (O–C–C=O), 177.9, 181.9 (C=O); MS (ESI): 389 $(M + Na)^+$; Anal. Calcd. for $C_{22}H_{22}O_3S$: C, 72.10; H, 6.05; S, 8.75%. Found C, 71.72; H, 6.03; S, 8.99%; Beilstein test [14]: Cl negative.

*4.2.1.5. 2,3-Bis((4-chlorobenzyl)thio)naphthalene-1,4-dione (**5a**).* Orange powder; yield 1.6 g (77%); m. p. 137.5–139 °C; R_f : 0.77 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3064 cm^{-1} (C–$H_{aromatic}$), 1591, 1496 (C=C), 1661 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 4.35 (s, 4H, S–CH_2), 7.10–7.22 (m, 4H, $CH_{aromatic}$), 7.59–7.62 (m, 4H, $CH_{aromatic}$), 7.90–7.93 (m, 4H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 37.39 (S–CH_2), 125.77, 126.22, 127.65, 129.37, 131.69, 132.54, 134.74 ($C_{aromatic}$), 146.27 (=C–S), 177.90 (C=O); MS (ESI): 471 $(M)^+$; Anal. Calcd. for $C_{24}H_{16}Cl_2O_2S_2$: C, 61.15; H, 3.42; S, 13.60. Found C, 58.83; H, 3.06; S, 10.21; Beilstein test [14]: Cl positive.

*4.2.1.6. 2,3-Bis((9-hydroxynonyl)thio)naphthalene-1,4-dione (**5b**).* Red powder; yield 0.74 g (42%); m. p. 72–72.2 °C; R_f: 0.6 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3313 (O–H), 2924 (C–$H_{aromatic}$), 2848 (C–$H_{aliphatic}$), 1665 (C=O), 1589 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 1.1–1.6 (m, 28H, CH_2), 3.2 (t, J = 7.28, 2H, -SCH_2), 3.5 (t, J = 6.83, 2H, -OCH_2), 7.60–8.0 (m, 4H, $H_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 32.98, 30.63, 29.62, 29.53, 29.25, 28.88, 25.92 (-CH_2), 35.17 (-S–CH_2), 63.23 (-O–CH_2), 148.11 (=C–S), 179.24 (C=O); MS (ESI): 506 $(M)^+$; Anal. Calcd. for $C_{28}H_{42}O_4S_2$: C, 66,36; H, 8.35; S, 12,65. Found C, 65.28; H, 8.10; S, 13.01; Beilstein test [14]: Cl negative.

*4.2.1.7. 2,3-Bis((4-hydroxybutyl)thio)naphthalene-1,4-dione (**5c**).* Red powder; yield 0.55 g (38%); m. p. 70.6–71 °C; R_f: 0.3 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3533 (O–H), 2936 (C–$H_{aromatic}$), 2862 (C–$H_{aliphatic}$), 1662 (C=O), 1590 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 1.6–1.8 (m, 8H, CH_2), 3.2–3.3 (t, 4H, J = 6.84 Hz, S–CH_2), 3.5–3.7 (t, 4H, J = 6.85, CH_2–OH), 7.95–8.05 (m, 4H, $H_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.92, (S–CH_2), 61.23 (CH_2–OH), 125.91, 131.99, 132.51 ($C_{aromatic}$, $CH_{aromatic}$), 146.84(=C–S), 177.97 (C=O); MS (ESI): 366 $(M)^+$; Anal. Calcd. for $C_{18}H_{22}O_4S_2$: C, 58.99; H, 6.05; S, 17.50. Found C, 58.35; H, 6.20; S, 17.25; Beilstein test [14]: Cl negative.

*4.2.1.8. 2,3-Bis((6-nitrobenzo[d]thiazol-2-yl)thio)naphthalene-1,4-dione (**5d**).* Orange powder; yield 1.5 g (60%); m. p. 148.5–150 °C; R_f : 0.50 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3090 cm^{-1} (C–$H_{aromatic}$), 1573, 1520 (C=C), 1678 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 7.69–8.67 (m, 10H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 116.76, 121.15, 121.77, 126.06, 127.01, 131.17, 133.82 ($C_{aromatic}$), 144.05 (=C–S), 147.85 (C–NO_2), 155.33 (C=N–C-), 166.75 (S–C–S), 176.57 (C=O); MS (ESI): 578 $(M)^+$; Anal. Calcd. for $C_{24}H_{10}N_4O_6S_4$: C, 49.82; H, 1.74; N, 9.68; S, 22.17. Found C, 49.15; H, 1.69; N, 8.56; S, 15.90; Beilstein test [14]: Cl negative.

*4.2.1.9. 2,3-Bis((2,4,6-trimethylbenzyl)thio)naphthalene-1,4-dione (**5e**).* Yelow powder; yield 0.73 g (34%); m. p. 252.1–254.4 °C; R_f : 0.80 [PET/CH_2Cl_2(1:1)]; IR (KBr): ν (cm^{-1}) 3004 (C–$H_{aromatic}$), 2968, 2913, 2859 (C–$H_{aliphatic}$), 1665 (C=O), 1590 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 2.17 (s, 6H, CH_3), 2.34 (s, 12H, CH_3), 4.50 (s, 4H, S–CH_2), 6.76 (s, 4H, $CH_{aromatic}$), 7.64–7.66 (m, 2H, $CH_{aromatic}$), 8.03–8.05 ppm (m, 2H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 18.6, 19.9 (CH_3), 33.8 (S–CH_2), 125.9, 128.1, 128.5, 132.0, 132.5, 136.4, 136.7, 147.1 ($CH_{aromatic}$, $C_{aromatic}$), 178.2 (C=O); MS (ESI): 509 $(M + Na)^+$; Anal. Calcd. for $C_{30}H_{30}O_2S_2$: C, 74.04; H, 6.21; S, 13.18. Found C, 73.75; H, 6.12; S, 13.10; Beilstein test [14]: Cl negative.

*4.2.1.10. 2,3-Bis((furan-2-ylmethyl)thio)naphthalene-1,4-dione (**5f**).* Red powder; yield 0.65 g (39%); m. p. 110–111.5 °C; R_f: 0.56 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3141, 3021 (C–$H_{aromatic}$), 1587, 1458 (C=C), 1648 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 4.41 (s, 4H, S–CH_2),

6.00 (d, $J = 0.98$, 2H, CH_{furan}), 6.10 (dd, $J = 1.95$, $J = 1.95$, 2H, CH_{furan}), 7.15 (d, $J = 0.98$, 2H, O–CH_{furan}), 7.60 (dd, $J = 3.42$ Hz, $J = 3.42$ Hz, 2H, $H_{aromatic}$), 7.95 (dd, $J = 3.42$ Hz, $J = 3.42$ Hz, 2H, $H_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.64 (S–CH_2), 122.35, 129.02 (C–$H_{aromatic}$), 128.43 ($C_{aromatic}$), 103.95, 105.94, 137.97 (CH_{furan}), 143.07 (=C–S), 146.21 (C_{furan}), 174.45 (C=O); MS (ESI): 382 $(M)^+$, 300 $(M-C_5H_5O)^+$, 259 $(M-C_5H_5OS)^+$; Anal. Calcd. for $C_{20}H_{14}O_4S_2$: C, 62.81; H, 3.69; S, 16.77. Found C, 63.25; H, 3.83; S, 18.14; Beilstein test [14]: Cl negative.

*4.2.1.11. 2,3-Bis(pyrimidin-2-ylthio)naphthalene-1,4-dione (**5g**).* Yelow powder; yield 1.24 g (75%); m. p. 161.5–163 °C; R_f : 0.16 ($CHCl_3$); IR (KBr): ν (cm^{-1}) 3029 (C–$H_{aromatic}$), 1554, 1524 (C=C), 1727, 1668 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 6.96 (t, $J = 4.88$ Hz, 2H, CH–$CH_{pyrimidine}$–CH), 7.69 (dd, $J = 2.93$ Hz, $J = 3.42$ Hz, 2H, $H_{aromatic}$), 8.04 (dd, $J = 3.42$ Hz, $J = 3.41$ Hz, 2H, $H_{aromatic}$), 8.40 (d, 4.88 Hz, 4H, N–$CH_{pyrimidine}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 118.09 (CH–$CH_{pyrimidine}$–CH), 127.72 (C–$H_{aromatic}$), 133.19 ($C_{aromatic}$), 134.15 (C–$H_{aromatic}$), 149.03 (=C–S), 157.84 (N–$CH_{pyrimidine}$), 170.43 (N–$C_{aromatic}$–N), 179.07 (C=O); MS (ESI): 401 $(M + Na)^+$, 267 $(M-C_4H_3N_2S)^+$; Anal. Calcd. for $C_{18}H_{10}N_4O_2S_2$: C, 57.13; H, 2.66; S, 16.95. Found C, 56.16; H, 3.58; S, 17.28; Beilstein test [14]: Cl negative.

*4.2.2. General procedure **2**: for the synthesis of cyclic 1,4-naphthoquinones*

2,3-dichloro-1,4-naphthoquinone (**1**) and thiol (**6** and **8**) were stirred in chloroform as solvent (50 mL). Triethylamine (1 mL) or sodium carbonate (1.52 g) was added to the reaction mixture slowly. Without heating, the mixture was stirred for 4 h. The color of the solution changed quickly, and the extent of the reaction was monitored by TLC. Chloroform was added to the reaction mixture to separate the organic layer. Then, the organic layer was washed with water (5 × 30 mL) and dried with Na_2SO_4. After filtering, the solvent was evaporated, and the residue was purified by column chromatography on silica gel.

*4.2.2.1. 2,2′,3,3′,11,11′,12,12′-octahydro-7,7′-spirobi[naphtho[2,3-j][1,5,9,12]dioxadithiacyclopentadecin]-4,4′,10,10′,14,14′,19,19′(6H,6′H,8H,8′H)-octaone (**7**).* Yellow powder; yield 1 g (57.5%); m. p. 205 °C (decomp.); R_f: 0.22 (CH_2Cl_2); IR (KBr): ν (cm^{-1}) 3044 ($CH_{aromatic}$), 1590, 1497 (C=C), 1739, 1662 (C=O); ^1H NMR (500 MHz, $CDCl_3$): δ 2.61–2.68 (m, 8H, (C=O)–CH_2), 3.45–3.49 (m, 8H, S–CH_2), 4.07 (s, 8H, O–CH_2), 7.81–7.84 (m, 2H, $CH_{aromatic}$), 7.95–7.99 (m, 2H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 29.79 (S–CH_2), 35.31 ((C=O)–CH_2), 35.65 (-C-), 63.64 (S–CH_2), 134.56, 133.59, 127.26 ($C_{aromatic}$), 147.29 (=C–S), 178.88, 171.72 (C=O); MS (ESI): 796 $(M)^+$; Anal. Calcd. for $C_{37}H_{32}O_{12}S_4$: C, 55.77; H, 4.05; S, 16.09. Found C, 54.70; H, 4.85; S, 14.19; Beilstein test [14]: Cl negative.

*4.2.2.2. 2-((2-((2-((1,4-Dioxo-3-((2-(propylthio)ethyl)thiwo)-1,4-dihydronaphthalen-2-yl)thio)ethyl)thio)ethyl)thio)-3-mercaptonaphthalene-1,4-dione (**9**).* Orange powder; yield 0.12 g (9%); m. p. 231.7–233.2 °C; R_f : 0.1 [PET/CH_2Cl_2(1:1)]; IR (KBr): ν (cm^{-1}) 2962, 2919, 2850 (C–$H_{aliphatic}$), 1663 (C=O), 1590 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 2.78–2.83 (m, 8H, S–CH_2), 3.38–3.43 (m, 8H, S–CH_2), 7.58–7.61 (m, 4H, $CH_{aromatic}$), 7.93–7.98 ppm (m, 4H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 30.5, 32.2, 33.7, 37.8 (CH_2), 125.9, 131.9, 132.6 ($CH_{aromatic}$, $C_{aromatic}$), 146.2 (S–C–C=O), 177.9 (C=O); MS (ESI): 639 $(M + Na)^+$; Anal. Calcd. for $C_{28}H_{24}O_4S_6$: C, 54.52; H, 3.92; S, 31.19. Found C, 54.35; H, 3.82; S, 31.29; Beilstein test [14]: Cl negative.

*4.2.2.3. 2,3,5,6-Tetrahydronaphtho[2,3-b][1,4,7]trithionine-8,13-dione (**10**).* Orange powder; yield 0.02 g (5%); m. p. 110.8–112.8 °C; R_f : 0.67 [PET/CH_2Cl_2(1:1)]; IR (KBr): ν (cm^{-1}) 2962, 2917, 2851 (C–$H_{aliphatic}$), 1659, 1644 (C=O), 1590 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 2.85 (t, $J = 5.86$ Hz 4H, S–CH_2), 3.74 (t, $J = 5.86$ Hz 4H, S–CH_2), 7.63–7.65 (m, 2H, $CH_{aromatic}$), 8.01–8.03 (m, 2H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 34.5, 37.6 (CH_2), 127.1, 132.3, 133.8 ($CH_{aromatic}$, $C_{aromatic}$), 146.2 (S–C–C=O), 180.7 (C=O); MS (ESI): 308 $(M)^+$; Anal. Calcd. for $C_{14}H_{12}O_2S_3$: C, 54.52; H, 3.92; S, 31.19. Found C, 54.25; H, 4.01; S, 31.38; Beilstein test [14]: Cl negative.

*4.2.2.4. 2,3,5,6,9,10,12,13-Octahydronaphtho[2,3-i][1,2,5,8,11,14]hexathiacyclohexadecine-15,20-dione (**11**).* Orange powder; yield 0.05 g (4%); m. p. 135.9–136.8 °C; R_f : 0.57 [PET/CH_2Cl_2(1:1)]; IR (KBr): ν (cm^{-1}) 2960, 2919, 2850 (C–$H_{aliphatic}$), 1661, 1650 (C=O), 1588 (C=C); ^1H NMR (500 MHz, $CDCl_3$): δ 2.80–2.83 (m, 12H, S–CH_2), 3.45–3.47 (m, 4H, S–CH_2), 7.65–7.66 (m, 4H, $CH_{aromatic}$), 7.99–8.02 ppm (m, 4H, $CH_{aromatic}$); ^{13}C NMR (125 MHz, $CDCl_3$): δ 31.3, 32.5, 34.2, 38.28 (CH_2), 126.1, 131.9, 132.7 ($CH_{aromatic}$, $C_{aromatic}$), 147.3 (S–C–C=O), 177.9 (C=O); MS (ESI): 460 $(M)^+$; Anal. Calcd. for $C_{18}H_{20}O_2S_6$: C, 46.92; H, 4.38; S, 41.76. Found C, 46.38; H, 4.03; S, 40.35; Beilstein test [14]: Cl negative.

4.3. Antifungal and antibacterial evaluation

4.3.1. Diffusion technique

Antimicrobial activity of compounds was evaluated by diffusion in peptone on nutrient medium (meat-extract agar for bacteria; wort agar for fungi). The microbial loading was 10^9 cells (spores)/1 mL. The required incubation periods were as: 24 h at 35 °C for bacteria and 48–72 h at 28–30 °C for fungi. The results were recorded by measuring the zones surrounding the disk. Control disk contained Vancomicine (for bacteria) or Nistatine (for fungi) as a standard.

4.3.2. Serial dilution technique

Testing was performed in a flat-bottomed 96-well tissue culture plate. The tested compounds were dissolved in DMSO, and arriving the necessary concentration. The exact volume of solution of compounds is brought in nutrient medium. The inoculum of bacteria and fungi was inoculated in nutrient medium (meat-extract agar for bacteria; wort agar for fungi). The duration of incubation was at 37 °C for bacteria and 30 °C for fungi during 24–72 h. The results were estimated according to the presence or absence of microorganism growth.

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