

SYNTHESIS AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF NEW 2-MERCAPTO-3-SUBSTITUTED-1,4-NAPHTHOQUINONES (I)

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Abstract

Compounds bearing the 1,4-naphthoquinone ring and sulphur containing derivatives had been the subject of much interest due to their antimicrobial, antiviral, anti-inflammatory and anti-tumoural activities. Design of new derivatives of naphthoquinones, in order to improve their pharmaceutical properties, is still a research area of utmost importance. In the present study, we describe the synthesis, physico-chemical properties and evaluation of antimicrobial activity of new 2-mercapto-3-substituted-1,4-naphthoquinones. For the synthesis we used different 2-chloro-3-substituted-1,4-naphthoquinones and thiourea. The new naphthoquinone derivatives were characterized by elemental analysis, UV-VIS and IR spectrometry, mass spectrometry and ¹H-NMR. All this methods confirmed the structure of the compounds. The antimicrobial activity was tested on several Gram-positive and Gram-negative bacteria using disk diffusion technique. In general, the compounds were active against tested Gram-positive bacteria. This encourages further studies for application of these compounds as antibiotic therapy.

Rezumat

Datorită activității antimicrobiene, antivirale și antitumorale dovedite pentru unii reprezentanți, derivații de 1,4-naftochinonă și numeroși compuși cu sulf continuă să prezinte interes. De aceea, proiectarea unor derivați tiolici de naftochinonă cu proprietăți farmaceutice îmbunătățite este încă o direcție de cercetare de importanță maximă. Pentru acest studiu ne-am propus sinteza, caracterizarea fizico-chimică și evaluarea activității antimicrobiene a unor noi derivați tiolici de 1,4-naftochinonă-3-N-substituită. Pentru sinteză am folosit amestecul 2-cloro-3-N-substituit-1,4-naftochinonelor corespunzătoare cu tiouree. Produsele brute au fost purificate prin recristalizare din metanol absolut, gradul de puritate fiind verificat printr-o metodă cromatografică. Randamentele reacțiilor au fost între 64–83%. Caracterizarea fizico-chimică a derivaților 2-mercapto-3-N-substituit-1,4-naftochinonă puri s-a făcut prin analiză elementală, spectrometrie UV-VIS și IR, spectrometrie de masă și ¹H-RMN. Metodele folosite au confirmat structurile compuşilor sintetizați. Activitatea antimicrobiană a fost testată pe germeni Gram-pozitivi și Gram-negativi folosind metoda difuziei, iar cea antifungică pe *Candida albicans*. În general, compuşii sintetizați s-au dovedit activi pe bacteriile Gram-pozitive testate. Rezultatele încurajează continuarea studiilor legate de posibila utilizare a derivaților tiolici ca antibiotice.

Keywords: naphthoquinone derivatives, thiols, antibacterial, antifungal

Introduction

Naphthoquinones are secondary metabolites widely distributed in plants, fungi and animals that possess various biological roles. Naphthoquinones are usually coloured in orange or brown and are important for pigmentation. Plants containing the naphthoquinone ring are world-wide used in the traditional medicine [2]. For this reason, biological activities of several natural compounds bearing a substituted 1,4-naphthoquinone ring have been intensely studied and have been found to possess several pharmacological effects such as

antibacterial, fungicidal, antiviral, antiparasitic, antitumor or anti-aggregative activities [1, 3, 8, 12]. 1,4-naphthoquinone moiety is the pharmacophore unit of several antibiotics, such as rifamycin, damavaricin [5] and anticancer drugs, for example anthracycline, doxorubicin and mitoxanthrone [4]. Thiol- containing compounds had been the subject of much interest due to their antimicrobial, antiviral, anti-inflammatory and antitumor activity. The combination of thiol groups with the naphthoquinone ring provide more labile bonds and are supposed to extend the spectrum of biological

action [9]. Several sulphur containing derivatives of 1,4-naphthoquinone are reported in literature [8 - 11, 13], green chemistry methods being used in the last years in order to obtain such compounds.

Due to their importance, design of new sulphur derivatives of naphthoquinones to improve their pharmaceutical properties is still a research area of the utmost importance.

The present study aimed the synthesis, physico-chemical characterization and evaluation of antibacterial and antifungal activity of new 2-mercapto-3-substituted-1,4-naphthoquinones.

Materials and Methods

Chemistry. All the reagents and solvents used in the present study were of analytical grade and purchased from Merck Co. The degree of purity was determined by standard procedures (melting point, elemental analysis). The structures were confirmed by UV-visible and infrared spectroscopy, proton nuclear magnetic resonance ($^1\text{H-NMR}$) and mass spectrometry. Elemental analysis has been performed on a CHNOS Vario El analyser. Melting points were measured in open capillary tubes with a Gallenkamp melting point apparatus and are uncorrected. UV-Vis spectra were recorded with a Jasco V-530 spectrophotometer, within the range 200–700 nm on substances solved in dimethylsulphoxide (DMSO). IR spectra have been recorded in potassium bromide (KBr) pellets with a BioRad FTS 135 FT-IR spectrophotometer, within the range 3500–400 cm^{-1} . Mass spectra have been obtained using a HPGC-MS 5890 MD 5971 spectrometer, at 70 eV, with helium as carrier gas at a flow rate 2 mL/min. To record $^1\text{H-NMR}$ spectra we used a Varian NMR-System 300 spectrometer, at 300 MHz, in $\text{CDCl}_3/\text{DMSO}-d_6$ or $\text{DMSO}-d_6$ with tetramethylsilane (TMS) as an internal standard.

General procedure for the synthesis of 2-chloro-3-substituted-1,4-naphthoquinones. A mixture of 13.2 mmol 2,3-dichloro-1,4-naphthoquinone and 13.2 mmol different amines was dissolved in 75 mL absolute methanol and heated for 3 hours. The solution obtained was concentrated by distillation of 20 mL methanol. Over hot solution was added cool water and then obtained precipitate was separated by vacuum filtration. The crude products were purified by recrystallization from absolute methanol to obtain 2,3-disubstituted-1,4-naphthoquinones with various colours.

General procedure for the synthesis of 2-mercapto-3-substituted-1,4-naphthoquinones. A mixture of 2-chloro-3-substituted-1,4-naphthoquinone and thiourea in molar ratio 1:2 was dissolved in 100 mL absolute methanol and heated for 3 hours. To the hot solution were added 200 mL NaOH 1M and heated under stirring for 1.5 h. Afterwards, solution

was acidified with glacial acetic acid. The mixture was cooled at room temperature and the crude product was separated by vacuum filtration. The crude products were purified by recrystallization from absolute methanol, obtaining the derivatives of 2-mercapto-3-substituted-1,4-naphthoquinone derivatives with various colours.

In vitro antibacterial activity. The compounds were evaluated for their *in vitro* antibacterial activity against Gram positive (*Staphylococcus aureus* ATCC[®]25923, *Enterococcus faecalis* ATCC[®]29212) and Gram negative (*Escherichia coli* ATCC[®]25922, *Klebsiella spp.* ATCC[®]700603) strains by diffusion method. All bacterial strains were grown in Mueller Hinton medium. The disks needed for testing (5 mm diameter) were prepared from Whatman filter paper no.1 and sterilized in a hot air oven. The disks were soaked with 5 μL solution 10 mg/mL of 1,4-naphthoquinone derivatives in DMSO. Then disks were put on exponentially growing plated cultures of previous mentioned bacterial strains, the inoculums for direct colony suspension being equivalent to a 0.5 standard from the McFarland turbidimetric scale [14]. The plates were incubated for 18 hours at 37°C. The results were recorded by measuring the diameter of the zones of complete inhibition, including the diameter of the disk. Disks containing DMSO, ampicillin and oxacillin were used as control in this assay.

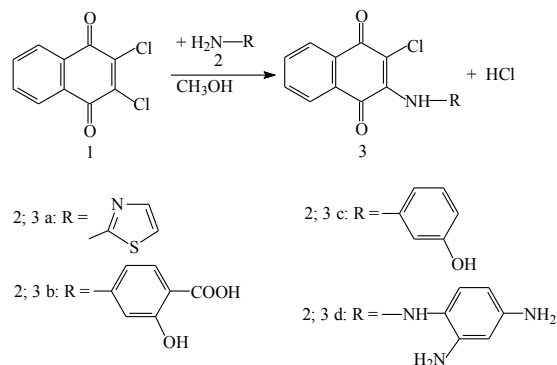
In vitro antifungal activity. The compounds were evaluated for their *in vitro* antifungal activity against *Candida albicans* ATCC[®]10231 by diffusion method. Fungi were grown in Sabouraud Dextrose Agar medium. The disks (5 mm diameter) were soaked with 5 μL solution 10mg/mL of 1,4-naphthoquinone derivatives in DMSO. Then disks were put on an exponentially growing plated culture with a dilution to 10^{-6} colony forming unit. The plates were incubated for 48 hours at 37°C. The results were recorded by measuring the diameter of the zones of complete inhibition, including the diameter of the disk. Disks containing DMSO and clotrimazole were used as blank and standard in this assay.

Results and Discussion

We synthesized several 2,3-disubstituted derivatives of 1,4-naphthoquinone. 2-Chloro-3-substituted-1,4-naphthoquinones were obtained in one-step reaction between dichlorone (2,3-dichloro-1,4-naphthoquinone) and one of the following amines: 2-amino-sulphathiazole, 4-aminosalicylic acid, 2,4-diaminophenylhydrazine and 3-amino-phenol (Figure 1). 2-chloro-3-(N-thiazole-2-ylamino)-1,4-naphthoquinone, 3a

mp. = 164–165°C, yield = 74%; brown powder; IR ν (cm^{-1}): 3249 (w, NH); 1680 (vi, C=O); 1587, 793

(m, m, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO}-d_6$): 4.00 (1H, s, NH); 6.56–7.53 (2H, m, CH thiazole); 7.72–8.00 (4H, m, CH aromatic ring); calculated, %: C 53.70; H 2.40; Cl 11.01; N 9.63; S 11.01; found, %: C 53.69; H 2.44; Cl 10.98; N 9.60; S 10.98; $\text{C}_{13}\text{H}_7\text{N}_2\text{O}_2\text{SCl}$; $M = 290.5$ g/mol.

**Figure 1.**

Scheme for the synthesis of 2-chloro-3-substituted-1,4-naphthoquinones

2-chloro-3-(N-(4-carboxy-3-hydroxy-phenyl)-amino)-1,4-naphthoquinone, 3b

mp. = 169.5 °C; yield = 83%; red-brown powder; IR ν (cm^{-1}): 3254 (w, NH); 1679 (vi, C=O); 3618, 1138 (i, OH); 2921, 1718 (m, i, COOH); 1587, 793 (m, m, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO}-d_6$): 3.98 (1H, s, NH); 4.98 (1H, s, OH); 10.96 (1H, s, COOH); 6.16–7.71 (3H, m, CH aromatic ring); calculated, %: C 59.38; H 2.91; Cl 10.33; N 4.07; found, %: C 59.40; H 2.92; Cl 10.30; N 4.18; $\text{C}_{17}\text{H}_{10}\text{NO}_5\text{Cl}$; $M = 343.5$ g/mol.

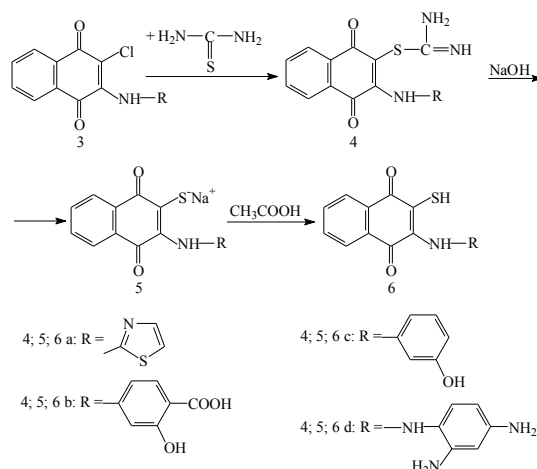
2-chloro-3-(N-3-hydroxy-phenylamino)-1,4-naphthoquinone, 3c

mp. = 166.5 °C; yield = 71%, red-brown powder; IR ν (cm^{-1}): 3255 (w, NH); 1678 (vi, C=O); 3619, 1136 (i, OH); 1588, 794 (m, m, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO}-d_6$): 3.96 (1H, s, NH); 4.95 (1H, s, OH); 5.93–6.84 (4H, m, CH aromatic ring); calculated, %: C 64.10; H 3.33; Cl 11.85; N 4.67; found, %: C 64.00; H 3.35; Cl 11.83; N 4.68; $\text{C}_{16}\text{H}_{10}\text{NO}_3\text{Cl}$; $M = 299.5$ g/mol.

2-chloro-3-(N²-(2,4-diaminophenyl)hydrazin)-1,4-naphthoquinone, 3d

mp. = 171–172 °C; yield = 73%, black powder; IR ν (cm^{-1}): 3258 (w, NH); 1670 (vi, C=O); 3495, 780 (m, NH₂); 1588, 794 (m, m, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO}-d_6$): 3.98 (1H, s, NH); 1.98 (1H, s, NH); 3.92 (2H, s, NH₂); 3.96 (2H, s, NH₂); 5.58–6.16 (3H, m, CH aromatic ring); 7.71–8.16 (4H, m, CH aromatic ring); calculated, %: C 56.72; H 3.84; Cl 10.48; N 16.54; found, %: C 56.68; H 3.89; Cl 10.41; N 16.57; $\text{C}_{16}\text{H}_{13}\text{N}_4\text{O}_2\text{Cl}$; $M = 338.5$ g/mol.

From 2-chloro-3-substituted-1,4-naphthoquinones we carried out the synthesis of 2-mercapto-3-substituted-1,4-naphthoquinones by a three-step-reaction with thiourea, sodium hydroxide and acetic acid (Figure 2).

**Figure 2.**

Scheme for the synthesis of 2-mercapto-3-substituted-1,4-naphthoquinones

2-mercapto-3-(N-thiazole-2-ylamino)-1,4-naphthoquinone, 6a

mp. = 178–179 °C; yield = 70%; black powder; IR ν (cm^{-1}): 3239 (w, NH); 1685 (vi, C=O); 2567 (w, SH); 1586, 795 (m, m, CH aromatic ring); ^1H NMR ($\text{DMSO}-d_6$): 3.99 (1H, s, NH); 1.48 (1H, s, SH); 6.56–7.53 (2H, m, CH thiazole); 7.73–8.00 (4H, m, CH aromatic ring); calculated, %: C 54.16; H 2.77; N 9.72; S 22.22; found, %: C 54.17; H 2.70; N 9.70; S 22.12; $\text{C}_{13}\text{H}_8\text{N}_2\text{O}_2\text{S}_2$; $M = 288$ g/mol.

2-mercapto-3-(N-(4-carboxy-3-hydroxy-phenyl)-amino)-1,4-naphthoquinone, 6b

mp. = 101–102 °C; yield = 72%; black powder; IR ν (cm^{-1}): 3256 (w, NH); 1680 (vi, C=O); 2568 (w, SH); 3612, 1209 (i, i, OH); 2922, 1718 (m, i, COOH); 1588, 794 (m, m, CH aromatic ring); ^1H NMR ($\text{DMSO}-d_6$): 3.98 (1H, s, NH); 1.50 (1H, s, SH); 4.97 (1H, s, OH); 10.95 (1H, s, COOH); 6.18–7.71 (3H, m, CH aromatic ring); 7.74–8.00 (4H, m, CH aromatic ring); calculated, %: C 59.82; H 3.22; N 4.10; S 9.38; found, %: C 59.85; H 3.13; N 4.00; S 9.30; $\text{C}_{17}\text{H}_{11}\text{NO}_5\text{S}$; $M = 341$ g/mol.

2-mercapto-3-(N-3-hydroxy-phenylamino)-1,4-naphthoquinone, 6c

mp = 126–127 °C; yield = 83%; brown powder; IR ν (cm^{-1}): 3258 (w, NH); 1679 (vi, C=O); 2569 (w, SH); 3610, 1211 (i, i, OH); 1587, 792 (m, m, CH aromatic ring); ^1H NMR ($\text{DMSO}-d_6$): 2.00 (1H, s, NH); 3.96 (1H, s, NH); 1.49 (1H, s, SH); 5.94–6.84 (4H, m, CH aromatic ring); 7.71–8.00 (4H, m, CH aromatic ring); calculated, %: C 64.64; H 3.70; N 4.71; S 10.77; found, %: C 64.60; H 3.68; N 4.75; S 10.80; $\text{C}_{16}\text{H}_{11}\text{NO}_3\text{S}$; $M = 297$ g/mol.

2-mercapto-3-(*N*²-(2,4-diaminophenyl) hydrazin)-1,4-naphthoquinone, 6d

mp. = 154°C; yield = 72%; black powder; IR ν (cm⁻¹): 3260 (w, NH); 1680 (vi, C=O); 2570 (w, SH); 3400, 689 (m, m, NH₂); 1590, 791 (m, m, CH aromatic ring); ¹H NMR (DMSO-d₆): 2.00 (1H, s, NH); 3.96 (1H, s, NH); 1.49 (1H, s, SH); 3.97 (2H, s, NH₂); 5.58–6.16 (3H, m, CH aromatic ring). 7.70–8.00 (4H, m, CH aromatic ring); calculated, %: C 58.89; H 4.29; N 17.17; S 9.81. found, %: C 58.92; H 4.28; N 17.10; S 9.78; C₁₆H₁₄N₄O₂S; M = 326 g/mol.

In vitro antibacterial and antifungal activity

1,4-naphthoquinone derivatives 3a-b and 6a-d were subjected to *in vitro* antimicrobial tests. Results reporting the complete inhibitory zones (mm) of tested compounds determined for several bacterial strains and fungi are presented in Table 1. As indicated in the table, the final products have potent antibacterial activity on standard *Staphylococcus*

aureus ATCC[®]25923 strain, comparable to oxacillin but slightly lower than ampicillin. Compound 6c has significant inhibitory activity with the widest inhibitory zones similar to that of ampicillin. In the same conditions, standard Gram negative strains *Escherichia coli* ATCC[®]25922 and *Klebsiella spp.* ATCC[®]700603 proved resistance to all tested compounds.

Regarding the antifungal activity, we observed that the intermediate compounds 3a-b have a more potent activity on *Candida albicans* ATCC[®]10231 than the final products 6a-d. Compound 3a has an inhibitory activity similar to that of clotrimazole.

The prevalence of microorganism strains resistant to conventional antibiotics, such as *Staphylococcus aureus* and *Escherichia coli*, has increased to high levels in many hospitals worldwide. So, the search for new antimicrobial agents is an important direction of many research teams because of the increased resistance acquired.

Table I

Antimicrobial activity of synthesized compounds assessed by diffusion technique

Compounds	The diameters (mm) of the inhibition zones				
	<i>S. aureus</i> ATCC [®] 25923	<i>E. faecalis</i> ATCC [®] 29212	<i>E. coli</i> ATCC [®] 25922	<i>Klebsiella spp.</i> ATCC [®] 700603	<i>C. albicans</i> ATCC [®] 10231
3a	11	10	-	-	28
3b	11	9	-	-	22
6a	19	12	-	-	10
6b	17	12	-	-	8
6c	26	16	-	-	20
6d	22	16	-	-	10
Ampicillin	28	18	17	16	*
Oxacillin	22	16	*	*	*
Clotrimazole	*	*	*	*	30

“*” not determined, “-” resistant

Previous studies regarding the antimicrobial activity of 1,4-naphthoquinones indicated that active compounds must possess at least a substitution, at position 2 or 3, which must be an electron-releasing for increased activity [6]. For the substitution at positions 2 or 3 in our compounds we used substituted amino groups which had acted as electron-releasing groups. But not all the synthesized compounds proved this effect. Because growth of *Staphylococcus aureus* was effectively inhibited by compounds 6c and 6d we suggest the applicability of these naphthoquinone derivatives against infections with staphylococcus. Meanwhile, the compound 3a, namely 2-chloro-3-(N-thiazole-2-ylamino)-1,4-naphthoquinone, could be tested further for its antifungal effect.

Conclusions

The antimicrobial effect of some 2,3-disubstituted-1,4-naphthoquinone derivatives has been reported. On the basis of their activity on *Staphylococcus aureus* further studies are required for these compounds to determine their *in vivo*

pharmacological properties and their potential use as antimicrobial agents.

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References.

1. Al-Hiari Y.M., Shakya A.K., Alzweiri M.H., Aburjai T., Abu-Dahab R., Synthesis and biological evaluation of substituted tetrahydro-1h-quinol[7,8-b][1,4]benzodiazepine-3-carboxylic derivatives. *Farmacia*, 2014; 62(3): 578-596.
2. Babula P., Adam V., Havel L., Kizek R., Noteworthy secondary metabolites naphthoquinones – their occurrence, pharmacological properties and analysis. *Curr. Pharm. Anal.*, 2009; 5: 47-68.
3. Babula P., Adam V., Kizek R., Sladky Z., Havel L., Naphthoquinones as allelochemical triggers of

- programmed cell death. *Environ. Exper. Bot.*, 2009; 65: 330-337.
4. Kanaan Y.M., Das J.R., Bakare O., Enwerem N.M., Berhe S., Beyene D., Williams V., Zhou Y., Copeland Jr. R.L., Biological evaluation of 2,3-dichloro-5,8-dimethoxy-1,4-naphthoquinone as an anti-breast cancer agent. *Anticancer Res.*, 2009; 29: 191-200.
 5. Parker K.A., Petraitis J.J., Synthesis of ansamycins: An approach to the naphthoquinone portion of the rifamycins and streptovaricins. *Tetrahedron Lett.*, 2009; 22: 397-400.
 6. Riffel A., Medina L.F., Stefani V., Santos R.C., Bizani D., Brandelli A., *In vitro* antimicrobial activity of a new series of 1,4-naphthoquinones. *Braz. J. Med. Biol. Res.*, 2002; 35: 811-818.
 7. Sasaki K., Abe H., Yoshizaki F., *In vitro* antifungal activity of naphthoquinones derivatives. *Biol. Pharm. Bull.*, 2002; 25: 669-670.
 8. Stasevych M.V., Plotnikov M.Yu., Platonov M.O., Sabat S.I., Musyanovych R.Ya., Novikov V.P., Sulfur containing derivatives of 1,4-naphthoquinone. Part 1. Disulfide synthesis. *Heteroatom Chem.*, 2005; 16: 205-211.
 9. Stasevych M.V., Chervetsova V.G., Plotnikov M.Yu., Platonov M.O., Sabat S.I., Musyanovych R.Ya., Novikov V.P., Synthesis and antimicrobial evaluation of novel 2-substituted-3-mercapto-1,4-naphthoquinones. *Ukrainica Bioorganica Acta*, 2006; 2: 33-39.
 10. Stasevych M., Semenjuk M., Mandzya I., Plotnikov M., Musyanovych R., Novikov V., Sulfen-naphthoquinones. *Ch&Ch T.*, 2007; 1(1): 35-40.
 11. Tandon V.K., Singh R.V., Yadav D.B., Synthesis and evaluation of novel 1,4-naphthoquinone derivatives as antiviral, antifungal and anticancer agents. *Bioorg. Med. Chem. Lett.*, 2004, 14: 2901-2904.
 12. Tandon V.K., Maurya H.K., Mishra N.N., Shukla P.K., Design, synthesis and biological evaluation of novel nitrogen and sulfur containing hetero-1,4-naphthoquinones as potent antifungal and antibacterial agents. *Eur. J. Med. Chem.*, 2009; 44: 3130-3137.
 13. Tandon V.K., Maurya H.K., Verma M.K., Kumar R., Shukla P.K., 'On water' assisted synthesis and biological evaluation of nitrogen and sulfur containing hetero-1,4-naphthoquinones as potent antifungal and antibacterial agents. *Eur. J. Med. Chem.*, 2010; 45: 2418-2426.
 14. Tataringa G., Stan C.D., Zbancioc A.M., Jitareanu A., Tuchilus C., Preliminary screening of biological activities of some new Schiff bases of isatins. *Farmacia*, 2014; 62(1): 14-22.