

SCREENING OF THIOALKANOIC SUBSTITUTED 1,4-NAPHTHOQUINONES AS POTENT ANTIMICROBIAL AGENTS

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Abstract

In the present study, there are described the synthesis and evaluation of physico-chemical properties and antimicrobial activity of some hetero-1,4-naphthoquinones containing thiol groups substituted with alkanolic acids. The synthesis of the new derivatives consists in a condensation between 2,3-dichloronaphthalen-1,4-dione and some thioalkanoic acids. The structures of the pure products were characterized by spectroscopic methods. The antimicrobial activity was tested on Gram-positive and Gram-negative bacteria and the antifungal activity on *Candida albicans* using the disk diffusion method. The compounds tested proved activity against *Candida albicans* and Gram-positive bacteria.

Rezumat

În lucrarea prezentă este descrisă sinteza și evaluarea proprietăților fizico-chimice și a activității antimicrobiene a unor hetero-1,4-naftochinone ce conțin grupe tiol substituite cu resturi de acizi alcanici. Sinteza noilor derivați a constat în reacția de condensare între 2,3-dicloronafthalen-1,4-dionă și unii acizi tioalcanici. Producții purificați au fost caracterizați prin metode spectroscopice. Activitatea antimicrobiană a fost testată pe bacterii Gram-pozitive și Gram-negative, iar activitatea antifungică pe *Candida albicans*, folosind metoda difuzimetrică. Compușii au dovedit activitate variabilă față de *Candida albicans* și tulpinile de bacterii Gram-pozitive testate.

Keywords: 1,4-naphthoquinone derivatives, thioalkanoic acids, antibacterial activity, antifungal activity

Introduction

Quinones are aromatic compounds widespread in nature including pigments, antibiotics, vitamins and coenzymes [6]. Many quinones represent drugs extensively used in studies regarding the oxidative stress [8]. Among them, 1,4-naphthoquinone derivatives, act as vital links in aerobic metabolism being involved in one and/or two electrons transfer chains [3]. They are of great pharmacological interest, due to the fact that many of them serve as chemotherapeutic agents proving antibacterial, antifungal, antimalarial and anticancer activities [10, 14, 17-19]. Two major mechanisms have been proposed to explain the cytotoxic effect of quinones: i) generation of the semiquinone radical after one-electron reduction of the quinone ring and its participation in a redox cycle to give potent reactive oxygen species, such as superoxide anion radical and hydrogen peroxide; ii) quinones, as potent electrophiles, are able to react with the thiol group of glutathione with depletion of its reduced form and enhancement of oxidative stress [6]. But there are also other bio-

chemical mechanisms related to the biological effects of quinones such as inhibition of special enzymes involved in DNA metabolism, DNA intercalation or alkylation [1, 13].

Besides naturally occurring compounds, the quinone ring is often incorporated into synthetic biologically active structures. Studies regarding the relationship structure-activity showed that the presence of amino, thio or chloro moieties on the quinones was an important factor for their antimicrobial and antifungal activities [9].

Research on thiol-containing derivatives generated wide interest due to their extended spectrum of biological actions. It is already known that some sulfide- and sulfoxide- quinones have antifungal activity [8]. Other synthetic thiol-containing derivatives of 1,4-naphthoquinone are reported as potent antimicrobial and anticancer agents [4, 5, 16-18].

Considering all these aspects, the design of 1,4-naphthoquinones derivatives with improved pharmacological effects is still important for the

identification of new antimicrobial, antiviral, antifungal and antitumoral agents.

The activities exhibited by compounds previously designed [15] prompted us to synthesize and perform the biological evaluation of hetero-1,4-naphthoquinones containing thiol groups substituted with residues of alkanic acids at 2- or 2,3-positions described in the present paper.

Materials and Methods

Chemistry. The reagents and solvents were of analytical grade and obtained from Merck (Germany). Melting points were measured with a Gallenkamp apparatus and are uncorrected. Elemental analysis has been made on a CHNOS Vario El analyser. The structures were confirmed by spectral (UV-VIS, IR, ^1H -NMR and GC-MS) studies.

Method for the synthesis of hetero-1,4-naphthoquinones (1-5). The synthesis of these five hetero-1,4-naphthoquinones containing thiol groups substituted with residues of alkanic acids at 2- or 2,3-positions consists in a condensation reaction between 2,3-dichloronaphthalene-1,4-dione (dichlone), thioacetic acid, 3-thiopropionic acid and ethyl thioacetate, respectively, heated in the presence of pyridine, in a ratio of 1:1 or 1:2.

For example, the synthesis of 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalene-2-yl-thio) acetic acid was performed in a reflux installation that comprises a round-bottomed flask with two necks, an ascending reflux condenser, a dropping funnel and a magnetic stirrer. In the round-bottomed flask it was put, under stirring, a mixture of 5.02 mmol of dichlone and 5.02 mmol of thioacetic acid diluted in 18 mL dry toluene and heated at 55°C. The mixing continued until the final dissolution of the reactants. Then, for 15 minutes, 10.12 mmol of pyridine were added drop wise and the temperature increased to 65°C. The mixture was then cooled to room temperature and the orange precipitate obtained was filtered under *vacuum* and washed with water. The crude product was purified by recrystallization from a mixture of methanol:acetic acid = 2:1. The same steps were used to synthesize all the compounds adjusting the amounts of reagents according to a ratio 1:1 or 1:2.

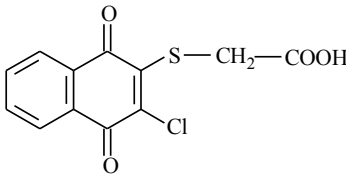
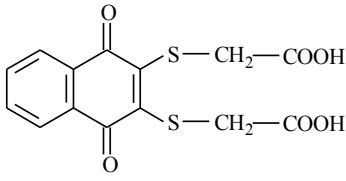
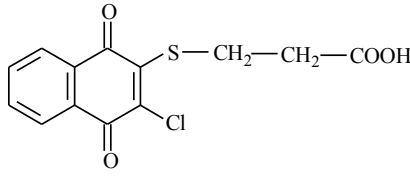
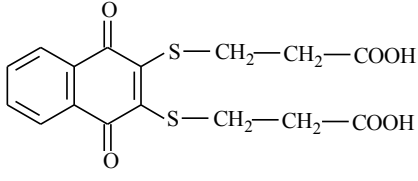
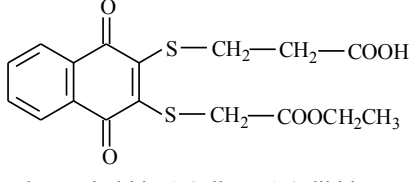
Antifungal and antibacterial evaluation. Screening of the antibacterial and antifungal activity of all compounds was assessed by disk diffusion method according to the recommendations of Clinical and Laboratory Standards Institute [7, 11]. All examined compounds were tested against the following pathogenic bacterial strains: *Staphylococcus aureus* ATCC®25923, *Escherichia coli* ATCC®25922, *Pseudomonas aeruginosa* ATCC®27853 and yeast *Candida albicans* ATCC®10231. Bacterial strains were grown in Mueller Hinton medium. Fungi were grown in Sabouraud Dextrose Agar medium. 5 mm diameter disks were prepared from Whatman filter paper no.1 and sterilized in a hot air oven. The disks were soaked in 5 μL solution 10 mg/mL of naphthoquinone derivatives solved in dimethyl sulfoxide (DMSO). Disks were put on exponentially growing plated cultures of previously mentioned strains prepared as previously reported [1] and incubated 24 hours (for the antibacterial activity) and 48 hours (for the antifungal activity) at 37°C. The results were recorded by measuring the diameter of the zones of complete inhibition, including the diameter of the disk. The antimicrobial activity was considered to begin when the diameter was higher or equal to 6 mm. The following scale of activity was stated: small activity for a diameter ≤ 10 mm; weak activity - diameter between 11 - 14 mm; medium activity - diameter between 15 - 20 mm; strong activity – diameter ≥ 21 mm. Disks containing vancomycin (30 μg /disk) were used as antibacterial control. Fluconazole (25 μg /disk) was used as antifungal control, dichlone as structural control and DMSO as blank.

Results and Discussion

We synthesized five hetero-1,4-naphthoquinones containing thiol groups substituted with alkanic acids residues at 2- or 2,3-positions and tested them against pathogenic bacteria and yeasts. These were obtained in one-step reaction between dichlone and one of the following: thioacetic acid, 3-thiopropionic acid and ethyl thioacetate, in a ratio 1:1 or 1:2. Table I contains synthetic data of these compounds.

Table I

Synthetic data of the thioalkanoic derivatives of 1,4-naphthoquinone

Nr. crt.	Structural formula	Molecular formula	Molecular weight (g/mol)	Colour	Melting point (°C)	η (%)
1.	 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio) acetic acid	C ₁₂ H ₇ O ₄ SCl	282.5	Orange powder	167.7	78.87
2.	 2,2'-[(1,4-dioxo-1,4-dihydronaphthalen-2,3-diyl)-dithio] diacetic acid	C ₁₄ H ₁₀ O ₆ S ₂	338	Yellow powder	193.5	78.87
3.	 3-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio) propanoic acid	C ₁₂ H ₇ O ₄ SCl	296.5	Red powder	172.2	86.57
4.	 3,3'-[(1,4-dioxo-1,4-dihydronaphthalen-2,3-diyl)-dithio] dipropanoic acid	C ₁₆ H ₁₄ O ₆ S ₂	366	Red powder	190	85.32
5.	 Ethyl 2-(3-carboxyethylthio-1,4-dioxo-1,4-dihydronaphthalen-2-ylthio) acetate	C ₁₇ H ₁₆ O ₆ S ₂	380	Red powder	177	85.78

Spectroscopic data of the five compounds and the results of the elemental analysis are shown below.

2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio) acetic acid (**1**)

IR ν (cm⁻¹): 3309, 3330, 1706 - 1773, 1424, (w, w, m-i, m-w, COOH); 1653 - 1696 (vi-i, C=O); 2920, 2851 (m, CH_{2antisym}, m, CH_{2sym}); 702, 747 (i, w, C-Cl); 603 - 641 (w, C-S); 3035 - 3057, 1830 - 1995, 1576 - 1612 (w, fw-w, m-w, CH aromatic ring); ¹H NMR (300MHz, DMSO-d₆) δ : 3.80 (2H, s, CH₂); 7.73 - 8.00 (4H, m, CH aromatic ring); 10.99 (1H, s, COOH); calculated, %: C 50.97; H 2.47; Cl 12.56; S 11.32; found, %: C 51.09; H 2.44; Cl 12.98; S 11.98.

2,2'-[(1,4-dioxo-1,4-dihydronaphthalen-2,3-diyl)-dithio] diacetic acid (**2**)

IR ν (cm⁻¹): 3301, 3332, 1701 - 1773, 1424, (m-w, m-w, m-i, m-w, COOH); 1653 - 1696 (i, C=O); 2918, 2854 (m-w, CH_{2antisym}, m-w, CH_{2sym}); 602 - 640 (w, C-S); 3019 - 3056, 1830 - 1996, 1576 - 1613 (m-w, fw-w, m-w, CH aromatic ring); ¹H NMR (300MHz, DMSO-d₆) δ : 3.80 (4H, s, CH₂); 7.73 - 8.00 (4H, m, CH aromatic ring); 10.99 (2H, s, COOH); calculated, %: C 49.70; H 2.95; S 18.93; found, %: C 48.95; H 2.44; S 19.43.

3-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio) propanoic acid (**3**)

IR ν (cm⁻¹): 3304, 3331, 1705 - 1773, 1419, (w, w, m-i, w, COOH); 1653 - 1696 (vi-i, C=O); 2923, 2859 (m, CH₂antisym, m-w, CH₂sym); 701, 746 (fi-i, w, C-Cl); 602 - 642 (w, C-S); 3035 - 3057, 1830 - 1995, 1576 - 1610 (w, m-w, m-w, CH aromatic ring); ¹H NMR (300MHz, DMSO-d₆) δ : 3.15 (2H, t, S-CH₂); 2.90 (2H, t, CH₂-COOH); 7.73 - 8.00 (4H, m, CH aromatic ring); 11.00 (1H, s, COOH); calculated, %: C 52.61; H 3.03; Cl 11.97; S 10.79; found, %: C 52.98; H 2.97; Cl 12.14; S 11.43.

3,3'-[(1,4-dioxo-1,4-dihydronaphthalen-2,3-diyl)-dithio] dipropionic acid (4)

IR ν (cm⁻¹): 3304, 3330, 1707 - 1762, 1419, (w, w, m-i, m-w, COOH); 1653 - 1696 (vi-i, C=O); 2925, 2859 (m-w, CH₂antisym, m-w, CH₂sym); 601 - 642 (w, C-S); 3035 - 3057, 1830 - 1995, 1576 - 1610 (w, m-w, m-w, CH aromatic ring); ¹H NMR (300Hz, DMSO-d₆) δ : 3.14 (4H, t, S-CH₂); 2.90 (4H, t, CH₂-COOH); 7.73 - 8.00 (4H, m, CH aromatic ring); 11.00 (1H, s, COOH); calculated, %: C 61.20; H 3.82; S 17.48; found, %: C 62.31; H 3.46; S 16.98.

2-(3-carboxiethylthio-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio) ethylacetate (5)

IR ν (cm⁻¹): 3306, 3329, 1707 - 1730, 1417, (w, w, m-i, m-w, COOH); 1672 - 1696 (vi-i, C=O); 2933, 2856 (i, CH₂antisym, m-i, CH₂sym); 609 - 644 (w-m, C-S); 3035 - 3057, 1830 - 1995, 1576 - 1610 (w, m-w, m-w, CH aromatic ring); 2933, 2874, 1465 (i, m, m, CH₃); 1730, 1271 (vi, vi, COOC₂H₅); ¹H NMR (300 MHz, DMSO-d₆) δ : 3.14 (4H, t, S-CH₂); 2.90 (4H, t, CH₂-COOH); 7.73 - 8.00 (4H, m, CH aromatic ring); 11.00 (1H, s, COOH); 4.12 (2H, q, CH₂CH₃); 1.30 (3H, t, CH₂CH₃); calculated, %: C 53.68; H 4.21; S 16.84; found, %: C 53.31; H 4.46; S 16.98.

Mono(thioalkanoic)-substituted and di(thioalkanoic)-substituted naphthoquinones were subjected to antimicrobial tests using representative Gram-positive and Gram-negative strains. Results reporting the complete inhibition zones of tested compounds on selected bacterial strains and fungi are presented in Table II.

Table II

Antibacterial and antifungal activity of the synthesized compounds assessed by disk diffusion method

	Diameter of inhibition zones (mm)			
	<i>S. aureus</i> ATCC®25923	<i>P. aeruginosa</i> ATCC®27853	<i>E. coli</i> ATCC®25922	<i>C. albicans</i> ATCC®10231
1	12	-	-	≤ 10
2	14	-	-	11
3	12	-	-	≤ 10
4	12	-	-	≤ 10
5	16	-	-	18
D	10	-	≤ 10	14
C*	17	-	-	19

Legend: C* - control - vancomycin for the antibacterial activity, fluconazole for the antifungal activity; D - dichlone; "-" resistant.

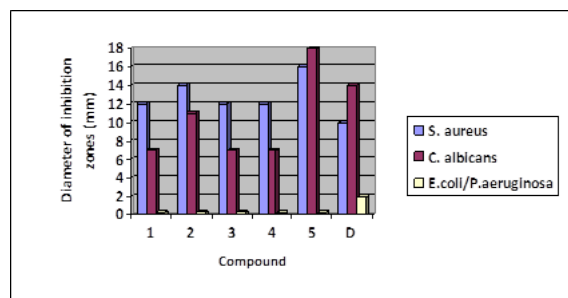


Figure 1.

Antimicrobial action of thioalkanoic substituted 1,4-naphthoquinones

Most of the compounds showed various antibacterial and antifungal activities some of them exceeding dichlone, used as a structural control. The majority of the compounds showed a higher activity on *S. aureus* than did dichlone. According to data reported in Table II and Figure 1, *S. aureus* has higher sensibility to compounds 2 and 5 which exerted antibacterial activity comparable to the antibiotic used as a control for this test, vancomycin.

The absence of inhibition zones of Gram-negative strains, *E. coli* and *P. aeruginosa*, has proven that the compounds studied have no antibacterial action at the concentration tested.

Regarding the antifungal activity on *C. albicans*, we observed that only the compound 5 proved a medium activity comparable to that of fluconazole, while the others were less efficient even than dichlone (Figure 1).

The search for new antimicrobial chemotherapeutics is an important direction of research because the resistance acquired by the bacterial strains to the antibiotics already in use. Some studies indicate that the antimicrobial activity of naphthoquinones is related to the presence of free keto groups at positions 1 and 4 [13] and that active compounds must possess at least a substituent at position 2 or 3, which must be an electron-releasing group (Gafner *et al.* cited by [12]). Naphthoquinone derivatives containing carboxylate groups at the extremity of a thio-alkyl side chain were previously reported to possess antiproliferative properties toward

HeLa cells [3]. Here we have reported that derivatives containing thio-alkanoic acid residues at positions 2 or 2, 3 of the naphthoquinone ring could act also as antimicrobial agents.

Conclusions

The antimicrobial effect of 2-mono(thio-alkanoic)-substituted and 2,3-di(thio-alkanoic)-substituted-1,4-naphthoquinones has been assessed. Among the tested compounds, 2-(3-carboxiethylthio-1,4-dioxo-1,4-dihydronaphthalen-2-yl-thio) ethyl acetate (compound 5) was the most effective against *S. aureus* as potent antibacterial and *C. albicans* as potent antifungal agent. This compound is promising as biologically active substance and requires further studies to determine its pharmacological potential *in vivo*.

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