SYNTHESIS AND ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF COMPOUNDS OF THE NAPHTHAZARIN SERIES

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A series of substituted naphthazarins (5,8-dihydroxy-1,4-naphthoquinones) were synthesized and their antimicrobial and antifungal activities were studied. The yeast *Saccharomyces carlsbergensis* was the most sensitive to these compounds. Among the compounds studied, the most marked antimicrobial and antifungal activities were obtained with unsubstituted naphthazarin, which was as active as juglone.

Key words: 5,8-Dihydroxy-1,4-naphthoquinone, naphthazarin, antimicrobial activity, *Saccharomyces carlsbergensis*, *Staphylococcus aureus*, *Escherichia coli*.

Substituted 1,4-naphthoquinones and juglones (5-hydroxy-1,4-naphthoquinones) are known to have antimicrobial activity [1 – 3]. This is clearly illustrated by juglone and plumbagin (2-methyljuglone), which have been recommended as preservatives to prevent spoiling of alcohol-free drinks and wines [2]. Previous studies have demonstrated that introduction of an α-hydroxy function into 1,4-naphthoquinones leads to increases in the biological activity of these compounds [4]. The literature contains occasional reports of the antimicrobial properties of naphthazarins (5,8-dihydroxy-1,4-naphthoquinones such as shikonin ((1-hydroxy-4-methylpent-3-en-1-yl)naphthazarin) and its analogs with modified side chains, which clearly established that the pharmacological actions of these compounds are due to the naphthazarin fragment [5]. However, the antimicrobial activity of other substituted naphthazarins thus far remains essentially unstudied, probably because of the lack of reliable synthesis methods.

With the aim of seeking further biologically active substances among naphthazarin derivatives, we report the first

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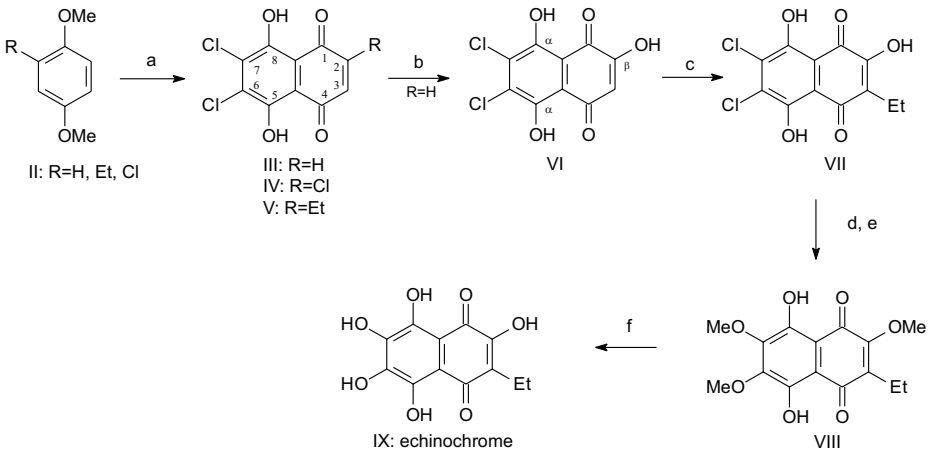
Scheme 1



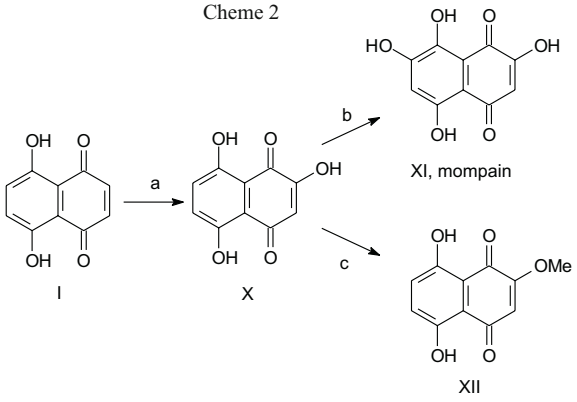
synthesis of a large collection of these compounds and studies of structure-activity relationships.

Naphthazarins I, III – V were prepared by double cycloacylation of the dimethyl esters of the corresponding hydroquinones II with maleic or dichloromaleic anhydrides (scheme 1) [6, 7]. Oxidation of dichloronaphthazarin (III) with MnO_2 in concentrated H_2SO_4 yielded product VI [8]. Radical acylation of the latter with propionylperoxide in *t*-BuOH yielded naphthazarin VII [9]; subsequent methylation of the β-hydroxy group [10] and substitution of the chlorine atom by a methoxy group using a system consisting of CsF, Al_2O_3, and MeOH in the resulting 2-methoxy-3-ethyl-6,7-dichloronaphthazarin gave trimethyl ester VIII [11]. Hydrolysis of the latter with $AlCl_3$ in nitrobenzene yielded echinochrome A (IX) [12].

Reagents and conditions: (a) maleic or dichloromaleic anhydrides, NaCl/$AlCl_3$ melt, 180°C; (b) MnO_2, concentrated H_2SO_4, room temperature; (c) $(EtCOO)_2$, *t*-BuOH, boiling; (d) $(MeO)_3CH$, boiling; (e) CsF, Al_2O_3, MeOH, 95°C; (f) $AlCl_3$, $PhNO_2$, 60°C.

Oxidation of naphthazarin (I) with MnO_2 in concentrated H_2SO_4, depending on the conditions for the reaction, yielded naphthopurpurine (X) or mompain (XI) (Scheme 2) [13]. Methylation of compound X with diazomethane formed methoxynaphthazarin XII [14].

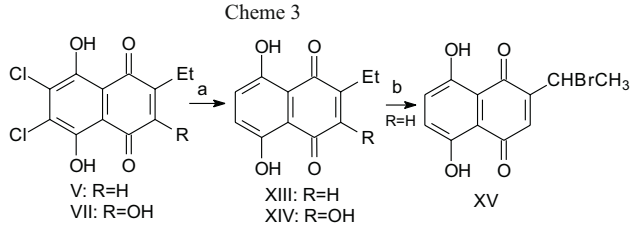
Cheme 2



Reagents and conditions: (a) MnO_2, concentrated H_2SO_4, room temperature; (b) MnO_2, concentrated H_2SO_4, ≈ 50°C; (c) CH_2N_2 in Et_2O, room temperature.

Ethyl- (XIII) and 2-hydroxy-3-ethylnaphthazarins (XIV) were prepared by dehaloidization of the corresponding dichloro derivatives V and VII using Fe in AcOH (Scheme 3) [15, 16]. Radical bromination of ethylnaphthazarin XIII yielded product XV [17].

Cheme 3



Reagents and conditions: (a) Fe, AcOH, boiling; (b) Br_2, CCl_4, room temperature.

Compounds were crystalline substances colored different shades of red; they were insoluble in water and soluble in ethanol, acetone, chloroform, and DMSO. The chemical structures and purities of the compounds synthesized were supported by ^1H NMR data, IR spectroscopy, and mass spectrometry. Elemental analysis data of the compounds synthesized corresponded to values calculated from atomic formulas. It should be noted that compounds I, IX, XI, and XIV synthesized here have previously been isolated from natural sources [18].

BIOLOGICAL METHODS

Biological tests consisted of 24-h cultures of the yeast *Saccharomyces carlsbergensis* and the Gram-positive and Gram-negative bacteria *Staphylococcus aureus* and *Escherichia coli*. Yeasts were cultured at 28°C in liquid growth medium containing 40 g/liter glucose and 10 g/liter peptone, pH 6. Bacterial cells were cultured at 37°C in liquid growth medium containing 5 g/liter glucose, 10 g/liter peptone, and 5 g/liter NaCl, pH 7.2. Incubation medium consisted of 2.0 ml of cell suspensions and 0.02 ml of test substance solutions. Compounds were dissolved in DMSO. The microbial load for assay of antimicrobial activity was 10^6 cells/ml. Multiplication of yeast and *E. coli* cells was monitored visually at 24 h, while *S. aureus* was monitored at 48 h. Activity was expressed as the minimum effective dose (MED), i.e., the maximal dilution leading to complete suppression of the growth of bacterial and yeast test cultures.

RESULTS AND DISCUSSION

Results obtained in studies of the antimicrobial and antifungal activities of the compounds are presented in Table 1.

The test organism most sensitive to the study compounds was *Saccharomyces carlsbergensis*. The compound with the greatest antifungal activity was unsubstituted naphthazarin (I).

Most of the test compounds had weak bacteriostatic actions in relation to *Escherichia coli* and *Staphylococcus aureus*. The most active of the study compounds were I, III, and V.

Thus, the results obtained here showed that among the study compounds, unsubstituted naphthazarin (I) had the greatest antimicrobial and antifungal activity.

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TABLE 1. Antimicrobial and Antifungal Activity of Substituted Naphthazarins.

Compound	Substituent				Antimicrobial activity, MED, μg/ml		
	R^1	R^2	R^3	R^4	*Escherichia coli*	*Staphylococcus aureus*	*Saccharomyces carlsbergensis*
I	H	H	H	H	25.00	12.50 – 25.00	1.56
III	Cl	Cl	H	H	25.00	25.00	3.10
IV	Cl	Cl	Cl	H	100.00	100.00	12.50
V	Cl	Cl	Et	H	25.00	25.00	3.10
VI	OH	H	Cl	Cl	> 100.00	> 100.00	> 100.00
VII	OH	Et	Cl	Cl	> 100.00	50.00	> 100.00
VIII	OMe	Et	OMe	OMe	> 100.00	> 100.00	3.10
IX	OH	Et	OH	OH	> 100.00	50.00	100.00
X	OH	H	H	H	> 100.00	> 100.00	> 100.00
XI	OH	H	H	OH	> 100.00	100.00	> 100.00
XII	OMe	H	H	H	100.00	25.00	12.50
XIII	Et	H	H	H	> 100.00	12.50	3.10
XIV	OH	Et	H	H	> 100.00	> 100.00	100.00
XV	$CHBrCH_3$	H	H	H	100.00	50.00	12.50
XVI	Juglone				25.00	25.00	1.56

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