Antileukemic Properties and Structure-Activity Relationships of *O*- and *S*-Glycosylated Derivatives of Juglone and Related 1,4-Naphthoquinones

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Abstract: Glycosylated derivatives of physiologically active natural compound juglone and related 1,4- naphthoquinones are known as antifungal, immunomodulatory, and antitumor substances. However, their antileukemic properties and structure-activity relationships have been studied insufficiently. Antileukemic effects and structure-activity relationships (SAR) of the 50 1,4- naphthoquinone derivatives were examined using HL-60 human promyelocytic leukemia cells and MTS method of the study of cell viability. As was shown, the substances inhibited viability of HL-60 cells at the wide range of concentrations. SAR study revealed the structure peculiarities which lead to increase or decrease of the antileukemic activity of the compounds studied. In conclusion, *O*- or *S*- glycosylated derivatives of juglone and related 1,4-naphthoquinones have potential for development of the new antileukemic agents and should be further investigated.

Keywords: Glycosides of 1,4-naphthoquinones, juglone, lawsone, phthiocol derivatives, antileukemic properties, SAR study.

INTRODUCTION

As early as in 1973 Cote and Goodman first reported the synthesis and isolation of four 1,4-naphthoquinone *O*-glycosides [1]. No biological activity for these compounds was published. In 1975-1977 a Brasilian scientific group published synthesis, structures, and biological activity of five other 1,4-naphthoquinone *O*-glycosides. The compounds were reported to be effective *in vivo* against rat tumor Walker 256 carcinosarcoma, mouse lymphocytic leukemia P-388, and Ehrlich ascitic tumor [2,3]. Since that time synthesis, isolation and cytotoxic, antitumor, immunomodulatory, and antifungal properties of *O*-glycosides of natural products juglone, lapachol, lawsone, shikonin, and related 1,4-naphthoquinones were reported [4-8].

In 1983 – 2010 years, we reported syntheses, structure elucidations, and biological activities of about 100 new *O*- or *S*- glycosides of 1,4-naphthoquinones and products of their intramolecular cyclisation [9-20]. Many of these compounds, in distinct from the earlier studied those, have a carbohydrate moiety attached to the quinone part of the molecule *via* sulfur atom (thioglycosyl group). The synthesized glycosides were shown to possess earlier known kinds of activity and some of them demonstrated new one, the induction of expression of heat-shock protein Hsp70 [21].

However, antileukemic properties and structure-activity relationships (SAR) of the glycosylated derivatives of 1,4-naphthoquinones have been studied insufficiently. In the present paper, antileukemic effects and SAR of the 50 of 1,4-naphthoquinone derivatives were examined using HL-60 human promyelocytic leukemia cells and MTS method of the study of cell viability.

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MATERIALS AND METHODOLOGY

Drugs and Chemicals

The systematic names of the compounds studied **1-50** are shown in Supplementary Material. These compounds were synthesized and purified as described previously [9-20] and were pure in accordance with chromatographic and NMR data. RPMI medium was from Gibco Invitrogen Corporation (Carlsbad, CA, USA). Fetal bovine serum (FBS) was from Gemini Bio-Products (Calabasas, CA, USA); penicillin and streptomycin were from Bio-Whittaker (Walkersville, MD, USA); L-glutamine was from Mediatech, Inc. (Herndon, Virginia, USA). The MTS (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl) tetrazolium, inner sault) reagent kit for the cell proliferation assay was from Promega (Madison, WI, USA).

Cell Culture

The human promyelocytic leukemia HL-60 cell line was obtained from the American Type Culture Collection (Rockville, MD, USA) and were cultured at 37°C and 5% CO_2 in RPMI medium containing 10% FBS, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. Information regarding the genetic background of HL-60 cell line is available online.

Cell Viability Assay

The effect of the glycosides on cell viability was evaluated using MTS reduction into its formazan product [22]. The HL-60 cells were cultured for 12 h in 96-well plates (6,000 cells/well in 50 μl of medium). Then 50 μl of medium containing glycosides at various concentrations were added and the cells were incubated for 22 h. Then 20 μl of the MTS reagent were added into each well and MTS reduction was measured 2 h later spectrophotometrically at 492 nm and 690 nm as background using the Multiskan MS microplate reader (Labsystems, Finland). Results are shown in Table **3** and represent the IC_{50} of the substances against HL-60 cells.

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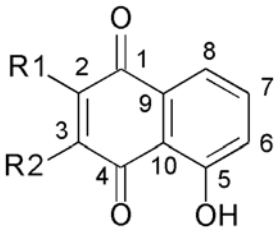
Statistics

The computer programs, Excel and Statistica 6.0 for Windows (StatSoft, Inc., Tulsa, OK, USA, 2001) were used for calculation of IC_{50} and other analyses of the obtained data.

RESULTS

We divided compounds into three structural groups: first, *O*- and *S*- glycosides of juglone (5-hydroxy-1,4-naphthoquinone) (**1-16**, Table **1**); second, *O*- and *S*- glycosides of other 1,4-napthoquinones (**17-40**, Table **2**); third, products of intramolecular cyclisation of the *S*-glycosides of 1,4-naphthoquinones (**41-50**).



Juglone, R1 = R2 = H

The structures of the sugar parts of the substances (**1-40)** from Tables **1** and **2** are given below the Table **2**:

Antileukemic effects of the compounds **1-50** and their structure-activity relationships were examined using HL-60 human promyelocytic leukemia cells and MTS method of the study of cell viability. The results are shown in Table **3** and represent the IC_{50} of the substances against HL-60 cells.

In accordance with the data presented in Table **3** the following structure-activity relationships for compounds **1-50** can be concluded:

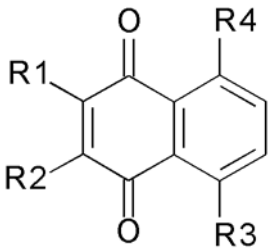
1. The most active compounds are glycosides of juglone **1-14** and products of intramolecular cyclisation of the *S*-glycosides of 1,4-naphthoquinones **41-49**;
2. Electronegative groups like –OH, –Cl, $-NH_2$, –OMe, –OAc at the α-position to the sugar moiety dramatically reduce antileukemic activity of the substances **29-31**, **33-40**;
3. Two sugar residues in the molecule significantly reduce activity of the substances (compare **18**, **19** with **32**, **20-24** or **3** with **1**, **2** or **8** with **7**). Furthermore, two –OAc groups at positions 5, 8 of the quinone moiety, renew the activity (compare **17** with **18**, **19**).
4. The presence of a disaccharide residue in the molecule also significantly reduces activity of compounds (**25**, **26**, **50**) as compared to compounds with a monosaccharide residue in the molecule (**20-24**, **32**, **41-49**);
5. Juglone derivatives possessing acetylated sugar moiety (**1-14**) are significantly more active than that possessing sugar moiety with free hydroxyl groups (**15**, **16**).
6. Juglone derivatives possessing various sugar moieties in the molecules (**1**, **2**, **4**, **5**, **7**, **10-14**) show almost equal activities.

DISCUSSION

Glycosides of 1,4-naphthoquinones are synthetically available biologically active compounds. Glycosylated derivatives of the natural compounds juglone, lawsone, phthiocol, shikonin, and echinochrome are among them. These compounds possess antitumor, immunomodulatory, antifungal and cytotoxic activities. Furthermore, lately some details of their mechanism of action became more pronounced, when their ability to induce the expression of heat-shock protein Hsp70 has been published [21]. Heat shock proteins are stress proteins and their upregulation is described as part of the stress response. Production of high levels of heat shock proteins can be triggered by infection, inflammation,

Table 1. Structures of the Juglone Glycosides 1-16

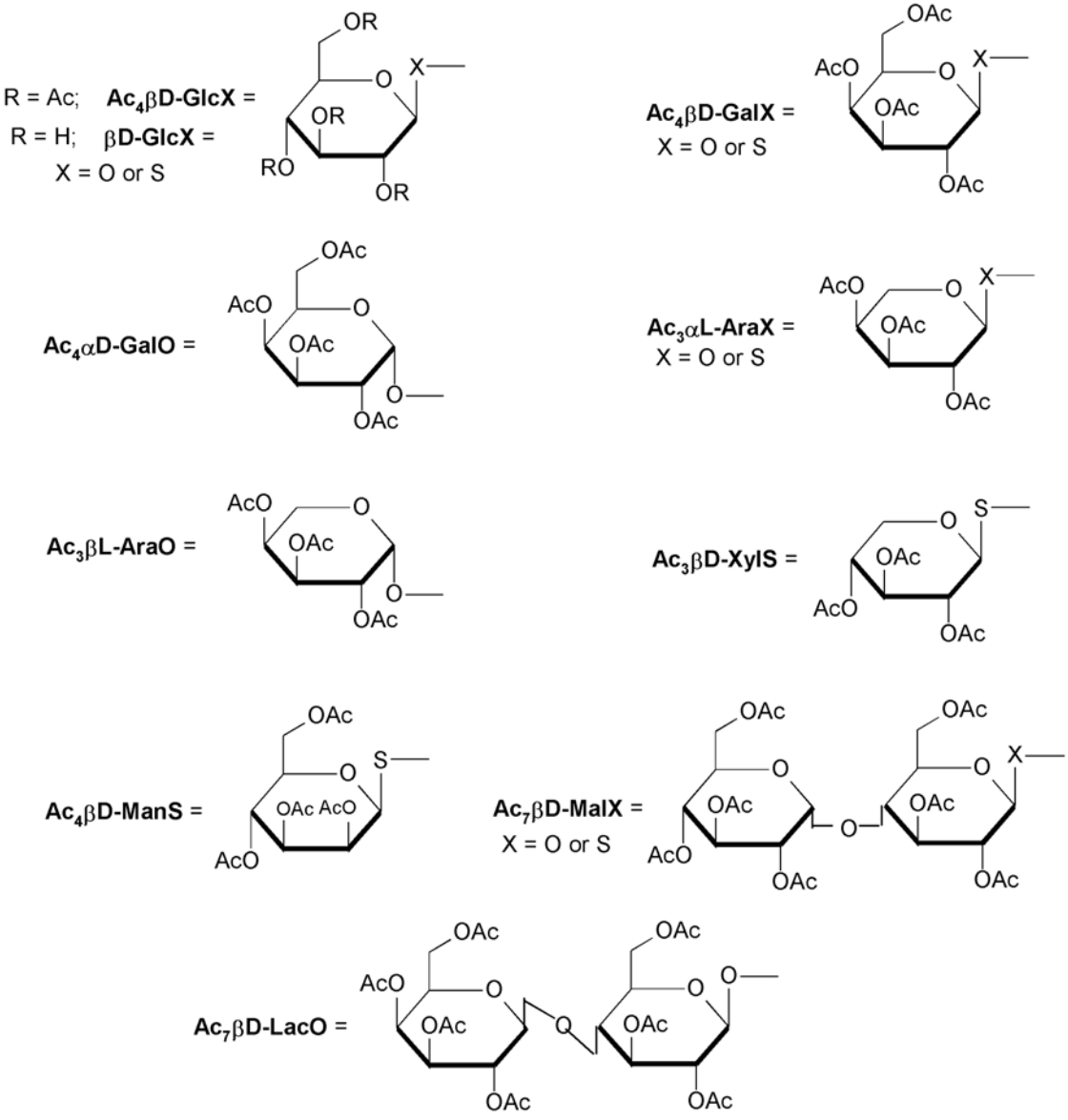
Glycoside number	Radicals	Glycoside number	Radicals
1	R_1 = Ac$_4$βD-GlcO; R_2 = H	**9**	R_1 = Ac$_4$βD-GlcS; R_2 = CH_3
2	R_1 = H; R_2 = Ac$_4$βD-GlcO	**10**	R_1 = H; R_2 = Ac$_4$βD-GalS
3	R_1 = R_2 = Ac$_4$βD-GlcO	**11**	R_1 = H; R_2 = Ac$_3$βD-XylS
4	R_1 = Ac$_7$βD-MalO; R_2 = H	**12**	R_1 = H; R_2 = Ac$_3$αL-AraS
5	R_1 = H; R_2 = Ac$_7$βD-MalO	**13**	R_1 = H; R_2 = Ac$_7$βD-MalS
6	R_1 = Ac$_4$βD-GlcS; R_2 = H	**14**	R_1 = H; R_2 = Ac$_4$βD-ManS
7	R_1 = H; R_2 = Ac$_4$βD-GlcS	**15**	R_1 = βD-GlcS; R_2 = CH_3
8	R_1 = R_2 = Ac$_4$βD-GlcS	**16**	R_1 = βD-GlcS; R_2 = $CH_2CH_2CH_3$



1,4-Naphthoquinone,
R1 = R2 = R3 = R4 = H

Table 2. Structures of the 1,4-Naphthoquinone Glycosides 17-40

Subst. #	Radicals	Subst. #	Radicals
17	R_1=R_2= Ac_4βD-GlcS; R_3=R_4=OAc	**29**	R_1=Ac_4βD-GlcO;R_2=OH;R_3=R_4=H
18	R_1=R_2= Ac_4βD-GlcS; R_3=R_4=H	**30**	R_1=Ac_4βD-GlcO;R_2=OMe;R_3=R_4=H
19	R_1=R_2= Ac_3βD-XylS; R_3=R_4= H	**31**	R_1=βD-GlcO; R_2=OH; R_3=R_4=H
20	R_1= Ac_4βD-GlcO; R_2=R_3=R_4=H	**32**	R_1= Ac_4βD-GlcS; R_2=R_3=R_4=H
21	R_1= Ac_4βD-GalO; R_2=R_3=R_4=H	**33**	R_1= Ac_4βD-GlcS; R_2=Cl; R_3=R_4=H
22	R_1= Ac_4αD-GalO; R_2=R_3=R_4=H	**34**	R_1=Ac_4βD-GlcS; R_2=OAc;R_3=R_4=H
23	R_1= Ac_3αL-AraO; R_2=R_3=R_4=H	**35**	R_1= Ac_4βD-GlcS; R_2=OH; R_3=R_4=H
24	R_1= Ac_3βL-AraO; R_2=R_3=R_4=H	**36**	R_1=Ac_4βD-GlcS; R_2=NH_2; R_3=R_4=H
25	R_1= Ac_7βD-LacO; R_2=R_3=R_4=H	**37**	R_1=Ac_4βD-GlcS;R_2=OMe; R_3=R_4=H
26	R_1=Ac_7βD-MalO;R_2=CH_3;R_3=R_4=H	**38**	R_1=Ac_3βD-XylS; R_2=OMe;R_3=R_4=H
27	R_1=Ac_4βD-GlcO;R_2=CH_3;R_3=R_4=H	**39**	R_1=Ac_4βD-ManS;R_2=OMe;R_3=R_4=H
28	R_1=Ac_4βD-GlcO;R_2=Et;R_3=R_4=H	**40**	R_1=Ac_3αL-AraS;R_2=OMe; R_3=R_4=H



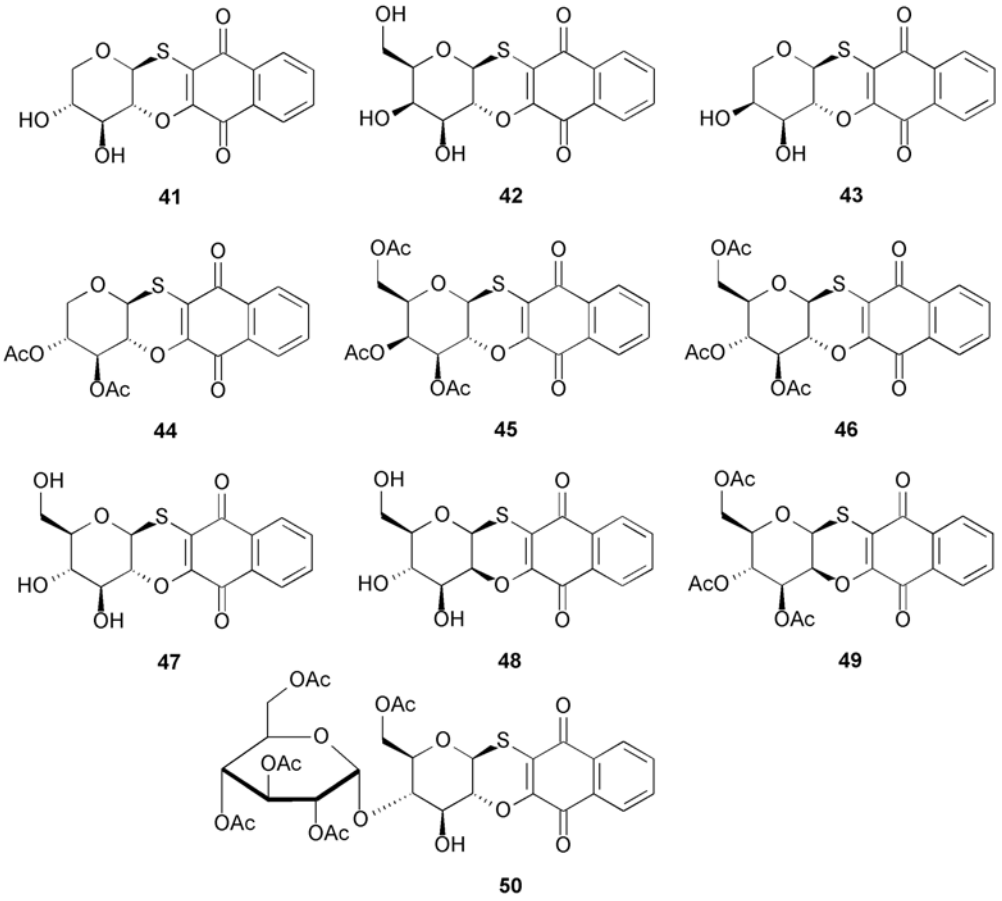


Table 3. IC_{50} of 1,4-Naphthoquinone Glycosides 1-50 Against HL-60 Cells

Subst. #	IC_{50}, μM	Subst. #	IC_{50}, μM	Subst. #	IC_{50}, μM	Subst. #	IC_{50}, μM
1	0.6	**14**	1.4	**27**	8.0	**40**	> 20.0*
2	0.7	**15**	13.3	**28**	8.9	**41**	1.0
3	2.4	**16**	10.0	**29**	> 100.0*	**42**	1.1
4	1.1	**17**	6.7	**30**	15.0	**43**	1.6
5	1.0	**18**	17.6	**31**	> 200.0*	**44**	1.6
6	2.3	**19**	18.8	**32**	3.5	**45**	1.8
7	1.3	**20**	7.0	**33**	48.0	**46**	1.5
8	4.8	**21**	6.9	**34**	> 20.0*	**47**	1.1
9	0.6	**22**	3.4	**35**	> 100.0*	**48**	5.0
10	1.6	**23**	4.6	**36**	22.8	**49**	1.5
11	1.1	**24**	8.8	**37**	20.2	**50**	> 20.0*
12	1.2	**25**	24.7	**38**	> 20.0*		
13	1.0	**26**	15.7	**39**	> 20.0*		

*Maximal dose studied.

and, in particular, by exposure to the cell toxins [23]. These proteins have a significant role in cardiovascular diseases, immunity, and carcinogenesis [24-26]. Thus, it was important to study SAR of a big row of 1,4-naphthoquinone glycosides in order to understand how to synthesize a compound with the desirable properties.

SAR study showed the juglone derivatives were among the most active compounds. This finding demonstrated the

importance of the hydroxyl group at the position 5 of the quinone moiety for the increase of the activity. Another one confirmation of this supposition was the fact that compound **17** possessing -OAc groups at the positions 5 and 8 was much more active than compounds **18** and **19**. Compounds **29-31** and **33-40** possessing –OH, –Cl, $-NH_2$, –OMe, or –OAc groups at the α-position to the sugar moiety showed dramatically reduced antileukemic activity. Presence of two monosaccaride moieties in the molecule also significantly reduced the activity of compounds **18**, **19**. These findings may be explained by the known effect of stabilization of the glycoside bond in the presence of –OH or relative groups [12] that in turn may lead to the stability of similar compounds inside the cells. We also concluded that the antleukemic activity of compounds does not depend on the kind of a monosaccharide residue presenting in the structures of the substances studied.

The search for glycosides of 1,4-naphthoquinones that possess more high antitumor, immunomodulatory, antifungal and Hsp70 upregulative activities will create opportunities for selection of new anticancer agents. We hope the revealed SAR can help to synthesize 1,4-naphtoquinone glycosides with needful activities.

CONCLUSION

In conclusion, *O*- or *S*- Glycosylated derivatives of juglone and related 1,4- naphthoquinones have potential for development of the new antileukemic agents and should be further investigated.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers Web site along with the published article.

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