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Molluscicidal activity of 2-hydroxy-[1,4]naphthoquinone and derivatives

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ABSTRACT

The toxic profile of lawsone (2-hydroxy-[1,4]naphthoquinone) and a series of [1,4]naphthoquinone derivatives was evaluated against the brine shrimp *Artemia salina* and against the mollusk *Biomphalaria glabrata*, the main transmitting vector of schistosomiasis in Brazil. Of the seventeen compounds tested nine fell below the threshold of 100 μg/mL set for potential molluscicidal activity by the World Health Organization. As a general rule derivatives with non-polar substituents presented the highest molluscicidal activities. These substances showed significant toxicity in *A. salina* lethality bioassay.

Key words: *Biomphalaria glabrata*, *Artemia salina*, toxicity, quinones, schistosomiasis, molluscicidal activity, lawsone, 1,4-naphthoquinone.

INTRODUCTION

Among human parasitic diseases, schistosomiasis (also called bilharziasis) remains one of the most prevalent parasitic infections, with significant economic and public health consequences (Chitsulo et al. 2000). Mortality due to schistosomiasis was estimated at 11,000 deaths per year (WHO 2005). The aquatic gastropod mollusk *Biomphalaria glabrata* (Perrett and Whitfield 1996, Verjovski-Almeida and DeMarco 2004) is the main intermediate host of schistosomiasis in South America. Infection usually occurs by contact with water containing infected snails. Permanent solutions to this problem include restriction of human contact with polluted water and prevention of further contamination of the environment (Chitsulo et al. 2000). The use of molluscicides as a prophylactic treatment involves breaking the life cycle of *Schistosoma mansoni* (the etiological agent of schistosomiasis) through the destruction of its intermediate host, the snail *B. glabrata* (Perrett and Whitfield 1996, Bezerra et al. 2002). This constitutes the weakest link in the transmission cycle and is the logical point of attack to control the disease. The search for molluscicides is of great interest for potential focal control of parasitary diseases, especially schistosomiasis, in endemic countries (Pointier and Giboda 1999, Capron 1998). New, safe, and effective molluscicides are urgently needed, but in order to be useful the products must be stable, inexpensive and easy to apply, and must show high selective toxicity to the target pest (Singh et al. 1996).

In our continuing search for bioactive substances,

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either natural (Silva et al. 2007a, b, 2006, 2005a) or synthetic (Silva et al. 2005b, Barbosa et al. 2005, Vasconcellos et al. 2006) we have recently described some effective molluscicide compounds prepared from natural 2-hydroxy-[3-methyl-(2-buten)]-1,4-naphthoquinone (lapachol). Studies on the amine and aza-antraquinones derivatives of lapachol and nor-lapachol (2-hydroxy-[3-methyl-(2-propen)]-1,4-naphthoquinone) showed that in general the compounds containing the most polar groups bonded to the nitrogen generally exhibited the lowest molluscicidal activities and that the lapachol derivatives were slightly more active than the nor-lapachol ones (Silva et al. 2005b, Barbosa et al. 2005). In order to find out whether a side chain on position 3 of the amino-naphthoquinone is necessary for activity, we have evaluated, and describe herein, both molluscicidal activity and brine shrimp toxicity of readily available 2-amino-derivatives of lawsone **1** (2-hydroxy-1,4-naphthoquinone) (Fieser and Martin 1955), whose structures are shown in Figure 1.

EXPERIMENTAL

Synthesis of the Compounds

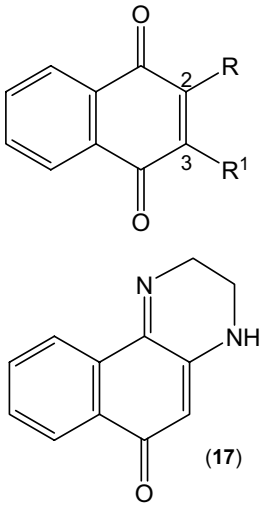
Seventeen derivatives of [1,4]naphthoquinone were prepared by know methods and their structures were confirmed by infrared spectroscopy, NMR, and mass spectrometry. Some of the title compounds are analogues of 2-amino-derivatives of lapachol and nor-lapachol reported elsewhere (Silva et al. 2005, Barbosa et al. 2005). All the nitrogen compounds were readily obtained by nucleophilic displacement of the methoxyl group of 2-methoxy-1,4-naphthoquinone **2** (Fieser and Martin 1955) with the appropriate amines. Except for compound **13**, all compounds were described previously in the literature: 2-bromo-3-methoxy-1,4-naphthoquinone **3** (Fieser and Brown 1949), 2,3-dibromo-1,4-naphthoquinone **4** (Kohn and Schwarz 1926), 2-azido-1,4-naphthoquinone **5** (Molina et al. 1995), 2-nitrogen derivatives **6-16** (Aristoff and Johnson 1992, Bowman et al. 1969, Couladouros et al. 1997, Fieser et al. 1948, Johnson et al. 1997) and cyclic derivative **17** (Kallmayer and Seyfang 1980). All compounds were purified by chromatographic techniques, especially flash chromatography, using silica-gel-60 (230-400 Mesh, Fluka), and fully characterized by analytical and spectroscopic methods. Melting points were obtained from an electrically heated metal block apparatus (Quimis) and compared with the literature data. FTIR spectra were obtained in a Bomen-Michelson spectrophotometer using KBr film, NMR spectra, in a Varian-Mercury 200 MHz for ^{1}H and 50.3 MHz for ^{13}C, with $CDCl_3$ or DMSO-*d*6 as solvents, and HR mass spectra, on a VG Autospec spectrometer (electron-impact at 70 eV).

Molluscicidal Assays

The bioassays were carried out as described previously (Silva et al. 2005b, 2007a, b) by dissolving the sample first in dimethyl sulfoxide (DMSO) and then adding dechlorinated water, to give a solution 0.1% in DMSO. Ten adult snails (9-16 mm in diameter) were placed in a beaker, containing 250 mL of the molluscicide suspension at four appropriate concentrations (1000, 100, 10 and 1 μg/mL). Each test concentration was set in duplicate. Snails were exposed to the potential molluscicide for 24 h, at room temperature, and were kept under normal diurnal lighting. After 24 h, the suspension was decanted; the snails were washed with water and offered lettuce leaves as food. The tested snails were then left in water for another 24 h, and at the end of this period were examined to assess mortality. Snails were considered dead if they either remained motionless or did not respond to the presence of food, or if the shell looked discolored. In order to verify the snails' susceptibility, two control sets were used: one with cupric carbonate at 50 ppm and the other containing 0.1% DMSO dechlorinated water. The concentrations that kill 90% (LC_{90}), 50% (LC_{50}) and 10% (LC_{10}) of the exposed snails (that would have survived in the negative-control cultures) was estimated by probit analysis, using the Origin 6.0 software package (Microcal Software, Northampton, MA). In this study, *B. glabrata* snails were grown in the laboratory (Laboratório de Tecnologia Farmacêutica, UFPB) from stock which originated from Universidade Federal de Pernambuco (Professor F.F. Amancio, CCB-UFPE). They were not infected by trematodes and were grown in transparent containers with appropriate sources of food, light and temperature.

Toxicity Against *Artemia salina*

The brine shrimp lethality bioassay was performed following the reported procedure (Barbosa et al. 2005).



1 R=OH, R^1=H
2 R=OCH_3, R^1=H
3 R=OCH_3, R^1=Br
4 R=R^1=Br
5 R=N_3, R^1=H
6 R=NH_2, R^1=H
7 R=NHPh, R^1=H
8 R=$NHCH_2Ph$, R^1=H
9 R=$NHCH_2CH_2Ph$, R^1=H
10 R=$NHCH_2CH_2NHCH_2Ph$, R^1=H
11 R=$NHCH_2CH{=}CH_2$, R^1=H
12 R=$NHCH_2CH_2CH_2CH_3$, R^1=H
13 R=$NHCH_2CH(OCH_3)_2$, R^1=H
14 R=$NHCH_2CH_2OH$, R^1=H
15 R=$NHCH_2CO_2H$, R^1=H
16 R=$N(CH_2CH_2)_2NCH_3$, R^1=H

Fig. 1 – Structures of lawsone **1** and derivatives **2-17**.

The growth medium was prepared with sea water of in a small tank divided into two compartments. The shrimp eggs were added to the covered compartment. A lamp was placed above the open side of the tank to attract hatched shrimps through perforations in the partition wall. After 48 h the shrimps are mature as nauplii and ready for the assay. Test compounds were dissolved in three drops of Cremophor®, 2 mL of DMSO and sea water to complete 5 mL of total volume. Appropriate volumes were then added to tubes with 5 mL of sea water containing 10 nauplii to afford the five desired concentrations, in quadruplicate for each concentration. The control samples containing Cremophor® and DMSO, under the same conditions, do not cause significant brine shrimp mortality. After 24 h incubation under light, the number of dead and survivor brine shrimps in each tube was counted. The LC_{50} values were calculated by graphics from drug concentration *vs.* lethality percentage using a Probit scale adjust. Data analysis was performed with Origin 6.0 software.

RESULTS AND DISCUSSION

All compounds, except for the glycine derivative **15**, showed toxicity in the brine shrimp lethality bioassay, with toxicities ranging from LC_{50} = 3.1 (**4**) to LC_{50} = 163.5 μg/mL (**12**), as summarized in Table I. Substitution at positions 2 and 3 of the naphthoquinone nucleus resulted in a series of compounds with increased toxicity compared with lawsone **1**. For example, substitution of the hydroxyl group in **1** (LC_{50} = 97.3 μg/mL) for a methoxyl group in **2** (LC_{50} = 14.6 μg/mL) led to a remarkable six fold enhancement of the toxicity. Introduction of a bromine atom at position 3 in compound **3** (LC_{50} = 10.2 μg/mL) and of two bromine atoms in compound **4** (LC_{50} = 3.1 μg/mL) resulted, respectively, in ten and thirty fold toxicity enhancement compared to compound **1**. The presence of an azido group in **5** (LC_{50} = 26.1 μg/mL) in place of the hydroxyl group in **1** also resulted in toxicity enhancement. Although the amino group in compound **6** (LC_{50} = 14.4 μg/mL showed similar profile to the methoxyl group of **2**, further substitution at the nitrogen atom led to an overall loss of activity, the toxicity in the 2-aminonaphthoquinone series ranging from LC_{50} = 10.1 μg/mL (amine **7**) to LC_{50} = 163.5 μg/mL (compound **12**). This series also includes a non-toxic derivative **15** with a glycine moiety. Decrease in polarity of the group attached to the nitrogen atom did not show any clear correlation with toxicity (*e.g.* compounds **10** and **9** presented similar activity profiles), and neither did the introduction of oxygen functionalities attached to the 2-amino groups [*e.g.* compounds **13** (LC_{50} = 21.7 μg/mL), **2** (LC_{50} = 14.6 μg/mL) and **6** (LC_{50} = 14.4 μg/mL)].

As shown in Table I of the seventeen compounds tested nine fell below the threshold of 100 μg/mL set for potential molluscicidal activity by the World Health

TABLE I
Molluscicidal activity (μg/mL) of lawsone and derivatives on *B. glabrata* and toxicity against *Artemia salina*.

	B. glabrata			*A. salina*
	LC_{10} (μg/mL)	LC_{50} (μg/mL)	LC_{90} (μg/mL)	LC_{50} (μg/mL)
1	14.4	28.3	41.9	97.3
2	3.3	10.2	17.0	14.6
3	0.1	2.1	4.2	10.2
4	4.9	16.7	28.4	3.1
5	1.7	7.4	13.1	26.1
6	9.8	20.0	29.9	14.4
7	–	–	Inactive[a]	10.1
8	3.5	23.8	44.1	83.1
9	–	–	Inactive[a]	61.9
10	35.8	64.3	92.7	69.2
11	15.0	32.9	50.8	27.2
12	–	–	Inactive[a]	163.5
13	–	–	Inactive[a]	21.7
14	–	–	Inactive[a]	89.3
15	–	–	Inactive[a]	Non toxic[b]
16	–	–	Inactive[a]	10.1
17	–	–	Inactive[a]	61.8

[a]Inactivity corresponds to a value of $LC_{90} > 100$ μg/mL. [b]Non toxicity corresponds to a value of $LC_{50} > 1000$ μg/mL.

Organization (WHO 1965). The results of the assays of compounds **1-17** indicated that the dibromonaphthoquinone **3** ($LC_{50} = 2.1$ μg/mL), which contains two non-polar groups, is the most active of the series, followed by the azido derivative **5** ($LC_{50} = 7.4$ μg/mL). These two compounds also figure as the most toxic in the brine shrimp assay for overall toxicity profile. As a general rule, derivatives with non-polar substituents (*e.g.*, **1-6** in Table I) present the highest molluscicidal activities. The presence of a glycine moiety results in inactive compounds, as found previously for analogous derivatives of lapachol (Silva et al. 2005b) and nor-lapachol (Barbosa et al. 2005). The appended moieties of derivatives **13** and **14** led to inactivity in the present study and in the nor-lapachol series (Barbosa et al. 2005). In the lapachol series, however, the corresponding compounds exhibit median to low activities ($LC_{50} = 54.9$ and 72.6 μg/mL, respectively) (Silva et al. 2005b).

In general, the amine derivatives of lapachol and nor-lapachol showed higher mosllusciciddal activity than those of lawsone **1** (Silva et al. 2005b, Barbosa et al. 2005), probably for being less polar than **1**, as a result of the presence of a lipophilic side chain; this result is in agreement with the general trend discussed above. Furthermore, the desired lowest toxicity in the brine shrimp assay/highest molluscicidal activity, although not verified for the most active compounds (**3** and **5**), was achieved in the nor-lapachol series (Barbosa et al. 2005) which suggests that the side chain is an important tool in tuning this selectivity.

Thus although several compounds containing polar substituents exhibited high toxicity in the brine shrimp assay, as a general rule these compounds did not show molluscicidal activity (*e.g.*, compounds **13** and **16**).

The bioactivity of lawsone **1** and derivatives **2-17** shows a clear correlation with the presence of non-polar groups appended to the 1,4-naphthoquinone nucleus of the tested compounds. These results are in agreement

with our previous findings with a similar series of 2-amino-naphthoquinones synthesized from lapachol and nor-lapachol.

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RESUMO

A toxicidade da lausona (2-hidroxi-1,4)-naftoquinona e de diversos derivados foi avaliada frente à *Artemia salina* e ao molusco *Biomphalaria glabrata*, o principal vetor de transmissão da esquistossomose no Brasil. Entre os dezessete compostos testados, nove apresentaram um perfil de toxicidade menor que 100 μg/mL, sendo potenciais agentes moluscicidas de acordo com as designações da Organização Mundial da Saúde. No presente estudo, os compostos contendo substituintes apolares exibiram as maiores atividades. Estes compostos também se mostraram significantemente tóxicos frente à *A. salina*.

Palavras-chave: *Biomphalaria glabrata*, *Artemia salina*, toxicidade, quinonas, esquistossomose, atividade moluscicida, lausona, 1,4-naftoquinona.

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