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Molluscicidal activity of synthetic lapachol amino and hydrogenated derivatives

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Abstract—A series of new amino derivatives and a new partially hydrogenated derivative of the natural naphthoquinone lapachol were assayed for molluscicidal activity against *Biomphalaria glabrata*. These derivatives showed low to medium LC_{50} values, and a 3.1 μg/mL value for the most potent derivative of the series. The toxicity is in agreement with the decrease of polar character of the tested compounds.

1. Introduction

Among human parasitic diseases, schistosomiasis (sometimes called bilharziasis) ranks second behind malaria in terms of socio-economic and public health importance in tropical and subtropical areas.[1] The disease is endemic in 74 developing countries, infecting over 200 million people in rural agricultural and *peri*-urban areas. Of these, 20 million suffer severe consequences from the disease and 120 million are symptomatic. In many areas, schistosomiasis infects a large proportion of under-14 children. An estimated 500–600 million people worldwide are at risk from the disease.[2] In Brazil, the worm *Schistosoma mansoni* is the ethyological agent, and it requires the aquatic snail *Biomphalaria glabrata* as the major intermediate host for transmission.[3] Molluscicides are of great interest for the potential focal control of schistosomiasis in endemic countries, and among these compounds of natural origin, toxic plant saponins have been investigated.[4,5] We report herein remarkable molluscicidal activity of some derivatives of lapachol **1** a natural naphthoquinone extracted in 2–5% yield from the bark of *Tabebuia* sp.[6] Amino derivatives **2a**–**h**,[7] 1-aza-anthraquinones **3a**–**c** and a partially hydrogenated derivative of lapachol,

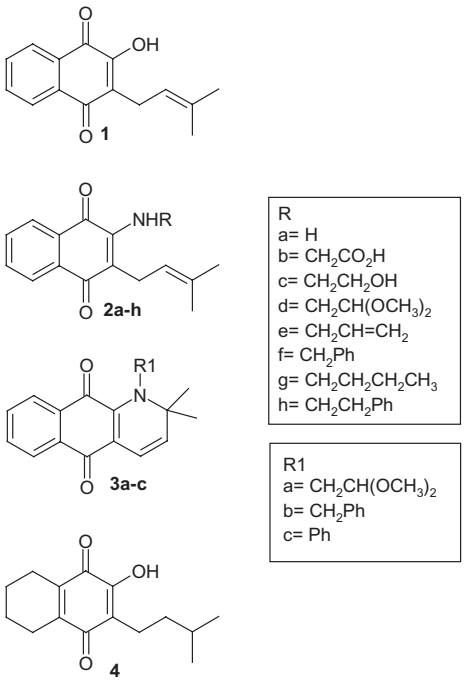

Figure 1. Derivatives of lapachol investigated.

Keywords: Lapachol; Molluscicide; Naphthoquinones.
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benzoquinone **4** were investigated (Fig. 1). Heterocyclic quinones and quinones containing nitrogen atoms are known to exhibit excellent antitumor[8] and other biological activities.[9]

2. Results and discussion

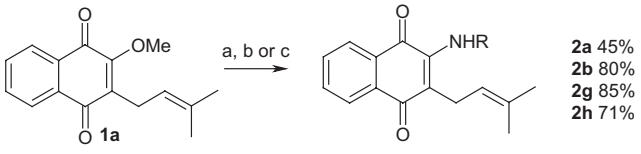
2.1. Synthesis of the compounds

The amino derivatives **2c–f** and 1-aza-anthraquinones **3a–c** were synthesized as described previously.[7] The new compounds **2a**, **2b**, **2g**, and **2h** were obtained by nucleophilic displacement of 2-methoxylapachol with 30% ammonium hydroxide solution (45% yield), glycine in KOH/MeOH[10a] (80% yield), *n*-butylamine in methanol (85%) and phenethylamine in methanol (71% yield), respectively (Scheme 1).

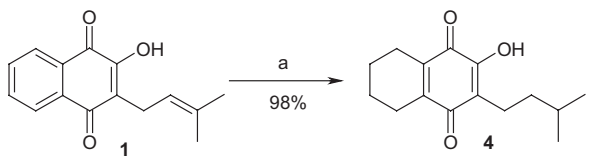
Compound **4** was obtained in high yield (>98%) from the catalytic hydrogenation of lapachol (5 bar) with 10% palladium on charcoal in acetic acid. Selective hydrogenating of the prenyl side chain of lapachol was monitored by ^{1}H NMR spectroscopy. Above 2 atm H_2 signals attributed to the hydrogenated quinone started to appear. The only other related hydrogenation reaction reported to date involves pyrano-quinones derived from lapachol, not the parent compound itself (Scheme 2).[11]

2.2. Molluscicidal assays

Table 1 summarizes the results of assays performed against adult snails of *B. glabrata*, with the LC_{10}, LC_{50}, and LC_{90} calculated values. In the bioassays compound **4** presented significant molluscicidal activity against the adult form of *B. glabrata*, with LC_{90} = 7.6 μmol/mL and LC_{50} = 12.5 μmol/mL. This activity is similar to that presented by molluscicide warburganal, with a reported LC_{50} = 2 ppm (8.0 μmol/mL).[12] Compound **4**, with LC_{90} = 12.5 μmol/mL, is two times more potent than lapachol **1** for which a



Scheme 1. Reagents and conditions: (a) R = H, NH_4OH, rt, 24 h; (b) R = CH_2CO_2H, 10% KOH/MeOH, rt, 12 h; (c) R = *n*-Bu and CH_2CH_2Ph, amine, MeOH, rt, 24 h.



Scheme 2. Reagents and conditions: (a) H_2, Pd/C 10%, 5.0 bar, 2 h, 65–70 °C, AcOH.

Table 1. Molluscicidal activity (μmol/mL) of the lapachol amino derivatives on *B. glabrata* (9–16 mm, 10 snails per concentration) under laboratory conditions and 24 h exposure

Compound	LC_{10} (μmol/mL)	LC_{50} (μmol/mL)	LC_{90} (μmol/mL)
2a	26.5	49.3	72.1
2b	86.9	206.6	330.1
2c	42.4	72.6	102.8
2d	25.8	54.9	75.9
2e	6.0	23.8	44.8
2f	Inactive[a]	Inactive[a]	Inactive[a]
2g	4.0	13.8	23.5
2h	33.6	45.2	57.6
3a	32.4	66.9	101.5
3b	53.8	89.0	13.6
3c	Inactive[a]	Inactive[a]	Inactive[a]
4	3.2	7.6	12.5

[a] The inactivity corresponds to a value >100 μg/mL.

LC_{90} value of 6.18 μg/mL (25.5 μmol/mL) has been reported.[4b] In the amino derivatives of lapachol series, **2a–h**, the most active compounds were **2e** and **2g**, whose activities are superior to that of molluscicide muzigadial (LC_{90} = 20–40 μmol/mL).[12] Compounds **2a** (LC_{50} = 49.3 μmol/mL), **2c** (LC_{50} = 72.6 μmol/mL), **2d** (LC_{50} = 54.9 μmol/mL) and **2h** (LC_{50} = 45.2 μmol/mL) presented activities similar or even superior to that of mukaadial (LC_{50} = 80.5 μmol/mL).[12] The least active compound of this series was the glycine derivative **2b** (LC_{50} = 206.6 μmol/mL), and compound **2f** was inactive. Among the 1-aza-anthraquinone derivatives **3a–c**, compounds **3a** with LC_{50} = 66.9 and **3b**, LC_{50} = 102.8 μmol/mL were less active than the corresponding amino derivatives of lapachol, and **3c** was inactive (Table 1).

These results indicate a correlation between the hydrophobic character of the aminoalkyl side-chain moiety of the compounds of series **2** and molluscicidal activity, compounds with the most polar groups generally exhibiting the lowest activities (**2b** and **2f**, Table 1).[13] Exceptions to this trend are compounds **2f**, that was inactive and **2c** with a hydroxyl group on the aminoalkyl side chain, which showed reasonable activity. These compounds are less active than lapachol **1**, and this might be associated to the more efficient conjugation of the vinylogous amide present in series **2** and **3** nitrogen compounds, when compared with the hydroxyl-substituted ones.[14] Series **3** of cyclic compounds showed a general loss of activity when compared with the parent *seco*-amino compounds of series **2** (except for compound **3b**, that is more potent than compound **2f**). In contrast, the partially hydrogenated lapachol derivative **4**, no longer a naphthoquinone, but a 2,3,5,6-tetra-substituted benzoquinone, is two times more potent than lapachol **1**. The increase of activity is probably due to the availability of the new Michael acceptor from a partially reduced aromatic moiety arising from the reduction of lapachol **1**.[13]

3. Conclusions

The molluscicidal activity of the amino derivatives of lapachol increased with the increasing lipophilic charac-

ter of the amino alkyl side chain. The partially hydrogenated lapachol derivative **4**, obtained from the catalytic reduction of lapachol **1**, showed molluscicidal activity significantly higher than **1**. These findings confirm the importance of lapachol as an important starting material for the production of biologically active compounds.

4. Experimental

Melting points are uncorrected and were determined on an electrically heated metal block apparatus. Column chromatography was performed on silica-gel G_{60} (70–230 mesh, ASTM, Merck), and thin-layer chromatography was performed on 0.2 mm plates (Merck), visualized with short wavelength UV light. ^1H and ^{13}C NMR spectra wee recorded on a Varian Mercury-200 MHz spectrometer. Values reported for coupling constants are first order. High-resolution mass spectra were obtained by electron impact on a VG Autospec spectrometer. 2-Methoxy-lapachol **1a** was obtained as described previously.[7a]

4.1. Synthesis of 2-amino-3-(3-methyl-but-2-enyl)-[1,4]naphthoquinone (2a)

A solution of 2-methoxy-lapachol (0.256 g, 1 mmol) in 10 mL of methanol PA was mixed with ammonium hydroxide 30% solution (10 mL), and the solution kept at room temperature for 24 h. The solvent was then removed by reduced pressure, and the aqueous solution extracted with dichloromethane (3×20 mL), the combined organic extracts were dried with anhydrous sodium sulfate, and the resulting red-brownish oil chromatographed in silica-gel with hexane–dichloromethane 95:5 affording the desired (**2a**) as red crystals (mp 130–132 °C, from hexane–CH_2Cl_2), in 45% yield. IR (KBr): ν_{max} 3426, 3315, 3289, 3241, 3064, 2916, 1676, 1625, 1594, 1552, 1437, 1391, 1368, 1277, and 726 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.71 (s, 3H), 1.80 (s, 3H), 3.27 (d, 2H, $J = 7.8$ Hz), 5.07 (m, 1H), 5.16 (br, NH), 7.67 (dt, 1H, 7.0/7.0/1.4 Hz), 7.60 (dt, 1H, 7.0/7.0/1.4 Hz), 8.02 (dd, 1H, 7.0/1.4 Hz) and 8.10 (dd, 1H, 7.0/1.4 Hz); ^{13}C NMR (50 MHZ, $CDCl_3$) δ 18.1, 23.1, 25.8, 116.4, 120.1, 125.9, 126.5, 126.9, 130.6, 132.1, 133.9, 134.4, 145.5, 181.8, 182.5. HRMS found: 241.1012. Calcd for $C_{15}H_{15}NO_2$: 241.1102.

4.2. Synthesis of [3-(3-methyl-but-2-enyl)-1,4-dioxo-1,4-dihydro-naphthalen-2-ylamino]-acetic acid (2b)

To a solution of 2-methoxy-lapachol **1a** (0.256 g, 1 mmol) and glycine (0.075 g, 1 mmol) in 20 mL of methanol PA was added dropwise a 6.2 mL of a KOH 10% aqueous solution with continuous stirring, and the resulting solution was stirred for 12 h. After TLC inspection, hydrochloric acid 10% aqueous solution was added until complete precipitation. The material was collected on a Büchner, washed with distilled water, and recrystallized from methanol, yielding red crystals, with mp 174–175 °C. IR (KBr): ν_{max} 3283, 2983, 2921, 2864, 1742, 1677, 1592, 1542, 1394, 1365, 1183, and 720 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.62 (s, 3H), 1.70 (s, 3H), 3.20 (d, 2H, 6.2 Hz), 4.22 (d, 2H, 6.3 Hz), 5.01 (m, 1H), 6.76 (t, 1H, 6.3 Hz), 7.66 (dt, 1H, 7.0/7.0/1.6 Hz), 7.76 (dt, 1H, 7.0/7.0/1.6 Hz), 7.87 (dd, 1H, 7.0/1.6 Hz), 7.91 (dd, 1H, 7.0/1.6 Hz); ^{13}C NMR (DMSO-d_6): δ 17.96, 22.52, 25.48, 46.06, 115.76, 122.15, 125.38, 125.64, 130.38, 131.79, 132.33, 132.35, 134.49, 146.09, 171.82, 181.31, 182.70. HRMS found: 299.1150. Calcd for $C_{17}H_{17}NO_4$: 299.1157.

4.3. Synthesis of 2-butylamino-3-(3-methyl-but-2-enyl)-[1,4]naphthoquinone (2g)

To a methanol solution of 2-methoxylapachol (0.254 g, 1 mmol) was added 0.146 g (2 mmol) of *n*-butylamine, and the resulting mixture was kept under stirring at room temperature for 24 h. The solvent was removed by reduced pressure and the resulting deep-brown oil chromatographed in a silica-gel column with hexane–CH_2Cl_2 98:2 to furnish **2g** as a red oil (253 mg, 85%). IR (KBr): ν_{max} 3298, 2976, 2914, 2854, 1751, 1682, 1590, 1522, 1391, 1364, 1180, and 710 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.91 (t, 3H, 7.4 Hz), 1.39 (m, 2H, 7.0/7.4 Hz), 1.58 (m, 2H), 1.67 (d, 3H, 1.2 Hz), 1.71 (d, 3H, 1.4 Hz), 3.36 (d, 2H, 6.0 Hz), 3.49 (m, 2H), 5.08 (dd, 2H, 7.4/1.2 Hz), 5.65 (NH, br s), 7.52 (dt, 1H, 7.4/7.4/1.4 Hz), 7.63 (dt, 1H, 7.4/7.4/1.4 Hz), 7.94 (dd, 1H, 7.4/1.4 Hz) and 8.04 (dd, 1H, 7.4/1.4 Hz); ^{13}C NMR ($CDCl_3$): δ 13.66, 18.06, 19.84, 22.94, 25.60, 32.80, 44.73, 114.95, 119.97, 123.31, 131.64, 133.12, 133.40, 133.60, 133.67, 134.27, 145.65, 182.69, 182.91. HRMS found: 297.1722. Calcd for $C_{19}H_{23}NO_2$: 297.1728.

4.4. Synthesis of 2-(3-methyl-but-2-enyl)-3-phenethylamino-[1,4]naphthoquinone (2h)

To a methanol solution of 2-methoxylapachol (0.254 g, 1 mmol) was added 0.242 g (2 mmol) of phenethylamine, and the resulting mixture was kept under stirring at room temperature for 24 h. The solvent was removed by reduced pressure and the resulting deep-brown oil chromatographed in a silica-gel column with hexane–CH_2Cl_2 98:2 to furnish 245 mg of red needles (71%), with a mp 80–81 °C. IR (KBr): ν_{max} 3290, 2958, 2915, 2834, 1740, 1684, 1582, 1533, 1389, 1359, 1180, and 715 cm^{-1}; ^{1} H NMR ($CDCl_3$): δ 1.67 (s, 3H), 1.67 (s, 3H), 2.91 (t, 2H, 7.2 Hz), 3.28 (d, 2H, 7.4 Hz), 3.80 (t, 2H, 7.2 Hz), 5.11 (ddt, 1H, 7.4/1.4), 5.70 (1H, br s), 7.22 (m, 1H), 7.52 (m, 1H), 7.64 (m, 1H), 7.65 (m, 1H), 7.67 (m, 1H), 8.05 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 22.82, 25.48, 25.63, 36.86, 46.00, 115.37, 119.90, 122.87, 125.98, 126.62, 128.58, 131.58, 132.15, 132.86, 133.42, 133.53, 134.11, 137.39, 145.32, 182.60, 182.73. HRMS found: 345.1728. Calcd for $C_{23}H_{23}NO_2$: 345.1729.

4.5. Biological assays

The bioassay was carried out as described by dos Santos et al.[4b] 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. 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 snail's susceptibility, two control sets were used: one with cupric carbonate at 50 ppm and the other containing 0.1% DMSO dechlorinated water. The collected data were computerized, and the LC_{10}, LC_{50}, and LC_{90} values determined by performing a probit analysis.[4b]

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