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New 1,2,3,4-tetrahydro-1-aza-anthraquinones and 2-aminoalkyl compounds from norlapachol with molluscicidal activity

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Abstract—New nitrogen derivatives from norlapachol, including four new diastereomeric 1,2,3,4-tetrahydro-1-aza-anthraquinones obtained from the Prins cyclization on suitable aminoacetaldehyde dimethylacetal derivatives with formic acid, were found to exhibit molluscicidal activity against *Biomphalaria glabrata*. These derivatives showed low to medium LC_{50} values, similar to those reported previously for the homologous series of nitrogen derivatives of lapachol. The toxicity profile against *Artemia salina* was also determined for all compounds.

1. Introduction

The search for molluscicides is of great interest for potential focal control of parasitary diseases in endemic countries, especially schistosomiasis,[1–3] a disease caused by *Schistosoma mansoni*. In this specific case, the aim is the control of the aquatic snail *Biomphalaria glabrata*, the major intermediate host for transmission of *S. mansoni*.[4,5] Norlapachol **1** is a semi-synthetic derivative of natural lapachol **2**,[6] a naphthoquinone readily extracted from a number of species of *Tabebuia sp.* (Bignoniaceae) abundant in Brazil and South America. Using Fieser's modified procedure for the Hooker oxidative degradation, norlapachol **1** can be obtained in good yield.[7,8]

The presence of a conjugated double bond in the isobutenyl side chain of **1** and the availability of cheap sources of **2**[9] prompted us to investigate the toxicity profile of a new series of nitrogen compounds obtained from norlapachol, which are the lower homologues of the lapachol derivatives reported previously[10a] and whose structures are shown in Figure 1. Heterocyclic quinones and quinones containing nitrogen atoms are known to exhibit excellent antitumor and other biological activities.[10b,c] The new 2-aminoalkyl compounds (**3a**–**h**) and the diastereomeric 1-aza-anthraquinones **4a/b** and **5a/b** (whose ring is formally named 1,2,3,4-tetrahydro-benzo[*g*]quinoline-5,10-dione or 1,2,3,4-tetrahydro-1-aza-anthraquinone) were obtained by means of a recently developed procedure involving the Prins cyclization reaction on the 2-aminoacetaldehyde dimethylacetal derivative **3d**.[9b]

2. Results and discussion

2.1. Synthesis of the compounds

The novel amino-derivatives **3a**–**h** and 1-aza-anthraquinones **4a-b** and **5a**–**b** were synthesized following the procedure reported for the syntheses of the analogous derivatives of **2**:[9,10a] (i) methylation of norlapachol **1** with dimethyl sulfate in acetone and potassium carbonate, which yields the methoxy derivative **1a** in 71% yield and (ii) nucleophilic displacement of **1a** with the appropriate amine that furnishes the novel compounds **3a**–**h**.

Keywords: Norlapachol; Molluscicidal; *Biomphalaria glabrata*; *Artemia salina*; Prins reaction.

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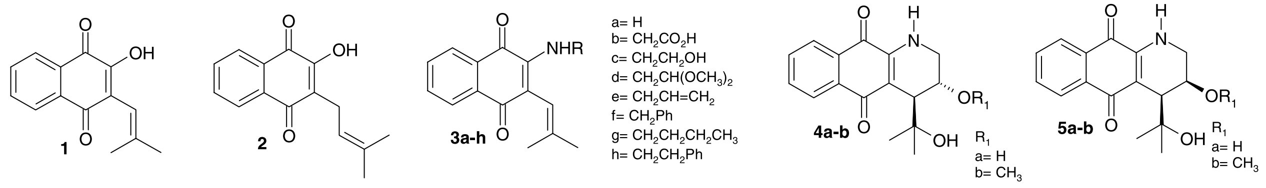
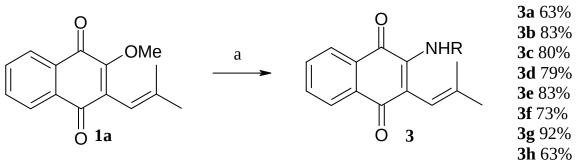


Figure 1. Structures of lapachol, norlapachol and synthetic norlapachol derivatives.

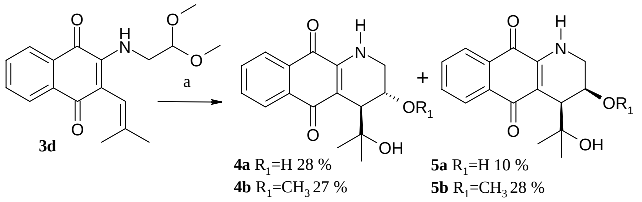


Scheme 1. Reagents and conditions: (a) RNH_2, rt, MeOH, 24 h.

Although these compounds were obtained in yields (Scheme 1) similar to those of the higher homologues of methoxylapachol,[9a,10a] longer reaction times are needed for total conversion of the methoxy derivative **1a** into the amino products.

Prins cyclization on **3d** was performed under the conditions previously described by our group:[9b] 88% formic acid at room temperature led to the formation of four diastereomeric compounds. The most polar aza-anthraquinones **4a** (*trans*) and **5a** (*cis*) were obtained in 28 and 10% yields, respectively, whereas the most apolar 3-methoxy derivatives **4b** (*trans*) and **5b** (*cis*) were isolated in 27 and 28% yields, respectively (93% total yield). These compounds were separated by flash chromatography and characterized as new compounds (Scheme 2).

Spectroscopic analysis of compound *trans*-**4a** (^{1}H NMR 200 MHz, $CDCl_3$) showed a signal at $\delta = 3.96$ ppm attributed to the C-3 carbinolic hydrogen (m, $J = 11.2$, 5.8, 5.4 and 5.2 Hz). This signal strongly couples in the COSY spectrum with a doublet at $\delta = 3.56$ ppm ($J = 11.2$ Hz), assigned to the C-4 methyne hydrogen. The 3-CH signal of *cis*-**5a** appeared as a multiplet at $\delta = 4.38$ ppm (m, $J = 2.0$, 1.8, 1.2 and 1.0 Hz), coupled with a doublet of doublets at $\delta = 3.30$ ppm ($J = 2.0$ and 1.1 Hz). The coupling constant values are compatible with the *trans* and *cis* configurations proposed for isomers **4a** ($J = 11.2$ Hz) and **5a** ($J = 2.0$ Hz), respectively. Similar coupling constant values were found for *trans*-**4b** (3-CH at $\delta = 3.97$ ppm, ddd, $J = 1.8$, 2.4 and 11.0 Hz; 4-CH at $\delta = 3.48$ ppm, d, $J = 11.0$ Hz) and *cis*-**5b** (3-CH at $\delta = 3.80$ ppm, m, $J = 3.0$, 2.4, 2.2 and 1.0 Hz and 4-CH at $\delta = 3.45$ ppm, dd, $J = 2.4$ and 1.0 Hz). The HMBC correlations (^3J) between the methoxyl hydrogens in **4b** with the 3-CH signal at $\delta = 76.8$ ppm, and in compound **5b** at $\delta = 70.9$ ppm, confirm the proposed structures.



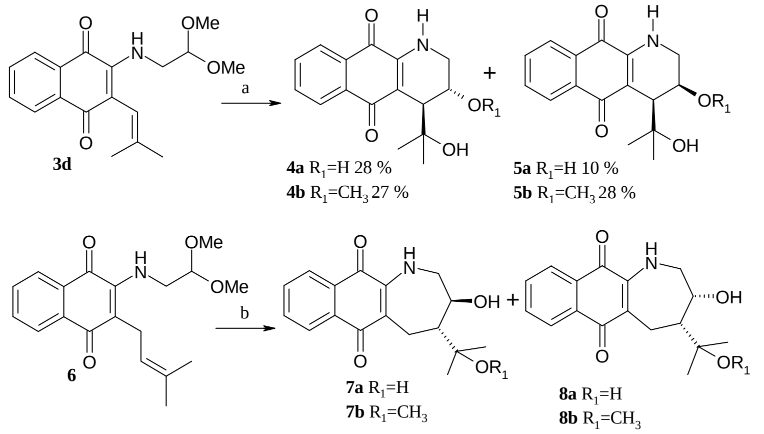
Scheme 2. Reagents and conditions: (a) HCO_2H 88%, rt, 2 h.

Cyclization of **3d** leads, therefore, to the methoxy group incorporation at position 3 in the newly formed six-membered ring of **4b** and **5b**, whereas that of the superior homologue of **3d**, lapachol derivative **6** (Scheme 3), results only in the 3-hydroxy seven-membered rings *trans*-**7a** and *cis*-**8a** (42%, 7:3 mixture) and methoxy derivatives *trans*-**7b** and *cis*-**8b** (8%, 7:3 mixture).[9b] A higher total yield of the six-membered ring products (93%) compared to the seven-membered ring ones (50%) can be rationalized in terms of a more favored cyclization process of **3d** than **6**.

The two 3-hydroxy diastereomeric derivatives **4a** and **5a** were formed in approximately the same diastereomeric ratio (7.3:2.7) as the seven-membered ring compounds **7a** and **8a**, and **7b** and **8b**,[9b] which suggests similar pathways for both processes, possibly involving cyclization after acidic hydrolysis of the acetal function, i.e., onto the unmasked aldehyde.[9b] In contrast, the 3-methoxy derivatives **4b** and **5b** were obtained in an approximate 1:1 ratio, suggesting that the cyclization step that leads to these compounds proceeds differently. Assuming that formation of the new bond is concerted with the departure of a protonated methoxyl group during the acetal hydrolysis (presumably at the oxygen-assisted carbocation resulting from the partial acetal hydrolysis), nucleophilic attack of the isobutenyl side-chain π-electrons probably occurs more easily in this case, leading to six-membered ring cyclized products (**4b** and **5b**) with an appended methoxyl group at C-3. The methoxyl group in compounds **7b** and **8b** results from nucleophilic quenching with methanol of the formed carbocation at the alkene side chain of **6**.

2.2. Molluscicidal assays

Table 1 summarizes the results of assays conducted on adult snails of *B. glabrata*, with the LC_{10}, LC_{50}, and LC_{90} calculated values. These results show some correlation between the hydrophobic character of the aminoalkyl side-chain moiety of compounds **3a**–**h** and molluscicidal activity, with the compounds containing the most polar groups generally exhibiting the lowest activities (Table 1, **3b** and **3c**).[11] A similar trend was observed for the aminoderivatives of lapachol.[10a] Thus, compounds **3g** (with a *n*-butylamino alkyl side chain) and **3e** (*n*-allylamino) are the most potent in the *seco*-series, with LC_{50} values of 23.07 and 13.65 μM,



Scheme 3. Reagents and conditions: (a) HCO_2H, rt, 2 h; (b) Ref. 9b.

Table 1. Molluscicidal activity (μM) of the norlapachol derivatives on *Biomphalaria glabrata* and toxicity against *Artemia salina*

Compound	*A. salina*		*B. glabrata*	
	LC_{50}	LC_{10}	LC_{50}	LC_{90}
3a	221.80	24.10	157.26	208.50
3b	862.21	40.99	224.77	305.75
3c	61.36	24.37	132.91	176.71
3d	27.65	25.00	111.11	143.23
3e	22.69	13.40	13.65	28.85
3f	87.22	24.97	113.05	148.95
3g	124.24	2.72	23.07	36.53
3h	90.99	13.95	89.03	135.95
4a	634.56		Inactive[a]	
4b	614.28	11.87	64.55	89.63
5a	3277.94		Inactive[a]	
5b	3475.08	99.00	99.00	164.18

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

respectively. Although the cyclic series does not show significant LC_{50} values, it is noteworthy that both dihydroxylated diastereoisomers **4a** and **5a** were inactive in the present study, whereas their 3-methoxy-substituted analogues **4b** and **5b** showed LC_{50} of 64.55 and 99.00 μM, respectively.

2.3. Toxicity against *Artemia salina*[12]

The data in Table 1 do not show a clear correlation between toxicity in *A. salina* and molluscicidal activity, except for the fact that in both cases increased lipophilicity of the aminoalkyl side chain results in increased toxicity. Furthermore, contrary to molluscicidal activity, toxicity of the cyclic compounds in *A. salina* seems to depend on the stereochemistry rather than on the nature of the substituent on C3, since *trans*-**4a** and **4b** are about fivefold more active than the parent *cis*-derivatives **5a** and **5b**.

3. Conclusions

The molluscicidal activity of the aminoderivatives of norlapachol shows a clear correlation with the lipophilic character of the amino-alkyl substitution. This corroborates the previous results obtained with a similar series of compounds derived from lapachol. The Prins cyclization reaction allows the synthesis of new 1-tetrahydro-1-aza-anthraquinone compounds and constitutes a new tool for the construction of interesting and biologically enriched compounds.[13]

4. Experimental

Melting points are uncorrected and were determined with an electrically heated metal block apparatus. Column chromatography (CC) 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 were 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. Norlapachol was obtained by Fieser's modification methodology of the original Hooker degradation procedure of lapachol **2**,[6a,7a] in 71% yield (mp 40.5–42 °C). The methoxy derivative **1a** is also a new compound and is described for the first time in the literature.

4.1. Synthesis of 2-methoxy-3-(2-methyl-propenyl)-[1,4]naphthoquinone (1a)

Norlapachol **1** (456 mg, 2 mmol) was added to a stirred mixture of potassium carbonate in acetone 1.38 g (10 mmol) in acetone (50 mL) at room temperature. Dimethyl sulfate (0.28 mL, 2.5 mmol) was slowly added to the mixture with stirring. After 2 h, tlc inspection showed no **1** left. The solvents were removed under reduced pressure, solids were extracted with ethyl acetate, washed with brine and then with water, and dried over anhydrous sodium sulfate. After removal of the solvent under vacuum, product **1a** was purified by flash chromatography with 99:1 hexane/ethyl acetate, yielding 71% of **1a**, which crystallizes at 4 °C as yellow crystals with (mp 40.5–42 °C). IR (KBr) ν_{max} 2943, 2912, 2853, 1670, 1652, 1596, 1574, 1446, 1378, 1329, 1310, 1296, 1265, 1215 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 1.57 (d, 3H, 1.2 Hz), 1.94 (d, 3H, 1.2 Hz), 3.96 (s, 3H), 5.99 (t, 1H, 1.2 Hz), 7.65 (m, 1H), 7.66 (m, 1H), 8.01 (m, 1H), 8.02 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 21.1, 26.3, 60.5, 114.7, 126.0, 126.2, 128.9, 131.3, 131.9, 133.1, 133.7, 143.0, 156.4, 181.6, 185.5.

4.1.1. General procedure for the reaction of 1a with amines (except for 3a and 3b).

To 1 mmol of **1a** dissolved in 10 mL methanol was added the appropriate amine (1.5–2 mmol), and the resulting solution was stirred at room temperature for 24 h. The solvent was removed by reduced pressure and the residue, a dark red oil in all cases, was subjected to CC using chloroform, chloroform–ethyl acetate or hexane–ethyl acetate as eluent.

4.2. Synthesis of 2-amino-3-(2-methyl-propenyl)-[1,4]naphthoquinone (3a)

Compound **3a** was obtained from the reaction of **1a** with a 30% ammonium hydroxide solution (10 mL) under stirring for 24 h. After CC using chloroform as eluent, **3a** was obtained as red crystals (mp 149–151 °C) in 63% yield. IR (KBr) ν_{max}, 3459, 3318, 2978, 2932, 2859, 1673, 1592, 1555, 1394, 1355 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.59 (d, 3H, 1.0 Hz), 1.92 (d, 3H, 1.4 Hz), 5.09 (m, 1H), 6.92 (br s, 2H), 7.57 (dt, 1H, 1.6/7.2/7.2 Hz), 7.66 (dt, 1H, 1.6/7.2/7.2 Hz), 7.99 (dd, 1H, 1.6/7.2 Hz), 8.05 (dd, 1H, 1.6/7.2 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.7, 25.8, 114.7, 115.9, 125.7, 126.2, 130.5, 132.0, 133.2, 134.3, 140.4, 144.2, 181.5, 182.6. HRMS Calcd for $C_{14}H_{13}NO_2$, 227.0946; found: 227.0941.

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

To a solution of **1a** (242 mg, 1 mmol) in 20 mL methanol was added 112.5 mg (1.5 mmol) glycine and the mixture was kept under stirring at room temperature, for 10 min. To this solution was added dropwise 6.2 mL of a KOH 10% aqueous solution and the resulting mixture was kept under continuous stirring for 24 h. After tlc inspection, the solvent was removed in vacuum and the resulting solution was neutralized with hydrochloric acid 10%. The precipitate was filtered off and purified by flash chromatography with silica gel, using chloroform 99:1 methanol increasing until 95:5 Compound **3b** was obtained in 83% as red crystals (mp 125–127 °C). IR (KBr) ν_{max}, 3347, 3090, 2978, 2941, 1758, 1743, 1667, 1613, 1600, 1571, 1510, 1339, 1305 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.41 (s, 3H), 1.85 (s, 3H), 3.95 (d, 2H, 6.0), 5.90 (s, 1H), 6.91 (br s, 1H), 7.80 (dt, 1H, 1.6/7.4/7.4 Hz), 7.84 (dt, 1H, 1.6/7.4/7.4 Hz), 7.90 (dd, 1H, 1.6/7.4 Hz), 7.94 (dd, 1H, 1.6/7.4 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) 19.9, 25.2, 45.3, 113.3, 117.6, 125.4, 125.7, 130.1, 132.4, 132.6, 134.7, 138.3, 144.9, 171.0, 181.7, 182.2. HRMS Calcd for $C_{16}H_{15}NO_4$, 285.1001; found: 285.1018.

4.4. 2-(2-Hydroxy-ethylamino)-3-(2-methyl-propenyl)-[1,4]naphthoquinone (3c)

Using chloroform/ethyl acetate 95:5 as eluent, **3c** was obtained as red crystals (mp 77–78.5 °C) in 80% yield. IR (KBr) ν_{max}, 3457, 3349, 3268, 2940, 2874, 1675, 1598, 1563, 1511, 1354, 1335 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) 1.47 (d, 3H, 1.0 Hz), 1.89 (d, 3H, 1.6 Hz), 2.46 (br s, 1H), 3.37 (q, 2H, 5.4 Hz), 3.73 (t, 2H, 5.4 Hz), 6.06 (dd, 1H, 1.0/1.6 Hz), 6.25 (br t, 1H, 5.4 Hz), 7.51 (dt, 1H, 1.4/7.6/7.6 Hz), 7.61 (dt, 1H, 1.4/7.6/7.6 Hz), 7.90 (dd, 1H, 1.4/7.6 Hz), 7.99 (dd, 1H, 1.4/7.6 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) 20.1, 25.4, 46.1, 61.3, 113.6, 117.7, 125.9, 126.1, 130.3, 131.9, 133.3, 134.4, 139.0, 144.8, 182.7, 183.4. HRMS Calcd for $C_{16}H_{17}NO_2$, 271.1208; found: 271.1169.

4.5. 2-(2,2-Dimethoxy-ethylamino)-3-(2-methyl-propenyl)-[1, 4]naphthoquinone (3d)

Using chloroform as eluent, **3d** was obtained as a red oil in 79%. IR (KBr): ν_{max} 3453, 3353, 2933, 2635, 1669, 1632, 1600, 1572, 1512, 1332, 1296, 1132 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.48 (d, 3H, 1.4 Hz), 1.91 (d, 3H, 1.4 Hz), 3.32 (t, 1H, 5.4 Hz), 3.35 (s, 6H), 4.38 (t, 1H, 5.4 Hz), 6.04 (t, 1H, 1.4 Hz), 6.09 (br s, 1H), 7.54 (dt, 1H, 1.0/7.0/7.0 Hz), 7.65 (dt, 1H, 1.0/7.0/7.0 Hz), 7.94 (dd, 1H, 1.0/7.0 Hz), 8.0 (dd, 1H, 1.0/7.0 Hz). ^{13}C NMR ($CDCl_3$, 200 MHz) 20.1, 25.4, 45.3, 54.0, 102.3, 113.9, 117.7, 125.9, 126.1, 130.3, 131.9, 133.3, 134.3, 139.0, 144.6, 182.5, 183.3. HRMS Calcd for $C_{18}H_{21}NO_4$, 315.1470; found: 315.1471.

4.6. 2-(2-Propenylamino)-3-(2-methyl-propenyl)-[1,4]naphthoquinone (3e)

Using hexane 1:9 chloroform as eluent, **3e** was obtained as red crystals in 83% yield (mp 63–65 °C). IR (KBr): ν_{max} 3315, 3012, 2973, 2905, 2848, 1673, 1595, 1563, 1505, 1331, 1316, 1301 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.47 (d, 3H, 1.0 Hz), 1.90 (d, 3H, 1.4 Hz), 3.82 (m, 2H), 5.11 (m, 1H), 5.16 (m, 1H), 5.79 (m, 1H) 5.97 (br s, 1H), 6.07 (br s, 1H), 7.54 (dt, 1H, 1.4/7.4/7.4 Hz), 7.64 (dt, 1H, 1.4/7.4/7.4 Hz), 7.96 (dd, 1H, 1.4/7.4 Hz), 8.02 (dd, 1H, 1.4/7.4 Hz). ^{13}C NMR ($CDCl_3$, 200 MHz) 20.0, 25.3, 46.3, 113.8, 117.0, 117.7, 125.8, 126.1, 130.2, 133.3, 134.1, 134.3, 138.8, 144.2, 182.7, 183.2. HRMS Calcd for $C_{17}H_{17}NO_2$, 267.1259; found: 267.1209.

4.7. 2-(Benzylamino)-3-(2-methyl-propenyl)-[1,4]naphthoquinone (3f)

Using hexane and increasing the polarity with chloroform as eluents, **3f** was obtained as red crystals (mp 94.5–96 °C) in 73% yield. IR (KBr) ν_{max} 3349, 3305, 3083, 3028, 2958, 2931, 2906, 1672, 1624, 1599, 1572, 1511, 1330, 1301, 1278 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.59 (d, 3H, 1.2 Hz), 1.93 (d, 3H, 1.2 Hz), 3.49 (m, 2H, 6.4/7.2 Hz), 4.38 (d, 2H, 6.0 Hz), 6.07 (br s, 1H), 6.20 (m, 1H), 7.27 (m, 5H), 7.58 (dt, 1H, 1.6/7.4/7.4 Hz), 7.68 (dt, 1H, 1.6/7.4/7.4 Hz), 8.01 (dd, 1H, 1.6/7.4 Hz), 8.07 (dd, 1H, 1.6/7.4 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.1, 25.4, 48.3, 114.1, 117.9, 125.9, 126.2, 127.4, 127.6, 128.7, 130.3, 131.9, 133.4, 134.4, 138.3, 138.9, 144.2, 182.7, 184.0. HRMS Calcd for $C_{21}H_{19}NO_2$, 317.1415; found: 317.1436.

4.8. 2-(Butylamino)-3-(2-methyl-propenyl)-[1,4]naphthoquinone (3g)

Using chloroform/hexane 9:1 as eluent, **3g** was obtained as a red oil in 92% yield. IR (KBr) ν_{max} 3343, 2972, 2959, 2935, 2875, 1669, 1626, 1601, 1571, 1508, 1334, 1299 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87 (t, 3H, 7.2 Hz), 1.20 (m, 2H), 1.44 (m, 2H), 1.45 (d, 3H, 1.0 Hz), 1.88 (d, 3H, 1.6 Hz), 3.13 (m, 2H, 5.4/6.6 Hz), 5.89 (m, 1H), 6.07 (dd, 1H, 1.0/1.6 Hz), 7.50 (dt, 1H, 1.6/7.6/7.6 Hz), 7.61 (dt, 1H, 1.6/7.6/7.6 Hz), 7.93 (dd, 1H, 1.6/7.6 Hz), 8.01 (dd, 1H, 1.6/7.6 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.5, 19.8, 19.9, 25.2, 31.9, 43.9, 112.9, 117.8, 125.7, 126.0, 130.2, 131.6, 133.4, 134.3, 138.2, 144.5, 182.7, 183.1. HRMS Calcd for $C_{18}H_{21}NO_2$, 283.1572; found: 283.1574.

4.9. 2-Phenethylamino-3-(2-methyl-propenyl)-[1,4]naphthoquinone (3h)

Using hexane and increasing the polarity with chloroform at 1% stepwise, **3h** can be obtained as a red-brownish oil in 63% yield. IR (KBr): ν_{max} 3461, 3348, 3067, 3027, 2957, 2929, 2868, 1670, 1598, 1569, 1510, 1334, 1298, 1277 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.49 (d, 3H, 1.2 Hz), 1.95 (d, 3H, 1.2 Hz), 2.78 (t, 2H, 7.2 Hz) 3.49 (dd, 2H, 6.4/7.2 Hz), 6.0 (br t, 1H, 6.4 Hz), 6.18 (t, 1H, 1.2 Hz), 7.15 (m, 1H), 7.25 (m, 2H), 7.33 (m, 2H), 7.57 (dt, 1H, 1.6/7.2/7.2 Hz), 7.68 (dt, 1H, 1.6/7.2/7.2 Hz), 7.99 (dd, 1H, 1.6/7.2 Hz), 8.07 (dd, 1H, 1.6/7.2 Hz). ^{13}C NMR ($CDCl_3$, 200 MHz) 20.1, 25.4, 36.2, 45.2, 113.6, 117.7, 125.9, 126.2, 126.6, 128.6, 128.6, 130.3, 131.8, 133.4, 134.4, 138.1, 139.0, 144.2, 182.7, 183.3. HRMS Calcd for $C_{22}H_{21}NO_2$, 331.1572; found: 331.1575.

4.10. Synthesis of 1,2,3,4-tetrahydro-1-aza-anthraquinones 4a–b and 5a–b by the Prins cyclization procedure

Compound **3d** (1 mmol) was dissolved in 5 mL of 88% formic acid in a water-ice bath with stirring, and the resulting solution was stirred for 2 h. After tlc inspection indicated the absence of starting material, the solution was neutralized using aqueous saturated sodium carbonate and extracted with ethyl acetate (3× 20 mL). The combined organic extracts were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The residue was submitted to flash CC using chloroform/ethyl acetate 98:2 and increasing to chloroform 80:20 ethyl acetate.

4.11. (±)-*trans*-3-Hydroxy-4-(1-hydroxy-1-methyl-ethyl)-1,2,3,4-tetrahydro-benzo[*g*]quinoline-5,10-dione (4a)

Compound **4a** was obtained in 28% yield as purple crystals (mp 251–252 °C). IR (KBr): ν_{max} 3432, 3341, 2970, 2919, 2850, 1675, 1602, 1591, 1556, 1527, 1369, 1337, 1257 cm^{-1}. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.06 (s, 3H), 1.23 (s, 3H), 3.35 (ddd, 2H, 5.2/5.4/17.0 Hz), 3.56 (d, 1H, 11.2 Hz), 3.96 (m, 1H, 5.2/5.4/5.8/11.2 Hz), 4.78 (br s, 1H), 5.68 (d, 1H, 5.2 Hz), 7.73 (m, 2H), 7.77 (m, 1H), 7.91 (m, 1H). ^{13}C NMR (DMSO-d_6, 50 MHz) 30.5, 31.3, 42.9, 44.6, 66.7, 74.9, 109.3, 125.3, 125.6, 130.2, 131.7, 133.7, 134.6, 145.7, 179.7, 180.9. HRMS (M^+-CH_3) Calcd for $C_{16}H_{17}NO_4$–CH_3, 272.0917; found: 272.0964.

4.12. (±)-*cis*-3-Hydroxy-4-(1-hydroxy-1-methyl-ethyl)-1,2,3,4-tetrahydro-benzo[*g*]quinoline-5,10-dione (5a)

Compound **5a** was obtained in 10% yield as red crystals (mp 99–101 °C). IR (KBr) ν_{max} 3362, 3324, 2972, 2934, 1681, 1599, 1562, 1480, 1345 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (s, 3H), 1.29 (s, 3H), 3.30 (dd, 1H, 1.1/2.0 Hz), 3.53 (ddd, $1H_{eq}$, 1.2/2.0/16.0 Hz), 3.65 (ddd, $1H_{ax}$, 1.0/1.8/16.0 Hz), 4.38 (m, 1H, 1.0/1.2/1.8/2.0 Hz), 5.28 (br s, 1H), 6.14 (br s, 1H), 7.58 (dt, 1H, 1.0/7.0/7.0 Hz), 7.67 (dt, 1H, 1.0/7.0/7.0 Hz), 8.00 (dd, 1H, 1.0/7.0 Hz), 8.07 (dd, 1H, 1.0/7.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 27.0, 29.7, 44.7, 47.9, 61.6, 72.6, 107.3, 125.9, 126.4, 130.1, 131.9, 133.7, 134.7, 145.1, 180.4, 183.5. HRMS (M^+-CH_3) Calcd for $C_{16}H_{17}NO_4$–CH_3 272.0917; found: 272.0994.

4.13. (±)-*trans*-3-Methoxy-4-(1-hydroxy-1-methyl-ethyl)-1,2,3,4-tetrahydro-benzo[*g*]quinoline-5,10-dione (4b)

Compound **4b** was obtained in 27% yield as purple crystals (mp 175–176 °C). IR (KBr) ν_{max} 3440, 3352, 2985, 2922.99, 2851, 1671, 1558, 1524, 1258 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.27 (s, 6H), 3.47 (s, 3H), 3.48 (d, 1H, 11.0 Hz), 3.75 (dddd, 2H, 1.8/2.4/3.0/17 Hz), 3.97 (ddd, 1H, 1.8/2.4/11.0 Hz), 6.02, (br s, 1H), 7.57 (dt, 1H, 1.0/7.0/7.0 Hz), 7.67 (dt, 1H, 1.0/7.0/7.0 Hz), 7.95 (dd, 1H, 1.0/7.0 Hz), 8.08 (dd, 1H, 1.0/7.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 30.0, 41.1, 42.2, 57.8, 75.1, 76.8, 110.0, 125.8, 126.6, 130.1, 131.8, 133.8, 134.7, 144.7, 180.7, 182.1. HRMS (M^+-CH_3) Calcd for $C_{17}H_{19}NO_4$–CH_3, 286.1073; found: 286.1076.

4.14. (±)-*cis*-3-Methoxy-4-(1-hydroxy-1-methyl-ethyl)-1,2,3,4-tetrahydro-benzo[*g*]quinoline-5,10-dione (5b)

Compound **5b** was obtained in 28% yield as red crystals (mp 142–143 °C). IR (KBr): $\nu_{max.}$ 3441, 3383, 3305, 2975, 2936, 1673, 1600, 1563, 1522, 1353, 1264 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.15 (s, 3H), 1.30 (s, 3H), 3.32 (s, 3H), 3.45 (dd, 1H, 1.0/2.4 Hz), 3.55 (dddd, $1H_{eq}$, 1.0/3.0/4.6/17.0 Hz), 3.65 (dddd, $1H_{ax}$, 1.0/2.2/4.0/17.0 Hz), 3.80 (m, 1H, 1.0/2.2/2.4/3.0 Hz), 6.04 (br s, 1H), 7.56 (dt, 1H, 1.0/7.0/7.0 Hz), 7.68 (dt, 1H, 1.0/7.0/7.0 Hz), 7.99 (dd, 1H, 1.0/7.0 Hz), 8.08 (dd, 1H, 1.0/7.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 29.9, 27.1, 42.2, 43.6, 56.1, 70.9, 72.2, 108.0, 125.7, 126.3, 130.2, 131.7, 133.7, 134.5, 144.9, 180.5, 183.1. HRMS Calcd for $C_{17}H_{19}NO_4$, 301.1314; found: 301.1311.

4.15. Molluscicidal assays

The bioassays were carried out as described by dos Santos et al.,[4b] by dissolving the sample first in dimethylsulfoxide (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 light. 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. 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 analyzed, and the LC_{10}, LC_{50}, and LC_{90} values determined by performing a probit analysis.[4b]

4.15.1. Toxicity against *Artemia salina*. The brine shrimp lethality bioassay was performed following the reported procedure,[12] with some modifications. The growth medium was prepared with a water solution 38 g/L of sea salt 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 mature as nauplii and are ready for the assay. Test compounds were dissolved in three drops of Cremophor®, 2 mL of DMSO, and saline solution to complete 5 mL of total volume. Appropriate volumes were then added to tubes with 5 mL saline solution containing 10 nauplii to afford final drug concentrations of 1000, 100, 10, and 1 μg/mL, 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 LD_{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.

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