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Synthesis and evaluation of the molluscicidal activity of the 5,6-dimethyl-dihydro-pyran-2,4-dione and 6-substituted analogous

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Abstract—Five dihydro-piran-2,4-diones, including 5,6-dimethyl-dihydro-piran-2,4-dione one of the intermediates of the synthesis of caloverticilic acid, were synthesized and submitted to molluscicidal bioassay. The compound's yields varied from moderate to good (42%- 80%) and were achieved through the preparation of the dianion of ethyl acetoacetate, reaction with and aldehyde followed by hydrolysis of the ester (NaOH, H_2O, 2 h, T.A.) and lactonization in acidic medium (HCl, 0 °C). The 5,6-dimethyl-dihydro-piran-2,4-dione and three analogous dihydro-piran-2,4-diones 6-substituted,-phenyl, (4-methoxy-phenyl), and -propenyl, showed significant activities against the *Biomphalaria glabrata* egg masses, while the analogous 6-(3,4-dimethoxy-phenyl) was inactive as molluscicide. This activity is reported for the first time, extending the range of biological activities of this group.

1. Introduction

Schistosomiasis (bilharziasis) is a major public health issue in tropical and subtropical regions of the world.[1] It is caused by the presence of the worm *Schistosoma mansoni* in the liver of the affected person. The fresh water mollusk *Biomphalaria glabrata* acts as an intermediate host. The incidence of the disease is still increasing in underdeveloped and developing countries due to poverty and lack of basic sanitation. It is estimated that 200 million people in 73 countries[2] or approximately 4–5% of the world's population[3] is infected with the *Schistosoma* worm.

The successful control of the disease should be based on an integrated approach including chemotherapy, control of the snail host using molluscicides, and ecological and biological control methods[4] to stop the transmission cycle. Mollusciciding is still considered the most important mean of control because the use of molluscicides in the prophylactic treatment promotes the rupture of the evolutionary cycle of the worm causing the destruction of its intermediate host.[5]

Many metabolites of different species of *Calophyllum* (Guttiferae) were already isolated. Some examples are the inophylloidic acid (**1**), isolated of *Calophyllum inophyllum* and caloverticilic acid (**2**), isolated of *Calophyllum verticillatum*, which showed intense molluscicidal activities concerning *Biomphalaria glabrata* (Fig. 1).[6] These compounds have as structural feature core the 5-hydroxy-2,3-dimethyl-2,3-dihydro-6H-chromene-4,7-dione (**3**), present in many natural biologically active products with important pharmacological activities.[7]

We have decided to investigate the use of (**2**) as a potential drug candidate against schistosomiasis by synthesing and studying the relationship between structure–activity of the fragments and whole molecule. In order to prepare (**2**), careful retrosynthetic analysis revealed the key steps to prepare (**2**), consisting of the condensation of the fragments β-ketoester (**3**) and dihydro-pyran-2,4-dione (**5**) and of the preparation of the R_1 lateral chain, since the other two branches, R_2 e R_3, are commercially available (Scheme 1).

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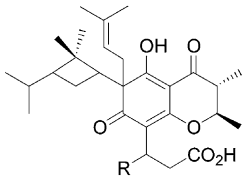
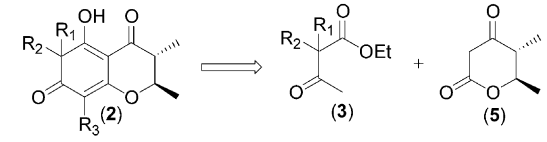
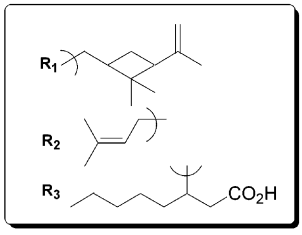


R = n-C_3H_7 Inophylloidic acid (**1**)
R = n-C_5H_{11} Caloverticilic acid (**2**)

Figure 1. Structure of compounds **1** and **2**.





Scheme 1. Retrosynthesis of caloverticilic acid (**2**).

As part of the study of the structure *vs*. the molluscicidal activity of caloverticilic acid (**2**), five 6-substituteds 5,6-dihydro-pyran-2,4-diones have been prepared and tested for their toxicity to *B. glabrata* (Fig. 2).

2. Results and discussion

2.1. Synthesis of compounds

According to Peterson et al.,[8] pyran-2,4-diones are easily accessed by aldol condensation of the methyl acetoacetate dianion with aldehydes or ketones affording good yields of the δ-hydroxy-β-ketoester. The aldolic adduct readily lactonized upon treatment with sodium hydroxide solution followed by acidification with hydrochloric acid solution (Scheme 2). This pathway seemed to us simple and very reliable.

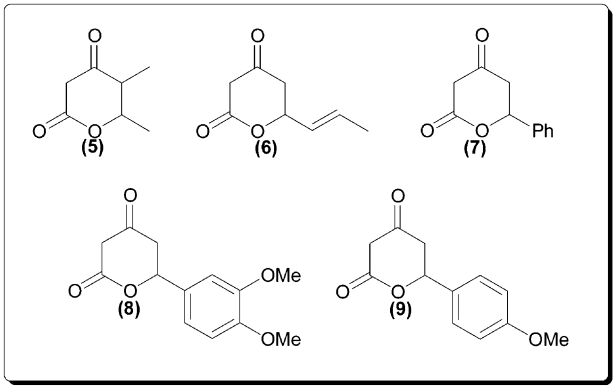
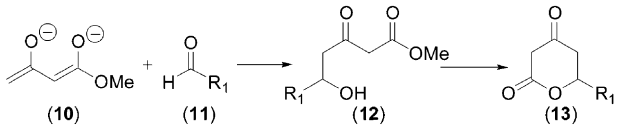


Figure 2. Structure of 6-substituted dihydro-pyran-2,4-diones **5**–**9**.



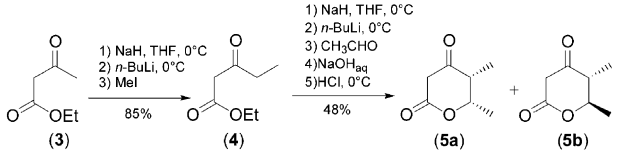
Scheme 2. Synthesis of dihydro-pyran-2,4-dione (**13**) from methyl acetoacetate dianion's (**10**).

Initially we prepared 5,6-dimethyl-dihydro-piran-2,4-dione (**5**), a synthetic precursor of (**2**), in 41% overall yield from the ethyl acetoacetate, by preparation of the ethyl acetoacetate dianion (NaH, *n*-BuLi, THF, −10 °C), alkylation with methyl iodide, another preparation of the dianion and reaction with acetaldehyde, followed by ester hydrolysis in basic medium (NaOH, 0.5 mol/L) and lactonization under acidic conditions (HCl 36%) (Scheme 3).

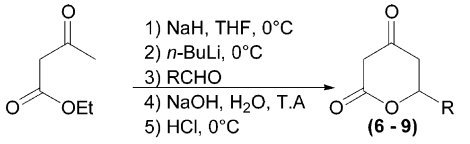
The 5,6-dimethyl-dihydro-piran-2,4-dione (**5**) was obtained as a mixture 1:1 of *cis* and *trans* isomers which was separated by careful column chromatography on silica gel. Based on the spin–spin coupling constant between the protons in C5 and C6, the isomer with smaller coupling constant ($J = 3.8$ Hz) was assigned the 5,6-*cis* configuration. This isomer was obtained from the more polar fraction. The isomer with larger coupling constant ($J = 10.5$ Hz), less polar fraction, was assigned to the 5,6-*trans* configuration.

This methodology was applied to the synthesis of analogous compounds to the 5,6-dimethyl-dihydro-pyran-2,4-dione (**5**) (Scheme 4).

In the proton nuclear magnetic resonance (^{1}H NMR) spectra in d_6-DMSO of the compounds (**6**–**9**), the signal of the C_3-olefinic proton, observed as a singlet at δ 4.96–5.12 suggests (see Experimental) that each compound exists mainly in the enol form. The ^{1}H NMR spectra in $CDCl_3$ shows that the 5,6-dimethyl-dihydro-pyran-2,4-dione (**5**) exists mainly in the keto form.



Scheme 3. Synthesis of dihydro-pyran-2,4-dione (**5**).



(6) R = $CH{=}CHCH_3$, 80%
(7) R = Ph, 61%
(8) R = 3,4-$(OMe)_2C_6H_3$, 69%
(9) R = p-(OMe)-C_6H_4, 42%

Scheme 4. Synthesis of compounds **6**–**9**.

2.2. Molluscicidal assays

The compounds (**5**–**9**) were evaluated for molluscicidal activities against *B. glabrata* egg masses, a vector of *S. mansoni*. The results of the molluscicidal assays are summarized in Tables 1 and 2.

In the biological assays, the activity on *B. glabrata* egg masses decreased in the order of (**7**) > (**6**) > (**9**) > > (**5**) with LC_{90} < 100 ppm, specifically 40.262 ppm and 39.399 ppm for (**6**) and (**7**), respectively (Table 2). These results suggest that the substitution of the methyl group in C-6 by propenyl or phenyl group increases its molluscicidal activity. Nevertheless the substitution of the propenyl group by phenyl group in the C-6 gives a relatively small change in activity.

It also was possible note that the introduction of groups methoxy in the phenyl group induces the decrease or even lose its of the molluscicidal activity as occurred in the compounds (**9**) and (**8**), respectively. Despite having a molluscicidal effect on the egg masses in the preliminary bioassay (Table 1), the (**9**) compound showed to be totally inactive in the accurate test (Table 2).

3. Conclusions

In this developmental study on a potential new class of molluscicides, we prepared a series of dihydro-piran-2,4-diones with different groups at the 6-position. The reported synthesis was carried out with success starting from ethyl acetoacetate dianion's. The compounds was synthesized with satisfactory yields and showed significant activities against the *B. glabrata* egg masses. We are continuing to search for more potent analogues and to elucidate their mechanism of action.

Table 1. Preliminary evaluation of the molluscicidal activity on *B. glabrata* Say

	Activity on *B. glabrata* egg masses—Afer 96 h		
Compd	Concentration ppm	Used embryos number	Death%
(**5**)	100	27	100
	10	46	0
	1	52	0
(**6**)	100	29	100
	10	49	2
	1	33	0
(**7**)	100	64	100
	10	44	0
	1	63	0
(**8**)	100	46	100
	10	42	0
	1	29	0
(**9**)	100	59	100
	10	71	0
	1	70	0

Table 2. Accurate evaluation of the molluscicidal activity on *B. glabrata* Say

Compd	Activity on *B. glabrata* egg masses, After 96 h		
	LC_{10} ppm	LC_{50} ppm	LC_{90} ppm
(**5**)	40.417	62.599	96.954
(**6**)	13.175	23.031	40.262
(**7**)	15.486	24.700	39.399
(**8**)	—	—	—
(**9**)	36.918	45.030	54.924

4. Experimental

Infrared spectra were recorded on a Perkin–Elmer 1600 Serie FTIR spectrophotometer using either a thin film or a KBr pellet technique. NMR spectra (^{1}H: 60, 300, 400 and 500 MHz; ^{13}C: 75, 100 and 125 MHz) were recorded on a Varian EM-360A, a Varian Gemini 300, a Bruker DRX-400 and a DRX-500 spectrometer respectively, using tetramethylsilane or solvent-d as internal standard. Infrared and NMR spectra of dihydro-pyran-2,4-diones **6**–**9** are referred to the enol form, with the exception of the 5,6-dimethyl-dihyidro-pyran-2,4-dione (**5**), whose spectra referred to the keto form.

Chemicals were purified previously. Tetrahydrofuran (THF) was distilled under nitrogen from sodium and benzophenone. The dianion condensations were conducted under a dry nitrogen atmosphere utilizing reaction septa and syringe techniques.

For column chromatography, silica gel (Merck, Kieselgel 90, 70–230 mesh) was used. TLC was performed on silica gel plates (Aldrich, Kieselgel 60 F_{254}, 2mm).

4.1. Procedure for preparation of ethyl 3-*oxo*-pentanoate (4)

To a cooled (0 °C) and stirred slurry of sodium hydride suspension (80% in paraffin oil −0.706 g–23.56 mmol) in THF (50 mL), under nitrogen, ethyl acetoacetate (2.00 mL- 15.70 mmol) was added dropwise. The mixture was stirred for 15 min, and then *n*-BuLi (2.0 mol/L in hexane, 11.78 mL, 23.56 mmol) was added dropwise. The solution was stirred for another 15 min, and then methyl iodide (2.41 mL, 25.92 mmol) was added dropwise. After 30 min at 25 °C, the reaction mixture was quenched with water and extracted with AcOEt (3×50 mL). The combined extracts were washed with water (2×100 mL) and brine (2×50 mL), dried under Na_2SO_4, and concentrated under reduced pressure. The crude product was distilled under reduced pressure (50 °C, 1 mmHg) to give a colourless oil, ethyl 3-*oxo*-pentanoate (1.924 g, 13.35 mmol, 85%); IR (film): $\nu_{máx}$ 2981, 2931, 1743, 1717, 1628, 1459, 1413, 1368, 1311, 1249, 1160, 1107, 1062 and 1028 cm^{-1}; ^{1}H NMR (60MHz, CCl_4): δ 0.8, 1.5 (m, 6H), 2.5 (q, 2H, J = 8 Hz), 3.4 (s, 1H), 4.2 (q, 2H, J = 6 Hz), 4.9 (s).

4.2. General procedure for preparation of dihydro-pyran-2,4-diones (5–9)

To a cooled (0 °C) and stirred slurry of sodium hydride suspension (80% in paraffin oil, 0.353 g, 11.78 mmol) in THF (25 mL) under nitrogen, β-keto ester (1.00 mL), ethyl 3-*oxo*-pentanoate (7.27 mmol), to synthesis of **5**,

or ethyl acetoacetate (7.85 mmol), to synthesis of compounds **6–9,** was added dropwise. The mixture was stirred for 15 min, and then *n*-BuLi (1.2 mol/L in hexane, 10 mL, 11.78 mmol) was added dropwise. The solution was stirred for another 15 min, and then an aldehyde (8.64 mmol) was added dropwise. After 30 min, the reaction mixture was poured into NaOH (0.5 mol/L, 500 mL). After 2 h at 25 °C, the reaction mixture was partitioned between Et_2O and water, and the aqueous phase was extracted with two additional portions of Et_2O. The aqueous phase was cooled with ice and acidified until pH 6 with concentrated HCl. The resulting precipitate was extracted with AcOEt (3×50 mL). The combined extracts were washed with water (2×100 mL) and brine (2×50 mL), dried under Na_2SO_4, and concentrated under reduced pressure. Recrystalization of these compounds using $CHCl_3$ provided the dihydropyran-2,4-diones.

4.3. Structural data for dihydro-pyran-2,4-diones (5–9)

4.3.1. 5,6-dimethyl-dihydro-pyran-2,4-dione (5). Compound **5** (0.496 g, 3.49 mmol, 48%) was obtained from ethyl 3-oxo-pentanoate (1.00 mL, 7.27 mmol) and acetaldehyde (0.43 mL, 8.64 mmol); mp: 112–114 °C (*cis*), 85–87 °C (*trans*) (from chloroform); IR: (KBr): *cis*- $\nu_{máx}$ 2980, 2668, 1649, 1572, 1383, 1284, 1227, 1087, 819 e 607 cm^{-1}; *trans*- $\nu_{máx}$ 2997, 2984, 2942, 2886, 2688, 2587, 2550, 1626, 1598, 1501, 1452, 1384, 1326, 1298, 1233, 1049, 1005 and 826 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): *cis*- δ 1.15 (d, 3H, $J=7.3$ Hz), 1.39 (d, 3H, $J=6.7$ Hz), 2.68, 2.74 (m, 1H), 3.40 (d, 1H, $J=19.9$ Hz), 3.54 (d, 1H, $J=19.9$ Hz) and 4.80–4.86 (m, 1H); *trans*- δ 1.19 (d, 3H, $J=7.3$ Hz), 1.53(d, 3H, $J=6.7$ Hz), 2.30–2.38 (m, 1H), 3.44 (d, 1H, $J=19.1$ Hz), 3.56 (d, 1H, $J=19.1$ Hz) and 4.41–4.48 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): *cis*- δ 9.1; 16.6; 45.5; 46.6; 74.9; 167.3; 203.0; *trans*- δ 10.6; 19.0; 45.8; 48.5; 76.9; 167.0; 202.4.

4.3.2. 6-propenyl-dihydro-pyran-2,4-dione (6). Compound **6** (0.968 g, 6.28 mmol, 80%) was obtained from ethyl acetoacetate (1.00 mL, 7.85 mmol) and crotonaldehyde (0.72 mL, 8.64 mmol); mp: 122–124 °C (from chloroform); IR (KBr) $\nu_{máx}$ 3080, 2963, 2640, 1886, 1841, 1690, 1587, 1449, 1418, 1370, 1357, 1276, 1256, 1186, 1100, 1057, 1012, 965, 937, 894, 875, 745 and 567 cm^{-1}; ^1H NMR (500 MHz, DMSO d_6) δ 1.70 (d, 3H, $J=6,5$ Hz), 2.38–2.24 (m, 2H), 2.48–2.54 (m, 2H), 4.96 (s, 1H), 5.60 (dd, 1H, $J=6.5$ and 15.4 Hz), 5.77–5.84 (m, 1H); ^{13}C NMR (125 MHz, DMSO d_6) δ 17.4; 32.7; 75.09; 90.8; 128.9; 129.0; 166.7; 172.3.

4.3.3. 6-phenyl-dihydro-pyran-2,4-dione (7). Compound **7** (0.910 g–4.78 mmol, 61%) was obtained from ethyl acetoacetate (1.00 mL, 7.85 mmol) and benzaldehyde (1.32 mL, 8.64 mmol); mp: 129–131 °C (from chloroform) (lit. Mp 132–134 °C); IR (KBr) $\nu_{máx}$ 3036, 2898, 2554, 1719, 1592, 1498, 1460, 1399, 1372, 1289, 1207, 1186, 1162, 1059, 1012, 817, 756 and 696 cm^{-1}; ^1H NMR (500 MHz, DMSO d_6) δ 2.59 (dd, 1H, $J=3.5$ and 17.0 Hz), 2.85 (dd, 1H, $J=11.9$ and 17.0 Hz), 5.07 (s, 1H), 5.45 (dd, 1H, $J=3.5$ and 11.9 Hz), 7.35–7.48 (m, 5H); ^{13}C NMR (125 MHz, DMSO d_6) δ 34.1; 76.1; 90.8; 126.3; 128.2; 128.4; 139.1; 166.7; 172.7. ^1H and ^{13}C NMR relative to enol form of (**7**).

4.3.4. 6-(4-methoxy-phenyl)-dihydro-pyran-2,4-dione (8). Compound **8** (0.731g, 3.32 mmol, 42%) was obtained from ethyl acetoacetate (1.00 mL, 7.85 mmol) and *p*-anisaldehyde (1.05 mL, 8.64 mmol); mp: 181–183 °C (from chloroform); IR(KBr) $\nu_{máx}$ 3059; 2949; 2839; 2545; 1881; 1712.5; 1608; 1427; 1390; 1345; 1172; 1012; 945; 911; 842; 771 and 662 cm^{-1}; ^1H NMR (300 MHz, DMSO d_6) δ 2.56 (dd, 1H, $J=3.0$ and 17.0 Hz); 2.89 (dd, 1H, $J=12.0$ and 17.0 Hz); 3.80 (s, 3H); 3.86 (s, 1H); 5.12 (s, 1H); 5.42 (dd, 1H, $J=3.0$ and 12.0); 6.98–7.07 (m, 2H); 7.42–7.45 (m, 2H); ^{13}C NMR (75 MHz, DMSO d_6) δ 55.2; 76.1; 90.9; 113.8; 127.9; 131.0; 131.4; 159.3; 166.9; 172.8.

4.3.5. 6-(3,4-dimethoxy-phenyl)-dihydro-pyran-2,4-dione (9). Compound **9** (1.355 g, 5.41 mmol, 69%) was obtained from ethyl acetoacetate (1.00 mL, 7.85 mmol) and 3,4-dimethoxy-benzaldehyde (1.434g, 8.64 mmol); mp: 150–153 °C (from chloroform); IR (KBr) $\nu_{máx}$ 3080, 3006, 2944, 2842, 1852, 1727, 1594, 1518, 1443, 1383, 1340, 1270, 1154, 1054, 1030, 1004, 925, 893, 846, 773, 754, 660 and 639 cm^{-1}; ^1H NMR (500 MHz, DMSO d_6) δ? 2.88–2.93 (m, 1H), 3.76 (s, 3H); 3.78 (s, 3H), 5.35–5.37 (m, 1H), 6.96 (d, 1H, $J=8.3$ Hz), 6.98 (dd, 1H, $J=1.5$ Hz and 8.3 Hz), 7.07 (d, 1H, $J=1.5$ Hz); ^{13}C NMR (125 MHz, DMSO d_6) δ 55.5; 76.3; 90.8; 110.4; 111.5; 118.9; 131.4; 148.8; 166.9; 172.9.

4.4. Founder Snail

Snails (shell diameter 19–25 mm) were isolated from a laboratory stock, originally derived from a, not infected by trematodes colony originated from Barreiro de Cima, a district of Belo Horizonte, Minas Gerais and reared in the laboratory for at least six years previously. The snails were maintained at 25–27 °C in a glass of aquarium (22×30×44 cm) containing dechlorinated current water system and were fed *ad libitum* with fresh lettuce and the medium was replenished every day. These snails provided egg capsules for subsequent experiments.

4.5. Molluscicidal activity for bioassay

B. glabrata egg masses were obtained from a colourless polyethylene sheet previously placed in the aquarium. In the test for evaluation of the ovicidal activity was used one egg mass aged 0–2 day-old for concentration. Egg masses were exposed to potential molluscicide for 24 h at room temperature and were kept under normal diurnal lighting. After 24 h, the suspension was decanted, the egg mass rinsed twice with aerated tap water. The test egg masses were then left in water for another 72 h (time of recovery). Of each 24 h the target organism were examined to assess mortality. The mass of each individual embryo was checked with a binocular microscopic. Egg mass were considered dead when their cells became opaque, dull or disaggregated. Three control sets were used in order to verify the snail susceptibility,

one with niclosamide at 3 ppm or cupric carbonate at 50 ppm and two white, consisting of dechlorinated water alone and dechlorinated water with 0.1% DMSO.[9,10]

For the preliminary bioassay with eggs mass adult, the stock solutions were prepared at 100 ppm, adding 1.2 mg of samples to 12 mL of dechlorinated water at 0.1% DMSO (v/v). Each test concentration was set in duplicate. The samples that cause the mortality of at least 40% at 100 ppm were submitted to the accurate bioassay with LC_{10}, LC_{50} and LC_{90} calculations using the probit analysis. In this test the evaluated concentrations were chosen according with the results obtained in the preliminary biological test. Each test concentration was set in quadruplicate.

4.6. Statistical methods

The LC_{10}, LC_{50} and LC_{90} values, as well as their 95% confidence intervals, were determined through probit analysis method[11] of the mortality data from the susceptibility assay. Because deaths occur in the control, percent death values were corrected using the Abbott's formula.[12]

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