Original article

Synthesis and cytotoxic activity of α-santonin derivatives

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ABSTRACT

Ten α-santonin derivatives were synthesized in moderate to high yields. Four derivatives namely 10α-acetoxy-3-oxo-1,7αH,6,11βH-guai-4-en-6,12-olide (**2**), isofotosantonic acid (**3**), 10α-hydroxy-3-oxo-1,7αH,6,11βH-guai-4-en-6,12-olide (**4**), and lumisantonin (**5**), were prepared by different photochemical reactions using α-santonin (**1**) as starting material. These transformations were carried out in either anhydrous acetic acid, acetic acid/water (1:1 v/v) or acetonitrile, using different types of reactors and ultraviolet light sources. Treatment of α-santonin (**1**) with lithium diisopropyl amide (LDA) followed by capture of the organolithium with phenyl selenium chloride produced the compound 3-oxo-7αH,6βH,11-(phenylselenyl)-eudesma-1,4-dien-6,12-olide (**6**). Subsequent treatment of compound **6** with hydrogen peroxide gave 3-oxo-7αH,6βH-eudesma-1,4,11-trien-6,12-olide (**7**). Photochemical reaction of compound **7** led to the formation of 11,13-dehydrolumisantonin (**8**) and 10α-acetoxy-3-oxo-1,7αH,6βH-guai-4,11-dien-6,12-olide (**9**). Sodium borohydride reduction of compounds **2** and **4** afforded the derivatives 10α-acetoxy-3β-hydroxy-1,7αH,6,11βH-guai-4-en-6,12-olide (**10**) and 3β,10α-hydroxy-1,7αH,6,11βH-guai-4-en-6,12-olide (**11**).

The cytotoxicity of the synthesized compounds were evaluated against the cancer cell lines HL-60 (leukemia), SF-295 (central nervous system), HCT-8 (colon), MDA-MB-435 (melanoma), UACC-257 (melanoma), A549 (lung), OVACAR-8 (ovarian), A704 (renal), and PC3 (prostate). The compounds with higher activity, possessing IC_{50} values in the range of 0.36–14.5 μM, showed as common structural feature the presence of an α-methylidene-γ-butyrolactone moiety in their structures. The biological assays conducted with normal cells (PBMC) revealed that the compounds are selective against cancer cell lines. The modified lactones seem to be interesting lead structures towards anticancer drug development.

1. Introduction

According to the World Health Organization (WHO), cancer is an important health problem that claims the lives of more than seven million people worldwide on an annual basis [1]. As a consequence, search for new anticancer drugs is highly demanding nowadays. However, cytotoxic agents have very little or no specificity, which leads to systemic toxicity, causing undesirable side effects. Therefore, the development of innovative and efficacious tumor-specific

effect on many thiol-containing enzymes, involved in the synthesis and processing of proteins, RNA and DNA [16–18]. In addition, sesquiterpene lactones exert their cytotoxic effects by triggering apoptosis in many types of cell lines [19,20].

It was recently found that a sesquiterpene lactone, parthenolide, can selectively kill primitive leukemia cells without affecting normal stem and progenitor hematopoietic cells [21]. These data indicate that SQLs and related compounds may represent a promising class of antileukemic agents.

In our continuous effort to discover novel cytotoxic compounds [22–26], we describe herein the preparation of several derivatives from α-santonin (**1**), a sesquiterpene lactone isolated from *Artemisia santonica* [27]. The evaluation of the cytotoxic effects of these lactones against tumor and normal cells is also described.

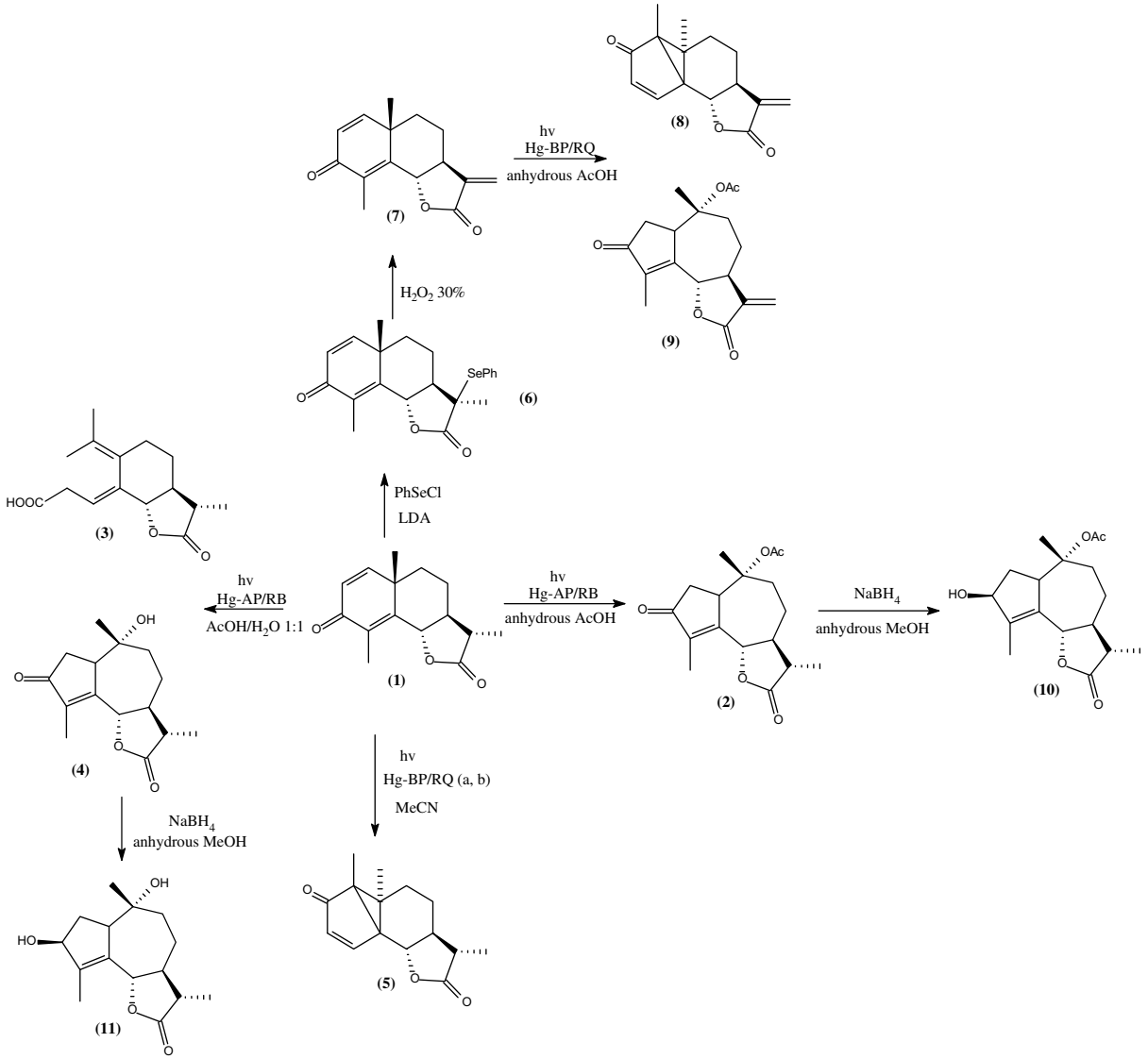
2. Results and discussion

2.1. Synthesis

The sesquiterpene lactones **2**–**11** were synthesized by a series of reactions as depicted in Scheme 1. The compounds were fully characterized by IR, ^{1}H and ^{13}C NMR spectrometry as well as mass spectrometry.

To prepare compound **2**, we utilized a reaction previously described in the literature [28–32]. Thus, irradiation of α-santonin (**1**) with high pressure mercury lamp, using anhydrous acetic acid as solvent in borosilicate reactor, afforded 10α-acetoxy-3-oxo-1,7αH,6,11βH-guai-4-en-6,12-olide (**2**) in 26% yield. The infrared spectrum of compound **2** showed a strong absorption at 1729 cm^{-1} due to C=O stretching of the acetyl group. The signals at δ_C 170.35 and δ_H 2.00 in the ^{13}C and ^{1}H NMR spectra respectively, confirmed the presence of the acetyl group.

Using acetic acid/water mixture (1:1 v/v), keeping the other conditions unchanged, isofotosantonic acid (**3**) and the 10α-hydroxy-3-oxo-1,7αH,6,11βH-guai-4-en-6,12-olide (**4**) were obtained in 44% and 32% yields, respectively. It should be pointed out that Barton and co-workers [28] carried out a similar reaction irradiating compound **1** in a mixture of acetic/water (9:11 v/v), from −5 °C to +5 °C, for 90 min. In this case, compounds **3** and **4** were obtained in 16% and 18% yields, respectively. In addition, Greene and Edgar [33] reported the isolation of compound **4** exclusively, in 31% yield, running the reaction in a mixture of



Scheme 1. Synthesis of lactone derivatives of α-santonin (**1**).

acetic acid/water (7:8 v/v) in a quartz reactor under refluxing conditions for 6.5 h.

The photochemical reaction of α-santonin (**1**) in a quartz reactor, using low pressure mercury lamp as source of ultraviolet radiation and acetonitrile as solvent, gave lumisantonin (**5**) in 83% yield.

Compounds **2** and **4** were further submitted to reduction reactions with sodium borohydride, affording 10α-acetoxy-3β-hydroxy-1,7αH,6,11βH-guai-4-en-6,12-olide (**10**) and the 3β,10α-hydroxy-1,7αH,6,11βH-guai-4-en-6,12-olide (**11**) in 86% and 72% yields, respectively. The stereochemistry of the resulting alcohols was proposed on the assumption of a preferred attack of the hydride from the less hindered side of the carbonyl group [31]. The infrared spectra of compounds **10** and **11** showed a broad band at 3400 cm^{-1} associated with the O–H stretching. The multiplets observed around δ_H 4.50 in the ^1H NMR spectrum and the signals around δ_C 78.00 in the ^{13}C NMR spectrum, along with other signals, helped to confirm the identity of the synthesized alcohols **10** and **11**.

The 3-oxo-7αH,6βH-eudesma-1,4,11-trien-6,12-olide (**7**) was obtained in 72% yield after reaction of α-santonin (**1**), with phenyl selenium chloride (PhSeCl), in the presence of lithium diisopropyl amide (LDA), followed by treatment of the selenyde **6** with hydrogen peroxide (H_2O_2). In the ^1H NMR spectrum of compound **7** the presence of a pair of doublets at δ_H 5.56 and δ_H 6.24, corresponding to hydrogens of the exocyclic double bond, assisted in confirming the formation of this substance. Subsequently, lactone **7** was submitted to a photochemical reaction in a quartz reactor, using anhydrous acetic acid as solvent, yielding compounds 11,13-dehydrolumisantonin (**8**) and 10α-acetoxy-3-oxo-1,7αH,6βH-guai-4,11-dien-6,12-olide (**9**) in 18% and 4.5% yields, respectively. The rational for the preparation of these derivatives **7**–**9** was the fact that many sesquiterpene with α-methylidene-γ-butyrolactone moiety in their structure have displayed cytotoxicity against several tumor cell lines [10–14].

2.2. Cytotoxicity assay

The cytotoxicity of the sesquiterpene lactones **2**–**11** was evaluated against HL-60 (leukemia), SF-295 (central nervous system), HCT-8 (colon), MDA-MB-435 (melanoma), UACC-257 (melanoma), A549 (lung), OVACAR-8 (ovarian), A704 (renal), and PC3 (prostate) human cancer cell lines, using a previously described MTT assay [20]. Doxorubicin was used as positive control. Table 1 summarizes the IC_{50} data (μM) for cytotoxic activity. The results indicated that compounds **7**–**9** exhibited relatively high cytotoxicity against all tumor cell lines tested, with IC_{50} values in the range of 0.36–14.5 μM. Cytotoxic effectiveness of these compounds was comparable to the well-known sesquiterpene lactones, parthenolide and helenalin [16,19]. Compound **5** exhibited low cytotoxic activity with IC_{50} values in the range of 49.84–91.46 μM. The other compounds investigated were not able to significantly inhibit cell growth under assay conditions ($IC_{50} > 100$ μM).

The cytotoxicity of compounds **7**–**9** was also evaluated against normal cells (PBMC). The results presented in Table 1 show that the effects of these lactones are less pronounced in normal cells.

Although the precise mechanism of action of sesquiterpene lactones as inhibitors of cell growth is still unclear, several experimental data indicate that cytotoxic activity of the more active compounds herein investigated can be related to the presence of the α-methylidene-γ-butyrolactone moiety in their structure. It is possible that the α-santonin derivatives can react via Michael addition reaction with bionucleophiles, especially thiol groups of cysteine [14].

3. Conclusion

The readily available α-santonin (**1**) was used as starting material for the preparation of ten sesquiterpene lactone derivatives which were evaluated against cancer cells. Compounds **7**–**9**, possessing the α-methylidene-γ-butyrolactone group in their structure, displayed significant cytotoxic activities. Thus, the results obtained reinforce the fact that the presence of such structural motif has a significant role in the mechanism by which these compounds exert their biological activities.

The biological assays revealed that compounds **7**–**9** are selective against normal cell lines (PBMC), an important feature towards the development of new drugs against cancer. These SQLs may represent a promising class of anticancer agents.

4. Experimental part

4.1. Synthesis

All reactions were carried out under a protective atmosphere of dry nitrogen. Methanol, acetic acid, diisopropylamine, tetrahydrofuran and acetonitrile were purified as described by Perrin and Armarego [34]. Commercial α-santonin (**1**) was purchased from Aldrich (Milwaukee, WI, USA) and utilized without further purification. Infrared spectra were recorded on a Perkin Elmer Paragon 1000 FTIR spectrophotometer, using potassium bromide (1% w/w) disk scanning from 500 to 4000 cm^{-1}. Flash column chromatography was performed using Crosfield Sorbil C60 silica gel (32–63 μm). Analytical thin layer chromatography analyses were conducted on precoated silica gel plates. Melting points were determined on an electrothermal digital apparatus model MQAPF-301 (Microquimica, Brazil), without correction. The ^1H and ^{13}C NMR spectra were recorded on Brucker AVANCE DRX 400 spectrometer at 400 and 100 MHz respectively using $CDCl_3$ as solvent and TMS as internal standard. Low resolution mass spectra were obtained on SHIMADZU GCMS-QP5050A instrument by direct injection using the following temperature program: 40 °C/min until temperature reaches 60 °C; then 80 °C/min until temperature reaches 300 °C; detector temp: 280 °C. Values are reported as a ratio of mass to charge (m/z) in Daltons and relative intensities are quoted as a percentage value. HRMS data were recorded under conditions of chemical ionization (CI) on a Fisons Autospec-oaTof (resolution = 10,000 FWHM) in CI^+ mode using NH_3 as the ionization gas.

4.1.1. 10α-Acetoxy-3-oxo-1,7αH,6,11βH-guai-4-en-6,12-olide (**2**)

A solution of α-santonin (0.50 g, 2.0 mmol) in anhydrous acetic acid (120 mL) was degassed with nitrogen for 30 min. The solution was then irradiated under high pressure mercury lamp (125 W) for 23 h in a pyrex vessel. The solvent was evaporated under reduced pressure to give a yellow oil which was dissolved in hot methanol and left in a freezer at −5 °C for 2 h. The white crystals formed were filtered under reduced pressure and washed with cold methanol to yield 24.3 mg (0.08 mmol) of lactone (**2**). The filtrate was purified by flash column chromatography eluted with hexane/ethyl acetate (3:2 v/v) yielding a further 139.7 mg (0.46 mmol) of compound **2**. The yield of this reaction was 26% (164 mg; 0.54 mmol). mp = 176.6–177.8 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 2976, 2934, 2876, 1783, 1729, 1707, 1645, 1459, 1371, 1246, 1178, 1045, 982, 969; ^1H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 3H, H14), 1.29 (d, 3H, $J_{13,11} = 6.7$, H13), 1.44–1.51 (m, 1H, H8′), 1.91 (s, 3H, H15), 2.00 (s, 3H, CH_3CO), 2.06–2.11 (m, 1H, H8), 2.15–2.25 (m, 2H, H7 and H9′), 2.33 (dq, 1H, $J_{11,7} = 12.2$, $J_{11,13} = 6.7$, H11), 2.41 (dd, 1H, $J_{2,2'} = 19.4$, $J_{2,1} = 2.1$, H2), 2.50 (dd, 1H, $J_{2',2} = 19.4$, $J_{2',1} = 6.2$, H2′), 2.62 (td, 1H, $J_{9,9'} = 13.6$, $J_{9,8} = J_{9,8'} = 4.1$, H9), 4.16 (m, 1H, H1), 4.80 (d, 1H, $J_{6,7} = 10.6$, H6); ^{13}C

Table 1
Cytotoxic activity (IC_{50} [μM]) of lactones derivatives of α-santonin.

Cell lines	Lactones											Doxorubicin
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	
Tumor cells												
HL-60	>100	>100	>100	>100	80.16 65.68–91.31	>100	1.14 0.23–2.77	2.30 1.87–2.84	1.60 1.09–2.35	>100	>100	0.04 0.03–0.05
SF-295	>100	>100	>100	>100	68.78 53.37–78.66	>100	2.70 0.67–1.90	2.15 1.90–2.40	3.06 2.52–3.70	>100	>100	0.48 0.34–0.72
HCT-8	>100	>100	>100	>100	59.19 46.54–65.25	>100	2.92 0.98–4.86	1.96 1.64–2.29	0.36 0.16–0.79	>100	>100	0.02 0.02–0.03
MDA-MB-435	>100	>100	>100	>100	91.46 82.23–99.6	>100	6.74 5.56–7.21	4.57 3.26–5.32	8.84 5.62–10.23	>100	>100	0.96 0.68–1.32
UACC-257	>100	>100	>100	>100	>100	>100	6.05 5.26–6.56	4.87 4.32–5.12	7.76 5.56–8.23	>100	>100	0.60 0.51–0.70
A549	>100	>100	>100	>100	69.35 55.26–74.23	>100	13.43 12.56–13.98	14.50 14.01–14.87	10.23 9.53–11.56	>100	>100	0.72 0.54–1.54
OVACAR8	>100	>100	>100	>100	49.84 45.55–56.28	>100	6.94 6.53–7.26	3.88 3.12–4.21	8.67 8.01–8.98	>100	>100	0.51 0.26–0.96
A704	>100	>100	>100	>100	85.43 69.23–95.23	>100	10.14 9.23–11.23	3.61 3.01–3.89	9.24 8.56–10.23	>100	>100	2.00 1.46–2.72
PC3	>100	>100	>100	>100	66.45 59.29–75.37	>100	13.04 12.56–13.78	5.07 4.85–5.79	14.41 14.02–14.97	>100	>100	1.32 0.62–1.56
Normal cells												
PBMC	Nd	Nd	Nd	Nd	Nd	Nd	3.23 1.64–5.33	13.36 5.74–29.10	13.45 5.92–29.60	Nd	Nd	1.77 0.92–3.13

Doxorubicin was used as positive control. Data are presented as IC_{50} values and 95% confidence interval from two independent experiments, performed two times. Nd: not determined.

NMR (100 MHz, $CDCl_3$): δ 9.49 (C15), 12.46 (C13), 20.04 (C14), 22.29 (CH_3CO), 25.37 (C8), 36.83 (C2), 37.95 (C9), 41.38 (C11), 47.27 (C1), 48.28 (C7), 81.25 (C6), 85.55 (C10), 143.30 (C4), 160.82 (C5), 170.35 (CH_3CO), 177.03 (C12), 207.00 (C3); MS, *m*/*z* (%): 306 [$M^{+\bullet}$], (0.4), 246 (100), 231 (51), 203 (15), 190 (25), 173 (64), 145 (34), 121 (18), 105 (33), 91 (61), 77 (47), 55 (95), 53 (40). HRMS (CI) $C_{17}H_{23}O_5$ (MH^+) requires 307.1540, found 307.1542.

4.1.2. Isofotosantonic acid (**3**) and the 10α-hydroxy-3-oxo-1,7αH,6,11βH-guai-4-en-6,12-olide (**4**)

A solution of α-santonin (2.0 g, 8.13 mmol) in a mixture of water (80 mL) and acetic acid (80 mL) in a pyrex vessel was degassed by a flow of nitrogen for 30 min. The reaction mixture was then irradiated with a high pressure mercury lamp (125 W) for 27 h. The solvent was evaporated under reduced pressure (60 °C) to give a yellow oil which was purified by flash column chromatography (hexane/ethyl acetate 1:2 v/v) yielding (**3**) as a white solid (0.95 g, 3.6 mmol) in 44% yield and compound **4**, also as a white solid (0.687 g, 2.6 mmol) in 32% yield.

Data for **3**. mp = 145.8–147.0 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 2979, 2931, 2600–3400, 1782, 1709, 1654, 1455,1373, 1240, 1182, 1137, 1007, 875, 743, 622; ^1H NMR (400 MHz, $CDCl_3$): δ 1.23 (d, 1H, $J_{13,11} = 6.9$, H13), 1.35 (dddd, 1H, $J_{7a,7e} \cong J_{7a,8a} \cong J_{7a,6} \cong 10.8$, $J_{7a,8e} = 4.1$, H7a), 1.63 (s, 3H, H14), 1.77 (s, 3H, H15), 1.78–1.88 (m, 2H, H6 and H8a), 2.00–2.06 (m, 1H, H7e), 2.34 (dq, 1H, $J_{11,6} = 12.6$, $J_{11,13} = 6.9$, H11), 2.85 (ddd, 1H, $J_{8e,8a} = 13.8$, $J_{8e,7a} = 4.1$, $J_{8e,7e} = 2.3$, H8e), 2.98 (ddd, 1H, $J_{2,2'} = 17.4$, $J_{2,3} = 7.8$, $J_{2,5} = 1.2$, H2), 3.03 (ddd, 1H, $J_{2',2} = 17.4$, $J_{2'3} = 6.5$, $J_{2'5} = 2.0$, H2′), 4.12 (dc, 1H, $J_{5,6} = 10.8$, H5), 5.67 (ddd, 1H, $J_{3,2} = 7.8$, $J_{3,2'} = 6.5$, $J_{3,5} = 1.4$, H3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.44 (C13), 19.97 (C15), 22.14 (C14), 27.43 (C7), 30.39 (C8), 33.74 (C2), 42.22 (C11), 54.20 (C6), 83.36 (C5), 110.40 (C3), 127.56 (C9), 130.84 (C10), 140.21 (C4), 177.50 (C1), 178.60 (C12); MS, *m*/*z* (%): 264 [$M^{+\bullet}$], (32), 246 (26), 218 (15), 191 (90), 175 (23), 145 (32), 131(47), 105 (33), 91 (61), 77 (38), 55 (100), 53 (35). HRMS (CI) $C_{15}H_{21}O_4$ (MH^+) requires 265.1434, found 265.1437.

Data for **4**. mp = 162.4–163.2 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3449, 3062, 2971, 2928, 2857, 1777, 1699, 1641, 1458, 1311, 1209, 1177, 1100, 1055, 991, 735, 707, 629; ^1H NMR (400 MHz, $CDCl_3$): δ 0.97 (s, 3H, H14), 1.29 (d, 3H, $J_{13,11} = 6.9$, H13), 1.42–1.47 (m, 1H, H8′), 1.75–1.85 (m, 1H, H9′), 1.90 (s, 3H, H15), 2.00–2.25 (m, 3H, H7, H8, H9), 2.32 (dq, 1H, $J_{11,7} = 12.2$, $J_{11,13} = 6.9$, H11), 2.54 (dd, 1H, $J_{2',2} = 19.7$, $J_{2',1} = 5.9$, H2′), 2.60 (dd, 1H, $J_{2,2'} = 19.7$, $J_{2,1} = 3.0$, H2), 3.23 (m, 1H, H1), 4.82 (d, 1H, $J_{6,7} = 11.0$, H6); ^{13}C NMR (100 MHz, $CDCl_3$): δ: 9.45 (C15), 12.50 (C13), 21.32 (C14), 25.89 (C8), 37.17 (C2), 41.44 (C11), 45.38 (C9), 48.51 (C7), 50.51 (C1), 74.46 (C10), 81.49 (C6), 143.12 (C4), 161.30 (C5), 177.15 (C12), 207.71 (C3); MS, *m*/*z* (%): 264, [$M^{+\bullet}$], (82), 221 (12), 206 (22), 193 (55), 169 (31), 149 (31), 133 (44), 105 (38), 91 (51), 77 (48), 55 (100), 53 (50). HRMS (CI) $C_{15}H_{21}O_4$ (MH^+) requires 265.1434, found 265.1439.

4.1.3. Photochemical synthesis of lumisantonin (**5**)

A solution of α-santonin (2.0 g, 8.13 mmol) in acetonitrile (120 mL) in a quartz tube was degassed by a flow of nitrogen for 30 min. The reaction mixture was then irradiated under four low pressure mercury lamps (4 × 15 W) for 2 h. The solvent was evaporated under reduced pressure (40 °C) to give a yellow solid which was recrystallized in a mixture of acetone and hexane (1:2 v/v) to give **5** as yellow crystals (0.416 g, 1.69 mmol) in 83% yield. mp = 145.8–147.3 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 2933, 2876, 1782, 1699, 1570, 1456, 1252, 1027, 998, 836; ^1H NMR (400 MHz, $CDCl_3$): δ 1.11 (s, 3H, H14), 1.12–1.21 (m, 1H, H8a), 1.22 (s, 3H, H15), 1.25 (d, 1H, $J_{13,11} = 6.9$, H13), 1.57–1.69 (m, 1H, H7), 1.79–1.98 (m, 3H, H8e, H9e, H9a), 2.30 (dq, 1H, $J_{11,7} = 12.3$, $J_{11,13} = 6.9$, H11), 3.81 (d, 1H, $J_{6,7} = 10.8$, H6), 6.01 (d, 1H, $J_{3,4} = 5.7$, H3), 7.59 (d, 1H, $J_{4,3} = 5.7$, H4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.68 (C15), 12.76 (C13), 17.45 (C14), 22.77 (C8), 29.88 (C9), 40.66 (C5), 41.48 (C11), 42.97 (C1), 48.97 (C7), 50.38 (C10), 77.89 (C6), 131.68 (C3), 157.99 (C4), 178.88 (C12), 206.91 (C2); MS, *m*/*z* (%): 246 [$M^{+\bullet}$], (35), 173 (49), 145 (24), 135 (60), 107 (38), 91 (81), 77 (52), 55 (100). HRMS (CI) $C_{15}H_{19}O_3$ (MH^+) requires 247.1329, found 247.1333.

4.1.4. 3-Oxo-7αH,6βH,11-(phenylselenyl)-eudesma-1,4-dien-6,12-olide (**6**)

To a mixture of anhydrous diisopropylamine (1.9 mL, 13.5 mmol) and anhydrous THF (10 mL) in a two necked round bottomed flask under nitrogen atmosphere at −78 °C, was added

n-BuLi (14.2 mL, 13.6 mmol). The mixture was stirred for 30 min and α-santonin (3.0 g, 12.2 mmol) in anhydrous THF (35 mL) was added. Phenyl selenium chloride (2.57 g, 13.4 mmol) in anhydrous THF was added to the reaction mixture after 30 min and stirred for a further 20 min at −78 °C. Distilled water (30 mL) was added to the mixture at 25 °C; the resulting mixture was transferred to a separatory funnel, and extracted with DCM (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried with anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluted with hexane/ethyl acetate (3:2 v/v), and recrystallized with hexane/ethyl acetate (2:1 v/v) to afford compound **6** as white crystals (1.9 g; 4.72 mmol; 39%). mp = 196.9–197.4 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3072, 2943, 2915, 2870, 1769, 1665, 1636, 1615, 1475, 1453, 1443, 1273, 1199, 1033, 742, 693; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (s, 3H, H14), 1.53 (m, 1H, H9e), 1.61 (s, 3H, H13), 1.92–2.00 (m, 4H, H7, H8e, H8a, H9a), 2.1 (s, 3H, H15), 5.22 (d, 1H, $J_{6,7} = 9.6$, H6), 6.25 (d, 1H, $J_{2,1} = 9.9$, H2), 6,68 (d, 1H, $J_{1,2} = 9.9$, H1), 7.35 (t, 2H, $J_{3',2'} = J_{3',4'} = 7.3$, H3′ and H5′), 7.45 (t, 1H, $J_{4',3'} = 7.3$, H4′), 7.65 (d, 2H, $J_{2',3'} = 7.3$, H2′ and H6′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.01 (C15), 20.6 (C8), 22.34 (C13), 25.08 (C14), 37.55 (C9), 41.34 (C10), 48.86 (C7), 57.58 (C11), 79.32 (C6), 123.94 (C1′), 126.04 (C2), 129.24 (C4, C2′, C6′),130.03 (C4′), 138.22 (C3′, C5′), 150.92 (C5), 154.64 (C1), 174.72 (C12), 186.15 (C3). MS, *m*/*z* (%): 402 [$M^{+\bullet}$], (1), 244 (100), 229 (48), 216 (65), 201 (52), 188 (22), 157 (15), 105 (20), 91 (50), 77 (63), 55 (39). HRMS (CI) $C_{21}H_{23}O_3Se$ (MH^+) requires 403.0807, found 403.0811.

4.1.5. 3-Oxo-7αH,6βH-eudesma-1,4,11-trien-6,12-olide (*7*)

To a solution of compound **6** (1.7 mL, 4.2 mmol) in anhydrous THF (10 mL), and acetic acid (0.65 mL) in a two necked round bottomed flask at 0 °C was added hydrogen peroxide 30% (3.0 mL). The mixture was stirred for 2 h at 0 °C, and the reaction was quenched with aqueous sodium bicarbonate (2 mol L^{-1}, 10 mL). The mixture was transferred into a separatory funnel, and extracted with diethyl ether (3 × 30 mL). The combined organic layers was washed with brine (30 mL), dried with anhydrous magnesium sulphate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography eluted with hexane/ethyl acetate (2:1 v/v) to afford compound **7** as a yellow solid (0.75 g; 3.1 mmol; 72%). mp = 148.7–149.6 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3044, 2937, 2870, 1777, 1663, 1635, 1615, 1458, 1254, 1041, 985, 963, 907, 834; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (s, 3H, H14), 1.56–1.63 (m, 2H, H8e, H9a), 1.79 (dddd, 1H, $J_{8a,8e} \cong J_{8a,9a} \cong J_{8a,7} \cong 11.6$, $J_{8a,9e} = 3.5$, H8a), 1.94 (dc, 1H, $J_{9e,8a} = 3.5$, $J_{9e,9a} = 13.4$, H9e), 2.17 (s, 3H, H15), 2.71 (tc, 1H, $J_{7,6} \cong J_{7,8a} = 11.6$, H7), 4.77 (d, 1H, $J_{6,7} = 11.6$, H6), 5.56 (d, 1H, $J_{13,13'} = 2.9$, H13), 6.24 (d, 1H, $J_{13',13} = 2.9$, H13′), 6.27 (d, 1H, $J_{2,1} = 9.9$, H2), 6.70 (d, 1H, $J_{1,2} = 9.9$, H1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.81 (C15), 21.68 (C8), 25.19 (C14), 37.68 (C9), 41.31 (C10), 50.30 (C7), 81.42 (C6), 119.61 (C13), 126.02 (C2), 129.05 (C4), 137.56 (C11), 150.61 (C5), 154.62 (C1), 169.04 (C12), 186.15 (C3); MS, *m*/*z* (%): 244 [$M^{+\bullet}$], (45), 216 (14), 201 (27), 173 (30), 145 (36), 105 (29), 91 (95), 77 (76), 65 (87), 53 (100). HRMS (CI) $C_{15}H_{17}O_3$ (MH^+) requires 245.1172, found 245.1179.

4.1.6. Photochemical synthesis of 11,13-dehydrolumisantonin (*8*) and 10α-acetoxy-3-oxo-1,7αH,6βH-guai-4,11-dien-6,12-olide (*9*)

A solution of compound **7** (0.65 g, 2.66 mmol) in anhydrous acetic acid (120 mL) in a quartz tube was degassed by a flow of nitrogen for 30 min. The reaction mixture was then irradiated under four low pressure mercury lamps (4 × 15 W) for 4 h. The solvent was evaporated under reduced pressure (50 °C) to give a yellow oil which was purified by flash column chromatography eluted with hexane/ethyl acetate (3:2 v/v) to afford compound **8** as a white solid (0.035 g, 0.12 mmol) in 4.5% yield and compound **9** as a yellow solid (0.12 g; 0.49 mmol) in 18%.

Data for ***8***. mp = 153.1–154.2 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 2929, 2876, 1776, 1698, 1568, 1381, 1258, 1134, 1039, 1073, 976, 838, 689; ^{1}H NMR (400 MHz, $CDCl_3$) δ (J/Hz): 1.17 (s, 3H, H14), 1.25 (s, 3H, H15), 1.30–1.40 (dddd, 1H, $J_{8a,8e} \cong J_{8a,9a} \cong J_{8a,7} \cong J_{6,7} = 11.2$, $J_{8a,9e} = 2.6$, H8a), 1.88–2.00 (m, 2H, H9a, H9e), 2.14–2.21 (m, 1H, H8e), 2.56–2.65 (m, 1H, H7), 3.82 (d, 1H, $J_{6,7} = 11.2$, H6), 5.50 (d, 1H, $J_{13,13'} = 3.1$, H13), 6.06 (d, 1H, $J_{3,4} = 5.8$, H3), 6.19 (d, 1H, $J_{13',13} = 3.1$, H13′), 7.65 (d, 1H, $J_{4,3} = 5.8$, H4); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 7.50 (C15), 16.92 (C14), 20.99 (C8), 28.62 (C9), 40.48 (C5), 43.23 (C1), 45.49 (C7), 50.13 (C10), 77.55 (C6), 119.24 (C13), 131.49 (C3), 138.36 (C11), 157.10 (C4), 170.00 (C12), 206.40 (C2); MS, *m*/*z* (%): 244 [$M^{+\bullet}$], (30), 229 (11), 215 (10), 201 (15), 173 (19), 145 (17), 105 (20), 84 (100), 77(32), 53 (72), 51 (98). HRMS (CI) $C_{15}H_{17}O_3$ (MH^+) requires 245.1172, found 245.1180.

Data for ***9***. mp = 139.7–141.0 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 2955, 2916, 2848, 1771, 1727, 1705, 1645, 1472, 1370, 1245,1103, 1058, 1018, 997, 963, 891; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 3H, H14), 1.46–1.56 (m, 1H, H8′), 1.95 (s, 3H, H15), 2.01 (s, 3H, CH_3CO), 2.22–2.32 (m, 2H, H8, H9′), 2.43 (dd, 1H, $J_{2,2'} = 19.3$, $J_{2,1} = 2.6$, H2), 2.54 (dd, 1H, $J_{2',2} = 19.3$, $J_{2',1} = 6.2$, H2′), 2.68 (td, 1H, $J_{9,9'} = 13.4$, $J_{9,8'} = J_{9,8} = 4.4$, H9), 3.14 (tc, 1H, $J_{7,6} = 10.9$, $J_{7,8} = J_{7,8'} = 1.4$, H7), 4.20 (dd, 1H, $J_{1,2'} = 6.2$, $J_{1,2} = 2.6$, H1), 4.81 (d, 1H, $J_{6,7} = 10.9$, H6), 5.61 (d, 1H, $J_{13,13'} = 3.2$, H13), 6.33 (d, 1H, $J_{13'}{}_{13} = 3.2$, H13′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.55 (C15), 20.03 (C14), 22.29 (CH_3CO), 24.48 (C8), 36.90 (C2), 37.54 (C9), 44.47 (C7), 47.32 (C1), 81.66 (C6), 85.49 (C10), 120.83 (C13), 137.58 (C11), 143.21 (C4), 160.30 (C5), 168.80 (C12), 170.35 (CH_3CO), 206.81 (C3); MS, *m*/*z* (%): 304 [$M^{+\bullet}$], (0.3), 262 (35), 244 (100), 229 (17), 201 (20), 187 (48), 159 (22), 91 (36), 67 (33), 53 (55). 51 (24). HRMS (CI), $C_{17}H_{21}O_5$ (MH^+) requires 305.1384, found 305.1379.

4.1.7. 10α-Acetoxy-3β-hydroxy-1,7αH,6,11βH-guai-4-en-6, 12-olide (*10*)

A mixture of sodium borohydride (0,056 g; 1.47 mmol), lactone **2** (0.225 g; 0.73 mmol), and anhydrous methanol (50 mL) in a round bottomed flask was stirred for 2 h. The reaction was quenched with saturated ammonium chloride aqueous solution (30 mL), filtered and the solid was washed with methanol. The filtrate was concentrated under reduced pressure (40 °C) and the aqueous residue transferred to a separatory funnel which was extracted with ethyl acetate (3 × 40 mL). The combined organic layers were washed with brine (40 mL), dried with anhydrous magnesium sulphate, filtered and concentrated under reduced pressure. The yellow solid was purified by flash column chromatography eluted with hexane/ethyl acetate (1:1 v/v) to give compound **10** as a white solid (195 mg, 0.63 mmol) in 86% yield. mp = 114.9–116.4 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3436, 2973, 2935, 2875, 1771, 1725, 1456, 1369, 1251, 1180, 850, 736; ^{1}H NMR (400 MHz, $CDCl_3$): δ (J/Hz): 1.20 (s, 3H, H14), 1.23 (d, 3H, $J_{13,11} = 6.5$, H13), 1.36–1.40 (m, 1H, H8′), 1.57 (ddd, 1H, $J_{2,2'} = 14.0$, $J_{2,1} \cong J_{2,3} \cong 6.4$, H2), 1.73 (s broad, 1H, OH), 1.89 (s, 3H, H15), 1.98 (s, 3H, CH_3CO), 1.99–2.25 (m, 4H, H7, H8, H9′, H11), 2.36–2.48 (m, 2H, H2′ and H9), 3.75–3.78 (m, 1H, H1), 4.54–4.59 (m, 1H, H3), 4.67 (dc, 1H, $J_{6,7} = 10.9$, H6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.81 (C13), 12.91 (C15), 20.56 (C14), 22.80 (CH_3CO), 25.61(C8), 35.05 (C2), 38.35 (C9), 41.84 (C11), 49.34 (C7), 51.66 (C1), 78.16 (C3), 81.92 (C6), 86.87 (C10), 131.59 (C5), 144.50 (C4), 170.65 (CH_3CO), 178.48 (C12); MS, *m*/*z* (%): 308 [$M^{+\bullet}$], 248 (67), 233 (100), 174 (49), 159 (34), 91 (44), 55 (74). HRMS (CI), $C_{17}H_{25}O_5$ (MH^+) requires 309.1697, found 309.1695.

4.1.8. 3β,10α-Hydroxy-1,7αH,6,11βH-guai-4-en-6,12-olide (*11*)

A mixture of sodium borohydride (0.05 g; 1.47 mmol), lactone **4** (0.12 g; 0.45 mmol), and anhydrous methanol (30 mL) in a round

bottomed flask was stirred for 3 h. The reaction was quenched and elaborated as described for the preparation of compound **10**, affording compound **11** as a white solid (86 mg, 0.32 mmol) in 72% yield; mp = 168.5–169.5 °C; IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3389, 2973, 2929, 2860, 1759, 1668, 1455, 1380, 1234, 1179, 1103, 985, 865, 735, 695; ^{1}H NMR (400 MHz, $CDCl_3$): δ: 1.06 (s, 3H, H14), 1.23 (d, 3H, $J_{13,11}$ = 7.0, H13), 1.30–1.45 (m, 1H, H8′), 1.60–1.69 (m, 4H, OH, OH, H2, H9′), 1.89 (s, 1H, H15), 1.93–1.99 (m, 3H, H7, H8, H9), 2.20 (dq, 1H, $J_{11,7}$ = 12.2, $J_{11,13}$ = 7.0, H11), 2.50 (dt, 1H, $J_{2',2}$ = 16.0, $J_{2',3} = J_{2',1}$ = 8.0, H2′), 2.90–2.95 (m, 1H, H1), 4.55 (m, 1H, H3), 4.70 (dc, 1H, $J_{6,7}$ = 10.9, H6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.45 (C15), 12.99 (C13), 21.55 (C14), 25.67 (C8), 34.74 (C2), 41.49 (C11), 44.83 (C9), 49.14 (C7), 54.66 (C1), 74.56 (C10), 77.62 (C3), 81.84 (C6), 131.80 (C4), 143.98 (C5), 178.20 (C12); MS, *m*/*z* (%): 266 [$M^{+\bullet}$], (0,1), 248 (55), 233 (8), 215 (17), 205 (12), 190 (83), 177 (30), 108 (54), 91 (44), 79 (57), 55 (100), 53 (38). HRMS (CI), $C_{15}H_{23}O_4$ (MH^+) requires 267.1591, found 267.1587.

4.2. Cytotoxic assays

4.2.1. Tumor cell assay

The cytotoxic effects of the synthesized compounds were evaluated against HL-60 (leukemia), SF-295 (central nervous system), HCT-8 (colon), MDA-MB-435 (melanoma), UACC-257 (melanoma), A549 (lung), OVACAR-8 (ovarian), A704 (renal), and PC3 (prostate) human cancer cell lines, all obtained from the National Cancer Institute, Bethesda, MD, USA. The cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 μg/mL streptomycin and 100 U/mL penicillin, and incubated at 37 °C with a 5% CO_2 atmosphere.

The tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to a purple formazan product [35]. For all experiments, the cells were seeded in 96-well plates (105 cells/well for adherent cells or 0.5×10^5 cells/well for suspended cells in 100 μL of medium). After 24 h, the compounds (0.09–25 μg/mL), dissolved in DMSO, were added to each well (using the HTS – high-throughput screening – Biomek 3000 – Beckman Coulter, Inc. Fullerton, California, USA) and incubated for 72 h. Doxorubicin (Sigma Aldrich Co., St Louis, MO, USA) was used as a positive control. At the end of the incubation, the plates were centrifuged and the medium was replaced by fresh medium (150 μL) containing 0.5 mg/mL MTT. After 3 h, the formazan product was dissolved in 150 μL DMSO and the absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc. Fullerton, Califórnia, USA). The drug effect was quantified as the percentage of control absorbance of reduced dye at 595 nm.

4.2.2. Normal cell assay

The cytotoxic effects of the synthesized compounds were evaluated against PBMC (Peripheral Blood Mononuclear Cells) from healthy donors, using Alamar blue assay. Heparinized blood (from healthy, non-smoker donors who had not taken any drug at least 15 days prior to sampling) was collected and PBMC were isolated by a standard method of density-gradient centrifugation over Ficoll-Hypaque. PBMC were washed and resuspended at a concentration of 3×10^5 cells mL^{-1} in RPMI-1640 medium supplemented with 20% fetal bovine serum, 2 mM glutamine, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin at 37 °C with 5% CO_2. Phytohemagglutinin (2%) was added at the beginning of culture. After 24 h of culture, cells were treated with the test compounds.

In order to investigate selectivity of the compounds towards a normal proliferating cell, the Alamar blue assay was performed with PBMC after 72 h drug exposure [36]. Briefly, PBMC were plated in 96-well plates (3×10^5 cells/well in 100 μl of medium). After 24 h, the compounds (0.09–25 μg mL^{-1}) dissolved in DMSO were added to each well (using the HTS – high-throughput screening-biomek 3000-Beckman Coulter, Inc. Fullerton, Califórnia, EUA) and incubated for 72 h. Doxorubicin was used as positive control. Twenty four hours before the end of the incubation, 10 μL of stock solution (0.312 mg mL^{-1}) of the Alamar Blue (resazurin – Sigma Aldrich Co. – St. Louis, MO/USA) were added to each well. The absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter, Inc. Fullerton, Califórnia, USA). The drug effect was quantified as the percentage of control absorbance at 570 nm and 595 nm.

4.3. Statistical analysis

The IC_{50} values and their 95% confidence intervals (CI 95%) were obtained by nonlinear regression using the GRAPHPAD program (Intuitive Software for Science, San Diego, CA).

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