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Synthesis, antiproliferative activity in cancer cells and theoretical studies of novel 6α,7β-dihydroxyvouacapan-17β-oic acid Mannich base derivatives

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ABSTRACT

Natural products are great prototypes for the design of new anticancer agents. The plant-derived natural product 6α,7β-dihydroxyvouacapan-17β-oic acid (**1**) is promising for the development of more potent antiproliferative agents against human cancer cells. Indeed, its lactone derivative 6α-hydroxyvouacapan-7β,17β-lactone (**2**), a non-natural furanoditerpene, exhibited higher anticancer activity than compound **1**. Herein, we describe the synthesis and antiproliferative activity of six new Mannich derivatives of compound **2** against nine cancer cell lines. Overall, our results revealed that Mannich derivatives **3**–**8** were more potent than compound **2** in inhibiting the proliferation of cancer cells. Theoretical studies also supported our findings, revealing the nucleophilic character of furan ring as an important feature for antiproliferative activity of the studied Mannich derivatives.

1. Introduction

Natural products are interesting sources of novel lead compounds for the design of new drugs. In fact, scientists have being taking advantage of natural products for such purpose; approximately 40% of the drugs approved for use in the past few years were somehow related to natural products.[1,2] Phytochemical studies of the genus *Pterodon* have resulted in the isolation and characterization of many compounds, such as alkaloids,[3] isoflavones,[4,5] and terpenes,[4,6,7] that exhibit a variety of biological activities. Furanoditerpenes have received considerable attention among the isolated terpenes. *Pterodon*-derived furanoditerpenes were shown to present analgesic,[8] plant growth regulatory,[9–12] anti-edematogenic,[13] photosystem II inhibitory and photosynthesis uncoupler,[14,15] larvicidal,[16] antinociceptive,[17] and antifungal[18] properties. Additionally, furanoditerpenes and their synthetic derivatives were reported to have expressive antiproliferative activity against various cancer cell lines.[19–21]

We have previously described that 6α-hydroxyvouacapan-7β,17β-lactone (**2**, Fig. 1), a synthetic compound derived from the natural product 6α,7β-dihydroxyvouacapan-17β-oic acid (**1**, Fig. 1),[22] was the most promising anticancer agent.[20] Our findings demonstrated the importance of 7β,17β-lactone ring and hydroxyl group at C-6 for the antiproliferative activity of compound **2**.[20] Thus, we designed and synthesized six novel compound **2**-derived furanoditerpene amines in an attempt to improve cytotoxicity against cancer cells. This work reports the antiproliferative activity of these novel furanoditerpene amines against nine cancer cell lines, as well as a theoretical study to explain and support the experimental results.

2. Material and methods

2.1. Instrumentation and chemicals

The natural product 6α,7β-dihydroxyvouacapan-17β-oic acid (**1**) was isolated from *Pterodon polygalaeflorus* Benth fruits as previously described.[23] All reactions were followed by analytical thin-layer chromatography analyses (TLC, Merck Silica Gel 60G, eluted with diethyl ether:dichloromethane, 2:3). Uncorrected melting points were determined by using a Mettler FP 82 HT apparatus. Elemental analyses were taken in a Perkin Elmer 2400 apparatus. Infrared (IR) spectra were recorded on a Mattson FTIR 3000 apparatus with samples prepared in KBr disks. NMR spectra were recorded in $CDCl_3$ on a Bruker DRX 400 AVANCE spectrometer using TMS as an internal standard. One-dimensional (1D) ^1H and ^{13}C NMR spectra were acquired under standard conditions using

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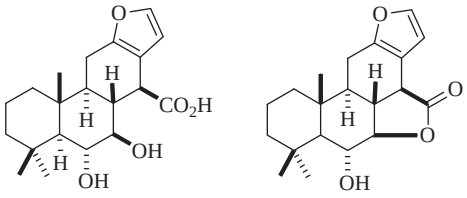


(1) (2)

Figure 1. Structure of 6α,7β-dihydroxyvouacapan-17β-oic acid (**1**) and its derivative 6α-hydroxyvouacapan-7β,17β-lactone (**2**).

a direct detection 5 mm $^{1}H/^{13}C$ dual probe with 90° pulse lengths of 11.3 and 8 μs for ^{1}H and ^{13}C, respectively. A relaxation delay of 2 s was used for all routine experiments. Standard pulse sequences were used for two-dimensional (2D) homonuclear and heteronuclear shift correlation spectra, employing a multinuclear inverse detection 5 mm probe with ^{1}H 90° pulse width of 11.3 μs.

2.2. Synthesis

2.2.1. Preparation of 6α-hydroxyvouacapan-7β,17β-lactone (2)

Compound **2** was prepared by reacting compound **1** with acetic anhydride and sodium acetate in tetrahydrofuran (THF) at 45 °C as reported elsewhere.[12,20] Mp 225.1–226.6 °C [lit.[22] 226.1–227.9 °C].

2.2.2. General experimental procedure for the preparation of Mannich bases (3–8)

Compounds **3–8** were obtained in 55–84% yields (see Table 1) through Mannich reaction of **2** with preformed *N*,*N*-dialkylmethyleniminium chloride salts (Fig. 2).[11] Ten milliliter of **2** (1 mmol in THF) was added to 10 mL of the respective preformed iminium salt (1 mmol in THF) at room temperature and inert atmosphere. The reaction mixture was stirred and heated to reflux from 2.8 to 3.6 h (see Table 1). Reaction mixtures were then cooled to room temperature, poured into 25 mL of water, following addition of NH_4OH until the product was precipitated.

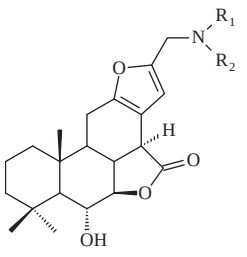
2.2.2.1. 16-(*N*,*N*-Diethylaminomethyl)-6α-hydroxyvouacapan-7β,17β-lactone (3). Mp 159.3–162.1 °C; IR (KBr, cm^{-1}) ν_{max}: 3421, 2970, 2923, 2873, 2849, 1781, 1460, 1386, 1367, 1208, 1140, 1096, 1050, 978, 946, 903, 862, 840, 771, 734, 703, 642, 580; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), *J* (Hz): δ_H = 1.01 (s, 3H, H-20), 1.06–1.10 (m, 1H, H-1ax), 1.08 (t, 3H, $J_{1''-2''}$ = 7.0, H-2″), 1.13 (s, 3H, H-19), 1.23 (s, 3H, H-18), 1.23–1.30 (m, 2H, H-3ax, H-5), 1.45 (br d, 2H, $J_{2ax-2eq}$ = $J_{3ax-3eq}$ = 11.4, H-2eq, H-3eq), 1.53–1.63 (m, 2H, H-1eq, H-2ax), 1.71–1.76 (m, 1H, H-9), 1.99–2.11 (m, 1H, H-8), 2.47–2.58 (m, 1H, H-11α), 2.64 (dd, 1H, $J_{11\beta-11\alpha}$ = 17.8, $J_{11\beta-9}$ = 8.2, H-11β), 2.11 (s, 1H, OH), 2.56 (q, 4H, $J_{1''-2''}$ = 7.0, H-1″), 3.21 (d, 1H, J_{14-8} = 13.2, H-14), 3.62 (s, 2H, H-1′), 4.07–4.14 (m, 2H, H-6, H-7), 6.39 (s, 1H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ_C = 11.5 (C-20), 15.5 (C-2), 18.1 (C-11), 21.9 (C-19), 22.9 (C-2″), 34.1 (C-4), 36.9 (C-18), 39.2 (C-1), 40.6 (C-10), 41.8 (C-14), 44.2 (C-3, C-9), 45.4 (C-8), 47.0 (C-1′), 48.9 (C-1″), 58.0 (C-5), 71.9 (C-6), 88.1 (C-7), 107.0 (C-15), 114.0 (C-13), 151.2 (C-16), 151.9 (C-12), 174.1 (C-17). Anal. Calcd for $C_{25}H_{37}NO_4$: C, 72.26; H, 8.97; N, 3.37. Found: C, 69.89; H, 8.25; N, 3.59.

2.2.2.2. 16-(*N*,*N*-Dipropylaminomethyl)-6α-hydroxyvouacapan-7β,17β-lactone (4). Mp 166.8–169.5 °C; IR (KBr, cm^{-1}) ν_{max}: 3476, 2951, 2931, 2871, 2850, 2817, 1780, 1467, 1383, 1366, 1096, 975, 954, 838, 817, 729, 581; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), *J* (Hz): δ_H = 0.87 (t, 3H, $J_{3''-2''}$ = 7.3, H-3″), 1.01 (s, 3H, H-20), 1.08–1.13 (m, 1H, H-1ax), 1.13 (s, 3H, H-19), 1.23 (s, 3H, H-18), 1.23–1.31 (m, 1H, H-5), 1.25–1.31 (m, 1H, H-3ax), 1.44–1.53 (m, 4H, H-2eq, H-3eq, H-2″), 1.57–1.64 (m, 2H, H-1eq, H-2ax), 1.73–1.78 (m, 1H, H-9), 2.00–2.09 (m, 1H, H-8), 2.21 (br, 1H, OH-6), 2.39 (t, 2H, $J_{1''-2''}$ = 7.7, H-1″), 2.50 (dd, 1H, $J_{11\alpha-11\beta}$ = 17.9, $J_{11\alpha-9}$ = 8.6, H-11α), 2.64 (dd, 1H, $J_{11\beta-11\alpha}$ = 17.9, $J_{11\beta-9}$ = 8.2, H-11β), 3.22 (d, 1H, J_{14-8} = 13.3, H-14), 3.58 (s, 2H, H-1′), 4.06–4.15 (m, 2H, H-6, H-7), 6.35 (s, 1H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ_C = 12.2 (C-3″), 15.9 (C-20), 18.4 (C-2), 18.7 (C-2″), 22.2 (C-11), 23.2 (C-19), 34.4 (C-4), 37.2 (C-18), 39.4 (C-1), 40.9 (C-10), 42.1 (C-14), 44.4 (C-3, C-9), 45.7 (C-8), 50.4 (C-1′), 56.0 (C-1″), 58.2 (C-5), 72.2 (C-6), 88.3 (C-7), 106.6 (C-15), 114.1 (C-13), 151.8 (C-12), 152.4 (C-16), 174.4 (C-17). Anal. Calcd for $C_{27}H_{41}NO_4$: C, 73.10; H, 9.32; N, 3.16. Found: C, 73.21; H, 8.63; N, 3.47.

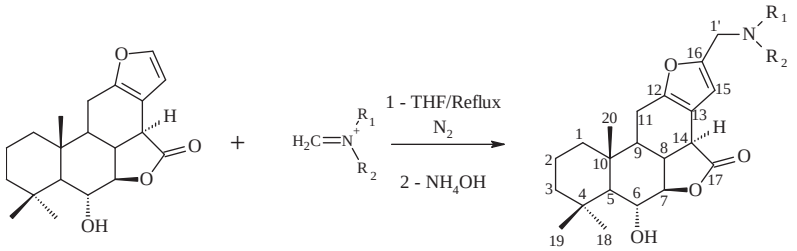
2.2.2.3. 16-(*N*,*N*-Diisobutylaminomethyl)-6α-hydroxyvouacapan-7β,17β-lactone (5). Mp 160.4–162.9 °C; IR (KBr, cm^{-1}) ν_{max}: 3423, 3011, 2922, 2868, 2849, 1782, 1459, 1354, 1298, 1205, 1115, 1093, 1049, 1007, 950, 919, 902, 861, 705, 644, 581; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), *J* (Hz): δ_H = 0.86 (d, 1H, $J_{3''-2''}$ = 6.6, H-3″), 1.01 (s, 3H, H-20), 1.11 (s, 3H, H-19), 1.11–1.13 (m, 1H, H-1ax), 1.23 (s, 3H, H-18), 1.23–1.26 (m, 1H, H-5), 1.27–1.31 (m, 1H, H-3ax), 1.44–1.47 (m, 2H, H-2eq, H-3eq), 1.53–1.57 (m, 1H, H-2ax), 1.61–1.64 (m, 1H, H-1eq), 1.69–1.79 (m, 2H, H-9, H-2″), 2.00–2.07 (m, 1H, H-8), 2.12 (d, 1H, $J_{1''-2''}$ = 7.3, H-1″), 2.13 (br, 1H, OH), 2.48 (dd, 1H, $J_{11\alpha-11\beta}$ = 17.8, $J_{11\alpha-9}$ = 8.8, H-11α), 2.63 (dd, 1H, $J_{11\beta-11\alpha}$ = 17.8, $J_{11\beta-9}$ = 8.3, H-11β), 3.24 (br d, 1H, J_{14-8} = 13.3, H-14), 3.53 (s, 2H, H-1′), 4.06–4.15 (m, 2H, H-6, H-7), 6.32 (s, 1H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ_C = 15.7 (C-20), 18.1 (C-2), 20.8 (C-3″), 21.9 (C-11), 22.9 (C-19), 26.5 (C-2″), 34.2 (C-4), 36.9 (C-18), 39.2 (C-1), 40.7 (C-10), 41.9 (C-14), 44.2 (C-3, C-9), 45.4 (C-8), 50.9 (C-1′), 58.0 (C-5), 63.0 (C-1″), 72.0 (C-6), 87.0 (C-7), 105.9 (C-15), 113.8 (C-13), 151.0 (C-12), 152.8 (C-16), 174.1 (C-17). Anal. Calcd for $C_{29}H_{45}NO_4$: C, 73.85; H, 9.62; N, 2.97. Found: C, 72.36; H, 9.05; N, 3.17.

2.2.2.4. 16-(1-Pyrrolidinylmethyl)-6α-hydroxyvouacapan-7β,17β-lactone (6). Mp 110.3–112.7 °C; IR (KBr, cm^{-1}) ν_{max}: 3423, 2927, 2874, 2846, 2806, 1785, 1627, 1560, 1460, 1389, 1365, 1348, 1211, 1119, 1096, 1049, 944, 901, 874, 860, 839, 816, 733, 704, 670, 657, 642, 581; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), *J* (Hz): δ_H = 1.00 (s, 3H, H-20), 1.07–1.12 (m, 1H, H-1ax), 1.12 (s, 3H, H-19), 1.17–1.30 (m, 1H, H-5), 1.23 (s, 3H, H-18), 1.23–1.30 (m, 1H, H-3ax), 1.43–1.46 (br d, 2H, $J_{2ax-2eq}$ = $J_{3ax-3eq}$ = 11.0, H-2eq, H-3eq), 1.53–1.62 (m, 2H, H-1eq, H-2ax), 1.72–1.80 (m, 2H, H-9, H-2″), 1.94–2.05 (m, 1H, H-8), 2.47–2.67 (m, 4H, H-11α, H-11β, H-1″), 2.93 (br, 1H, OH), 3.20 (d, 1H, J_{14-8} = 13.0, H-14), 3.60 (s, 2H, H-1′), 4.10 (br, 2H, H-6, H-7), 6.40 (s, 1H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ_C = 15.9 (C-1), 18.1 (C-2), 21.8 (C-11), 22.9 (C-19), 23.4 (C-2″), 34.1 (C-4),

Table 1
Yield and reaction time for the preparation of the furanditerpenes **3–8**



Compound	R_1	R_2	Yield (%)	Reaction time (h)
3	$-CH_2CH_3$	$-CH_2CH_3$	71	2.8
4	$-(CH_2)_2CH_3$	$-(CH_2)_2CH_3$	68	3.0
5	$-CH_2CH(CH_3)_2$	$-CH_2CH(CH_3)_2$	62	3.3
6	$-CH_2CH_2CH_2CH_2-$		66	3.3
7	$-CH_2CH_2CH_2CH_2CH_2-$		84	3.5
8	$-CH_2CH_2OCH_2CH_2-$		55	3.6



3 - $R_1 = R_2 = -CH_2(1'')-CH_3(2'')$

4 - $R_1 = R_2 = -CH_2(1'')-CH_2(2'')-CH_3(3'')$

5 - $R_1 = R_2 = -CH_2(1'')-CH(2'')-(CH_3)_2(3'')$

6 - R_1 and $R_2 = -CH_2(1'')-CH_2(2'')-CH_2(2'')-CH_2(1'')-$

7 - R_1 and $R_2 = -CH_2(1'')-CH_2(2'')-CH_2(3'')-CH_2(2'')-CH_2(1'')-$

8 - R_1 and $R_2 = -CH_2(2'')-CH_2(1'')-O-CH_2(1'')-CH_2(2'')-$

Figure 2. Structure of the furanoditerpenes derivatives **3**–**8**.

36.9 (C-18), 39.1 (C-1), 40.6 (C-10), 41.7 (C-14), 44.1 (C-3, C-9), 45.4 (C-8), 52.1 (C-1′), 53.8 (C-1″), 57.9 (C-5), 71.8 (C-6), 87.0 (C-7), 106.2 (C-15), 113.9 (C-13), 151.6 (C-16), 151.9 (C-12), 174.0 (C-17). Anal. Calcd For $C_{25}H_{35}NO_4$: C, 72.61; H, 8.53; N, 3.39. Found: C, 72.24; H, 8.41; N, 3.42.

2.2.2.5. 16-(1-Piperidinylmethyl)-6α-hydroxyvouacapan-7β,17β-lactone (7). Mp 117.7–118.9 °C; IR (KBr, cm^{-1}) ν_{max}: 3417, 3005, 2933, 2869, 2846, 2810, 2767, 1785, 1444, 1391, 1365, 1344, 1302, 1209, 1112, 1095, 1046, 977, 946, 903, 860, 786, 706, 643, 582; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), J (Hz): δ_H = 1.01 (s, 3H, H-20), 1.08–1.11 (m, 2H, H-1ax, H-3ax), 1.13 (s, 3H, H-19), 1.24 (s, 3H, H-18), 1.24–1.30 (m, 1H, H-5), 1.43–1.46 (m, 4H, H-2eq, H-3eq, H-3″), 1.53–1.56 (m, 1H, H-2ax), 1.60–1.64 (m, 5H, H-1eq, H-2″), 1.71–1.78 (m, 1H, H-9), 1.99–2.08 (m, 2H, H-8, OH), 2.46–2.54 (m, 5H, H-11α, H-1″), 2.66 (dd, 1H, $J_{11\beta-11\alpha}$ = 17.4, $J_{11\beta-9}$ = 8.0, H-11β), 2.79 (br, 1H, OH), 3.22 (d, 1H, J_{14-8} = 13.3, H-14), 3.51 (s, 2H, H-1′), 4.10–4.13 (m, 2H, H-6, H-7), 6.42 (s, 1H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ_C = 15.7 (C-20), 18.1 (C-2), 21.9 (C-11), 22.9 (C-19), 23.9 (C-3″), 25.5 (C-2″), 34.1 (C-4), 36.9 (C-18), 39.2 (C-1), 40.6 (C-10), 41.8 (C-14), 44.1 (C-9), 44.2 (C-3), 45.4 (C-8), 54.0 (C-1″), 55.5 (C-1′), 58.0 (C-5), 71.9 (C-6), 88.0 (C-7), 107.7 (C-15), 114.1 (C-13), 150.4 (C-16), 152.2 (C-12), 174.0 (C-17). Anal. Calcd for $C_{26}H_{37}NO_4$: C, 73.03; H, 8.72; N, 3.28. Found: C, 70.42; H, 8.01; N, 3.18.

2.2.2.6. 16-(4-Morpholinylmethyl)-6α-hydroxyvouacapan-7β,17β-lactone (8). Mp 112.4–114.7 °C; IR (KBr, cm^{-1}) ν_{max}: 3491, 3019, 2958, 2932, 2872, 2846, 2809, 2741, 1774, 1468, 1461, 1389, 1366, 1309, 1288, 1208, 1189, 1166, 1137, 1116, 1096, 1046, 1020, 972, 946, 905, 892, 862, 837, 813, 725, 706, 664, 644, 583, 556, 521, 485; ^{1}H NMR (400 MHz, $CDCl_3$, ppm), J (Hz): δ_H = 1.01 (s, 3H, H-20), 1.08–1.13 (m, 1H, H-1ax), 1.13 (s, 3H, H-19), 1.23 (s, 3H, H-18), 1.23–1.30 (m, 2H, H-3ax, H-5), 1.45 (br d, 2H, $J_{2ax-2eq}$ = $J_{3ax-3eq}$ = 11.4, H-2eq, H-3eq), 1.53–1.63 (m, 2H, H-1eq, H-2ax), 1.71–1.78 (m, 1H, H-9), 1.98–2.07 (m, 1H, H-8), 2.31 (br, 1H, OH), 2.48–2.54 (m, 5H, H-11α, H-2″), 2.65 (dd, 1H, $J_{11\beta-11\alpha}$ = 18.2, $J_{11\beta-9}$ = 7.8, H-11β), 3.21 (d, 1H, J_{14-8} = 13.1, H-14), 3.48 (s, 2H, H-1′), 3.73 (br, 4H, H-2″), 4.07–4.15 (m, 2H, H-6, H-7), 6.42 (s, 1H, H-15); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ_C = 15.9 (C-20), 18.4 (C-2), 22.2 (C-11), 23.2 (C-19), 34.4 (C-4), 37.2 (C-18), 39.5 (C-1), 40.9 (C-10), 42.0 (C-14), 44.4 (C-3, C-9), 45.7 (C-8), 53.5 (C-2″), 55.7 (C-1′), 58.2 (C-5), 67.0 (C-1″), 72.1 (C-6), 88.3 (C-7), 107.7 (C-15), 114.3 (C-13), 150.5 (C-16), 152.5 (C-12), 174.3 (C-17). Anal. Calcd for $C_{25}H_{35}NO_5$: C, 69.90; H, 8.21; N, 3.26. Found: C, 68.12; H, 7.65; N, 3.25.

2.3. Antiproliferative activity

The human tumor cell lines UACC-62 (melanoma), MCF-7 (breast), NCI-ADR/RES (ovarian expressing the resistance phenotype for adryamycin), 786-0 (kidney), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-03 (ovarian), HT-29 (colon), and K562 (erythromyeloblastoid leukemia) were kindly provided by Frederick Cancer Research & Development Center, National Cancer Institute, Frederick, MA, USA. Stock cultures were grown in 5 mL of RPMI 1640 medium (GIBCO BRL, Life Technologies) supplemented with 5% fetal bovine serum. Penicillin/streptomycin (100 μg/mL:1000 U/mL, 1 mL/L) was added to experimental cultures. Cells in 96-well plates (100 μL cells/well), at 37 °C and 5% CO_2, were exposed for 48 h to various concentrations of **3–8** (0.25, 2.5, 25, and 250 μg/mL diluted in DMSO at final concentrations of 1%). DMSO at 1% did not affect cell viability. Then, a solution of trichloroacetic acid (50% w/v) was added to each well following incubation for 30 min at 4 °C. Cells were washed, dried and the proliferation was determined by using sulforhodamine B assay with measurements carried out at 540 nm.[24] To assess the effect of **3–8** on cell growth, three measurements were obtained as it follows: at time zero (T_0 values) for all cells at the beginning of incubation; 48 h post-incubation for both control (C values, untreated cells) and test (T values). The formula $100[(T - T_0)/(C - T_0)]$ was used to calculate cytostatic effect (when $T \geqslant T_0$) while the cytocidal effect (when $T < T_0$) was verified by using the formula $100[(T - T_0)/T_0]$. The GI_{50} (test compound concentration that elicits cell growth inhibition by 50%) and TGI (test compound concentration that elicits cell growth inhibition by 100%) values were determined by non-linear regression analyses using Origin software, version 7.5. Results are representative of experiments done in triplicate, with standard errors lower than 5%.

2.4. Theoretical calculations

Electronic density, molecular orbital energies (OM) and orbital population calculations were performed to investigate chemical structure–antiproliferative activity relationships. Only contributions of functionalized carbon and hydrogen atoms, as well as heteroatoms were considered. The software package GAUSSIAN03W[25] was used for such purpose. Semi-empirical AM1-optimized geometries[26] were used as initial models for optimizations by Density

Functional Theory (DFT)[27] with the functional BLYP[28,29] and set of bases 6-31G* (BLYP/6-31G*).[30–34] The geometries obtained by theoretical calculations were characterized as true energy minima only when all calculated frequencies (PES) were positive, considering the absence of intermolecular interactions and gaseous state.

Calculations (BLYP/6-31G*) of energy and atomic contributions (orbital population) of occupied and virtual molecular orbitals were made after geometry optimization at the same calculation level, as previously described elsewhere.[35] Electronic charge density calculations were made by using Mulliken's method.

3. Results and discussion

3.1. Synthesis

Figure 2 exhibits the synthetic approach adopted in this study. The use of a preformed iminium salt was a key step for the synthesis of the compounds **3**–**8** with good yields (see Table 1).

All compounds (**2**–**8**) were characterized by elemental, IR and NMR analyses. NMR analyses were supported by DEPT, COSY, HMBC, and HSQC experiments. Compound **2** NMR data were

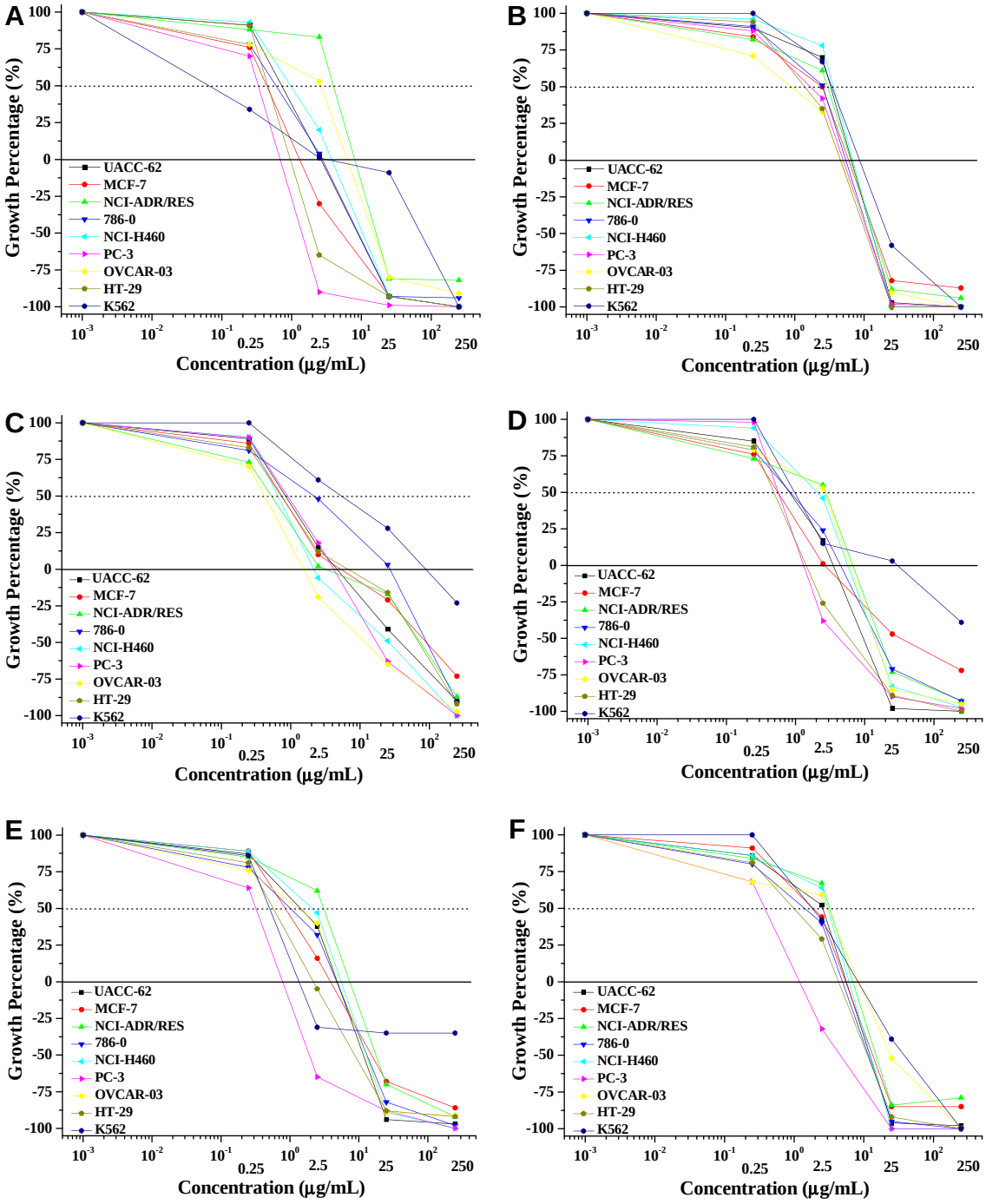


Figure 3. Effect of novel furanoditerpenes on cancer cells proliferation. Cells were exposed to compounds **3** (**A**), **4** (**B**), **5** (**C**), **6** (**D**), **7** (**E**) or **8** (**F**) for 48 h. Cells growth was determined as described in Material and methods section. UACC-62, melanoma cells; MCF-7, breast cancer cells; NCI-ADR/RES, adryamycin-resistance ovarian cancer cells; 786-0, kidney cancer cells; NCI-H460, lung, non-small cancer cells; PC-3, prostate cancer cells; OVCAR-03, ovarian cancer cells; HT-29, colon cancer cells; and K562, erythromyeloblastoid leukemia cells. Data are means of a representative experiment done in triplicate.

compatible to those reported in literature.[12] The Mannich reaction efficiency was assessed by comparing signals of aromatic hydrogen of **2** with those of **3**–**8**. Indeed, H-16 signal of **2** (δ_H 7.51, *J* 2.0 Hz)[12] was absent in the corresponding spectra of **3**–**8**. ^{1}H and ^{13}C NMR data attributed to diterpene backbone of all compounds are very similar. The main difference among these compounds resulted from respective resonances of methylamino groups at C-16.

3.2. Antiproliferative activity

The effect of **3**–**8** on proliferation of cancer cells of various histological origins was evaluated. Cell proliferation was determined by spectrophotometric measurements using sulforhodamine B as a protein-binding dye and doxorubicin (DOX; 0.025–25 μg/mL) as a reference drug. Compound concentrations that elicit cell growth inhibition by 50% (GI_{50}) or 100% (TGI) were determined after 48 h of cells treatment.

All novel synthesized furanoditerpenes exhibited antiproliferative activity against the tested cancer cell lines in a concentration-dependent fashion (Fig. 3). Compounds **3** and **7** presented higher selectivity against all tested cells when evaluated at 2.5 μg/mL (Fig. 3A and 3E, respectively). In contrast, compound **4** was the less selective (Fig. 3B).

Table 2 shows that compound **3** was the most potent furanoditerpene derivative against breast (MCF-7; GI_{50} = 0.27 μg/mL) and melanoma (UACC-62; GI_{50} = 0.57 μg/mL) cancer cells. Notably, compound **3** was as potent as the reference drug DOX (GI_{50} = 0.28 μg/mL) against prostate cancer cells (PC-3). The GI_{50} value for **3** against colon (HT-29) cancer cells was 0.32 μg/mL. This compound also inhibited by 50% the growth of erythromyeloblastoid leukemia (K562) cells when used at a concentration as low as 0.25 μg/mL. Compound **5** exhibited the broadest spectra of antiproliferative activity (Table 2). In fact, GI_{50} values for **5** against all cancer cells, except 786-0 and K562 cells, were lower than 1.0 μg/mL (Table 2). This compound was as effective as DOX in inhibiting the growth of adryamycin-resistant ovarian (NCI-ADR/RES) cancer cells. At concentrations lower than 1.0 μg/mL, compound **6** inhibited by 50% the growth of UACC-62, 786-0, PC-3 and colon (HT-29) cancer cells. In the case of prostate cancer cells (PC-3), compound **6** was as potent as DOX (Table 2). Compound **7** was able to inhibit by 50% the growth of MCF-7, PC-3 and K562 cancer cells when used at concentrations lower than 1.0 μg/mL. Compounds **7** and **8** were as potent as DOX against PC-3 cancer cells (Table 2). Promising results were also obtained for **4**, in which GI_{50} values were lower than 3.0 μg/mL for all tested cancer cell lines (Table 2).

In general, the novel furanoditerpenes **3**–**8** were far more potent than **2** in inhibiting cancer cells growth, with exception of NCI-ADR/RES cells.[20] Compound **3** was 164-, 110-, 100- and 9-fold more potent than **2** against HT-29, PC-3, MCF-7 and UACC-62 cancer cells, respectively.[20] For PC-3 cancer cells, compounds **6**–**8** were 90-, 110- and 110-fold more potent than **2**. Compound **5** was 42- and 2-fold more potent than **2** in inhibiting by 50% the growth of lung non-small (NCI-H460) and ovarian (OVCAR-03) cancer cells, respectively. Compound **6** was 175- and 11-fold more potent than **2** in inhibiting by 50% the growth of 786-0 and HT-29 cancer cells growth, respectively, while compound **7** was 6-fold more potent than **2** against K562 cancer cells (Table 2).

Additionally, complete inhibition of cancer cells growth (TGI values) was achieved when compounds **3**–**8** were employed at concentrations lower than those by which compound **2** was effective (Table 2).

3.3. Theoretical calculations

Figure 4 shows the orbitals HOMO−1, HOMO, LUMO, and LUMO+1 of compound **2**, calculated at BLYP/6-31G* level. Occupied orbitals (HOMO−1 and HOMO) of **2** have high contribution of the hydroxyl group at C-6, lactone group and furan ring. The hydroxyl group at C-6 and the furan ring are considered nucleophilic sites that could account for the antiproliferative activity of **2** against the studied human cancer cells. Lactone and furan ring, but not hydroxyl at C-6, significantly contributed to virtual frontier molecular orbitals, pointing them as possible electrophilic sites for the antiproliferative activity of **2** and derivatives against cancer cells. The importance of both hydroxyl at C-6 and lactone groups corroborates the experimental results previously reported.[20]

Theoretical studies also suggest that the *N*-alkyl group at C-16 in **3**–**8** plays critical roles in the inhibitory activity of such furanoditerpenes derivatives on cancer cells.

The orbital populations of backbone hydrogen and carbon atoms of **2** are very similar to those of **3**–**8**. No orbital population variations on OMs frontier were verified when the hydrogen atom at C-16 was substituted for amino groups (data not shown). Nitrogen atom, however, significantly contributed to HOMO of **3**–**8** suggesting it as an additional nucleophilic site for their activities. These calculations also indicate that the nucleophilic property of furanoditerperne derivatives is a critical feature for the antiproliferative activity of these compounds.

With exception of electronic density on C-15 and C-16 in the furan ring, **2** and derivatives **3**–**8** share similarities in other chemical properties. Indeed, C-15 in **2** presents lower electronic density than its counterpart in **3**–**8**. The opposite is observed for C-16 in **2**.

Table 2
Cytotoxicity activity (GI_{50}[a] and TGI[b], in μg/mL) of furanoditerpenes **3**–**8** against human cancer cell lines

Cell line	Compound															
	3		**4**		**5**		**6**		**7**		**8**		DOX[c]		**2**[d]	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
UACC-62	0.57	2.55	2.76	4.96	0.86	5.27	0.78	3.05	2.44	3.96	2.50	4.36	0.09	0.33	5.19	30.01
MCF-7	0.27	1.44	2.50	5.31	0.69	7.40	1.81	3.86	0.88	3.82	2.45	4.77	0.03	3.30	27.66	213.67
NCI-ADR/RES	3.12	8.15	2.64	5.53	0.39	5.39	2.50	6.18	2.65	7.10	2.73	6.34	0.33	6.60	0.35	>250
786-0	2.23	2.49	2.50	3.72	2.50	16.63	0.76	3.98	2.31	4.11	2.37	4.04	0.04	2.67	8.87	37.28
NCI-H460	1.35	3.23	2.96	6.01	0.29	3.20	2.50	4.94	2.50	4.64	2.69	4.05	<0.03[e]	0.90	2.53	12.45
PC-3	0.28	0.58	2.40	3.83	0.98	4.06	0.34	1.69	0.27	0.78	0.27	1.27	0.28	5.85	30.82	91.86
OVCAR-03	2.50	5.62	2.36	3.90	0.28	1.86	2.50	5.28	2.37	4.36	2.60	8.05	0.08	2.95	0.52	8.82
HT-29	0.32	1.14	2.33	3.35	0.67	6.91	0.30	1.61	1.76	2.23	1.68	3.68	0.07	3.90	52.53	>250
K562	<0.25[e]	3.01	2.77	8.87	5.06	86.07	1.58	23.18	0.32	4.55	2.39	8.24	0.40	20.48	2.08	>250

[a] GI_{50} is the concentration of compound (μg/mL) that inhibits cancer cells growth by 50%.
[b] TGI is the concentration of compound (μg/mL) that inhibits cancer cells growth by 100%.
[c] DOX is the reference drug doxorubicin.
[d] Data previously reported by our group is listed for comparison.[20]
[e] The lowest concentration tested was 0.25 μg/mL. Data are means of a representative experiment done in triplicate.

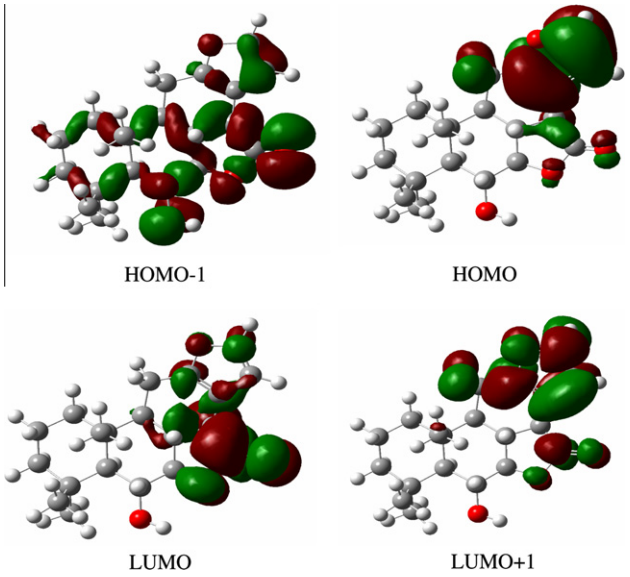


Figure 4. Calculated orbital population (BLYP/6-31G*) from HOMO−1 to LUMO+1 of compound **2**.

4. Conclusion

Six novel amino-derivatives (**3**–**8**) of 6α-hydroxyvouacapan-7β,17β-lactone (**2**) were synthesized and evaluated against nine human cancer cell lines. Such derivatives were found to be promising lead compounds against the majority of the studied cancer cell lines, exhibiting GI_{50} values much lower than those previously reported for the furanoditerpene **2**. Notably, compounds **3** and **5**–**8** showed to be as potent as the reference drug doxorubicin (DOX) against adryamycin-resistant ovarian (NCI-ADR/RES), non-small lung (NCI-H460), and erythromyeloblastoid leukemia (K562) cancer cells. Theoretical calculations showed that amino groups at C-16 could be important for the antiproliferative activity of these furanoditerpene derivatives.

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