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Synthesis of novel anticancer agents through opening of spiroacetal ring of diosgenin

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ABSTRACT

Diosgenin has been modified to furostane derivatives after opening the F-spiroacetal ring. The aldehyde group at C26 in derivative **8** was unexpectedly transformed to the ketone **9**. The structure of ketone **9** was confirmed by spectroscopy and finally by X-ray crystallography. Five of the diosgenin derivatives showed significant anticancer activity against human cancer cell lines. The most potent molecule of this series i.e. compound **7**, inhibited cellular growth by arresting the population at G_0/G_1 phase of cell division cycle. Cells undergo apoptosis after exposure to the derivative **7** which was evident by increase in sub G_0 population in cell cycle analysis. Docking experiments showed caspase-3 and caspase-9 as possible molecular targets for these compounds. This was further validated by cleavage of PARP, a caspase target in apoptotic pathway. Compound **7** was found non-toxic up to 1000 mg/kg dose in acute oral toxicity in Swiss albino mice.

1. Introduction

Cancer is a major heath menace. Over the period, cancer has become a challenge to public healthcare system. The morbidity and mortality of cancer is so high that it is an economic concern to the society nowadays. There are more than 100 types of cancers. Worldwide lung, stomach, liver, colon and breast cancer cause the most deaths each year. About 70% of all cancer deaths occur in low- and middle-income countries. Tobacco use is the single largest preventable cause of cancer in the world causing 20% of cancer deaths. Cancers of major public health relevance such as breast, cervical and colorectal cancer can be cured if detected early and treated adequately. One fifth of all cancers worldwide are caused by a chronic infection, for example human papillomavirus (HPV) causes cervical cancer and hepatitis B virus (HBV) causes liver cancer [1]. Despite continued efforts of researchers to combat cancer, this disease presents about 13% of total deaths.

Development of cancer therapeutics from steroids has been an attractive choice for medicinal chemists and many active molecules have emerged. Steroids have been developed either as antiproliferative or cytotoxic agents. Withaferin A (**1**), Gymnasterol (**2**), 24-hydroxyperoxide desmosterol (**3**), timosaponin A-III (**4**) etc. are some of the notable plant based cytotoxic steroidal leads [2,3]. Semisynthetic modification of some these natural products have yielded better cytotoxic analogs. Several synthetic analogs of withaferin are much better cytotoxic compounds [2].

Diosgenin (**5**) is a C_{27} spiroacetal steroidal sapogenin abundantly available in nature. It is obtained mainly in saponin form from *Smilax* spp., *Dioscorea* spp., *Costus speciosus* etc. The molecule exhibits significant activity against colon and leukemia cells by inducing apoptosis [4a–c]. In the present communication, we modified diosgenin at spiroketal position to get few anticancer analogs. While transforming C_{29} aldehyde to Schiff's bases a C_{28} ketone was formed which was confirmed by spectroscopy. All the derivatives were evaluated for cytotoxicity by Sulphorhodamine assay against five human cancer cell lines. The best analog of the series was further evaluated for cell cycle analysis and *in-vivo* acute oral toxicity in Swiss-albino mice.

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2. Experimental

2.1. General

Melting points were determined in open capillaries using E-Z Melt automated melting point apparatus, Stanford Research System, USA and were uncorrected. The starting substrate diosgenin was procured for Sigma, USA. Dry solvents were prepared as per standard methods. Reactions were monitored on aluminium thin layer chromatography (TLC, UV_{254nm} plates), E. Merck Germany. Further, visualization was accomplished by spraying with a solution of 2% ceric sulfate in 10% aqueous sulfuric acid and charring at 80–100 °C. Column chromatography was carried out on silica gel (100–200 mesh, Avra Chemicals, India). NMR spectra were obtained on Bruker Avance-300 MHz instrument with tetramethylsilane (TMS, chemical shifts in δ ppm) as an internal standard. ESI mass spectra were recorded on API 3000 LC-MS-MS, Applied Biosystem, USA after dissolving the compounds in methanol or acetonitrile. The best compound **7** was analyzed for high resolution mass (ESI-HRMS) also in Agilent 6520 Q-TOF. FT-IR spectra were recorded on Perkin-Elmer SpectrumBX. X-ray diffraction data were collected on a Bruker AXS SMART APEX CCD diffractometer using MoK_α radiation ($\lambda = 0.71073$ Å). Nomenclature of steroid derivatives has been given as per the recommendations published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC [5].

2.2. Chemical synthesis

2.2.1. Synthesis of (22β,25R)-spirost-5-en-3β-yl-3-acetate (6)

Acetylation of diosgenin (**5**) was done as per reported method [6a] with a little modification using dry chloroform as a co-solvent.

6: Yield = 1.01 g (91%), mp = 193–96 °C [195 °C, 6b]; ^{1}H NMR ($CDCl_3$), δ 0.77 (s, 3H,18-CH_3), 0.96 (d, 3H, 27-CH_3), 1.02 (s, 3H, 19-CH_3), 1.11–2.31 (m, 25H, rest of the 1 × CH_3, 8 × CH_2 and 6 × CH of steroidal ring), 2.01 (s, 3H, CH_3COO, Acetate), 2.24-2.31 (bd, 2H, 7-CH_2), 3.38 (m, 2H, 26-CH_2), 4.37 (bs, 1H, 3-CH), 4.42 (bd, 1H, 16-CH), 5.36 (s, 1H, 6-CH). ^{13}C NMR ($CDCl_3$, 75 MHz); δ 14.89 (C21), 16.64 (C18), 17.51 (C11), 19.69 (C19), 21.20 (C11), 21.74 (acetate CH_3), 28.12 (C24), 29.19 (C2), 30.66 (C25), 31.78 (C23), 31.80 (C8), 32.21 (C7), 32.41 (C15), 37.10 (C10), 37.34 (C1), 38.47 (C12), 40.10 (C4), 40.63 (C13), 42.00 (C20), 42.68, 50.35 (C9), 56.82 (C14), 62.52 (C17), 67.19 (C26), 74.26 (C3), 81.16 (C16), 109.60 (C22), 122.72 (C6), 140.05 (C5), 170.82 (acetate ester). ESI Mass (MeOH): 457.3 $[M+H]^+$, 479.3 $[M+Na]^+$, 495.4 $[M+K]^+$. IR (KBr, cm^{-1}): 2907, 1724, 1451, 1231.

2.2.2. Synthesis of (22β,25R)-3β,26-dihydroxyfurost-5-en-3β-acetate (7)

Compound **7** was synthesized as per reported method [6a].

7: Yield = 164 mg (81%), mp = 108-110 °C [6a]. ^{1}H NMR ($CDCl_3$): δ 0.83 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3), 1.03-1.90 (m, 28H, rest of the 2 × CH_3, 8 × CH_2 and 6 × CH of steroidal ring), 2.05 (s, 3H, CH_3COO, acetate), 2.35 (bd, 2H, 7-CH_2), 3.36 (bs, 1H, 22-CH), 3.48 (m, 2H, 27-CH_2OH), 4.34 (bs, 1H, 3-CH), 4.63 (bs, 1H, 16-CH), 5.40 (s, 1H, 6-CH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.80 (C18), 17.00 (C20), 19.30 (C27), 19.69 (C19), 21.03 (C11), 21.77 (acetate CH3), 28.13 (C24), 30.46 (C2), 30.82 (C25), 31.94 (C8), 32.36 (C7), 32.59 (C15), 36.08 (C23), 37.07 (C10), 37.37 (C1), 38.28 (C4), 38.46 (C12), 39.78 (C13), 41.07 (C20), 50.39 (C9), 57.28 (C14), 65.48 (C17), 68.27 (C26), 74.28 (C3), 83.57 (C16), 90.71 (C22), 122.74 (C6), 140.04 (C5), 170.91 (acetate ester); ESI Mass (MeOH): 459.4 $[M+H]^+$, 481.3 $[M+Na]^+$, 497.4 $[M+K]^+$; ESI-HRMS: 459.3467 for $C_{29}H_{47}O_4$, cal: 459.3474; 481.3282 for $C_{29}H_{46}O_6Na$, cal: 481.3294; IR (KBr, cm^{-1}): 3423, 2934, 1731, 1456, 1376, 1248, 1035.

2.2.3. Synthesis of (22β,25R)-3β-hydroxy,26-formyl-furost-5-en-3β-acetate (8)

Alcohol **7** (200 mg, 0.43 mmol) was dissolved in dry dichloromethane (10 mL) and stirred at room temperature. To this pyridinium chlorochromate (PCC) (200 mg, 0.93 mmol) was added and further stirred for an hour. Solvent was evaporated and residue was dissolved in ethyl acetate (30 mL). It was acidified with dil. HCl (5%, 10 mL) and washed with water. The organic layer was dried over anhydrous sodium sulfate and dried *in vacuo*. The crude mass was recrystallised with chloroform-hexane (1:3) to get aldehyde **8** as brown crystalline solid.

8: Yield = 182 mg (91%), mp = 119-123 °C; ^{1}H NMR ($CDCl_3$): δ0.80 (s, 3H, 18-CH_3), 0.93 (s, 3H, 19-CH_3), 1.16-1.97 (m, 28H, rest of the 2 × CH_3, 8 × CH_2 and 6 × CH of steroidal ring), 2.16 (s, 3H, CH_3COO, Acetate), 2.46 (bd, 2H, 7-CH_2), 3.45 (bs, 1H, 22-CH), 4.44 (bs, 1H, 3-CH), 4.73 (bd, 1H, 16-CH), 5.50 (s, 1H, 6-CH), 9.75 (s, 1H, 26-CHO). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.78 (C21), 16.79 (C18), 19.22 (C19), 19.71 (C27), 21.03 (C11), 21.77 (acetate CH_3), 28.15 (C24), 30.07 (C2), 31.11 (C23), 31.96 (C20), 32.37 (C7), 32.59 (C15), 37.09 (C10), 37.39 (C1), 38.26 (CH), 38.48 (C12), 39.77 (C4), 41.08 (C13), 46.72 (C25), 50.41 (C9), 57.29 (C14), 65.44 (C17), 74.28 (C3), 83.28 (C16), 90.11 (C22), 122.73 (C6), 140.09 (C5), 170.91 (acetate ester), 205.54 (C26); ESI Mass (MeOH): 457.3 $[M+H]^+$, 479.3 $[M+Na]^+$, 495.4 $[M+K]^+$; IR (KBr, cm^{-1}): 2833, 1739, 1254.

2.2.4. Synthesis of (22β)-3β-hydroxy,25-oxo-27-nor-furost-5-en-3β-acetate (9)

Aldehyde **8** (200 mg, 0.44 mmol) was taken in ethanol (10 mL) and stirred at ambient temperature (30-35 °C). To this 3,4,5-trimethoxyaniline (200 mg, 1.09 mmol) was added and further stirred for 2 h. The solvent was evaporated, residue was dissolved in ethyl acetate (30 mL) and washed with water. The organic phase was dried over anhydrous sodium sulfate and dried *in vacuo*. The crude mass was purified through silica gel column eluting with ethyl acetate:hexane. The ketone **9** was obtained at 8–10% ethyl acetate hexane as creamish white solid.

9: Yield = 163 mg (84%), mp = 138-40 °C; ^{1}H NMR ($CDCl_3$): δ 0.79 (s, 3H, 18-CH_3), 0.98 (s, 3H, 19-CH_3), 1.02-1.87 (m, 23H, rest of the 1 × CH_3, 8 × CH_2 and 4 × CH of steroidal ring), 1.95 (s, 3H, CH_3COO, acetate), 2.13 (s, 3H, 26-CH_3CO), 2.32 (d, 1H, 7-CH_2, J = 6.3 Hz), 2.51–2.63 (bd, 2H, 24-CH_2), 3.26–3.29 (bs, 1H, 22-CH), 4.24–4.29 (bs, 1H, 16-CH), 4.57 (bd, 1H, 3-CH), 5.35 (s, 1H, 6-CH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.80 (C18), 19.01 (C19), 19.71 (C21), 21.02 (C11), 21.80 (acetate CH_3), 27.44 (C23), 28.13 (C1), 30.33 (C26), 31.95 (C8), 32.37 (C7), 32.55 (C15), 37.10 (C10), 37.38 (C2), 38.24 (C20), 38.48 (C12), 39.75 (C4), 41.09 (C13), 41.28 (C24), 50.38 (C9), 57.27 (C14), 65.39 (C17), 74.28 (C3), 83.69 (C16), 89.53 (C22), 122.72 (C6), 140.12 (C5), 170.95 (Acetate ester), 209.24 (C25); ESI Mass (MeOH): 443.3 $[M+H]^+$, 465.4 $[M+Na]^+$, 481.3 $[M+K]^+$; IR (KBr, cm^{-1}): 2927, 1724, 1453, 1372, 1241.

2.2.5. Wittig reaction on aldehyde 8

Synthesis of (22β)-(E)-26-Benzylidene-3β-yl-furost-5-en-3-acetate (10): Benzyltriphenylphosphonium bromide (Wittig salt, 200 mg) was taken in dry toluene (10 mL). To this stirred solution pre-washed sodium hydride (200 mg, 8.33 mmol) added and stirred for 20 min. Aldehyde **8** (100 mg, 0.22 mmol) was added and the reaction mixture was further stirred for 2 h. Toluene was evaporated under vacuum and residue was taken in ethyl acetate