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Studies on novel D-ring substituted steroidal pyrazolines as potential anticancer agents

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ABSTRACT

An efficient and facile synthesis of 17-pyrazolinyl derivatives of pregnenolone and their evaluation as potential anticancer agents against various human cancer cell lines are reported. The scheme involves the transformation of the starting pregnenolone acetate into pregnenolone, conversion of pregnenolone to the corresponding benzylidine derivatives and finally the conversion of this derivative to the stable steroidal 17-pyrazoline. Various compounds **4b**, **4c**, **4e**, **4f**, **4h** and **4j** showed significant cytotoxic activity especially against HT-29, HCT-15, 502713 cell lines.

1. Introduction

Recent years have seen an extensive focus of research directed towards the rational modification of steroid molecules. This is probably because of the various advantages associated with steroid based chemotherapeutics. These compounds turn out to be non-toxic, less vulnerable to multi-drug resistance (MDR) and highly bioavailable because of being capable of penetrating the cell wall. This is pertinently true of modified steroids bearing heterocyclic systems as a part of their skeleton. This has been proved by different ring modification studies of steroidal molecules involving the A- and D-ring whereby incorporation of heteroatom (N or O) has been reported to enhance the biological activities of these molecules. Such systems have shown a lot of different biological activities such as anti-microbial, anti-inflammatory, hypotensive, hypocholesterolemic and diuretic activities [1–5]. As a result, a number of different heterocyclic systems have been introduced into the core structure of steroids with pyrazoles, pyrazolines, isoxazoles, isoxazolines, thiazoles, thiadiazoles, pyridines, pyrimidines, imidazoles, etc. as the notable ones. Amongst these heterocycles, pyrazolines are an interesting group of compounds, many of which possess wide spread pharmacological properties such as analgesic, antipyretic and antiandrogenic activities [6,7]. Pyrazolines also possess antidepressant, anti-inflammatory and antirheumatic activities [6,8,9]. Besides Pyrazolines are also used as potent antidiabetic agents [10,11]. Recently, pyrazolines were reported as a DP-IV inhibitors and antitumor agents [12–14]. Nitrogen heterocycles have also recently been reported to exhibit antiparkinsonian [15] anticancer [16–18], antimicrobial [19–21] activities.

In view of the therapeutic importance of these steroidal heterocycles, we, in continuation of our programme on the synthesis of D-ring substituted steroidal heterocyles, aimed to investigate the synthesis of steroidal pyrazolines starting from readily available 20-keto pregnenanes [22]. In spite of few preliminary reports about the synthesis of steroidal diphenyl pyrazolines, no literature precedants were found describing the synthesis of steroidal pyrazolines through the strategy we have employed. Keeping all these facts into consideration, we herein report a very facile and high yielding approach for the synthesis of steroidal D-ring substituted pyrazolines starting from 20-keto pregnenanes involving the intermediacy of the corresponding benzylidines prepared by condensation with various aromatic aldehydes.

2. Experimental

2.1. General methods

Melting points were recorded on Buchi Melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. NMR spectra were recorded on Bruker DPX200 instrument in $CDCl_3$ with TMS as internal standard for

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protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants are given in Hz. Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. The progress of all reactions was monitored by TLC on 2 cm × 5 cm precoated silica gel 60 F254 plates of thickness of 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine.

2.2. Chemical synthesis

2.2.1. General procedure for the synthesis of pyrazoline derivatives

To a solution of pregnenolone **1** (0.316 g, 1 mmol, 1 equiv.) in ethanol (10 ml) was added a conc. aq. solution of KOH (2 equiv.). Then aldehyde **2** (1.2 equiv.) was charged into the reaction mixture to get the corresponding benzylidine derivative **3**. After completion, the reaction mixture was precipitated with water. The precipitate was filtered, dried and recrystallized from EtOAc:hexane to give product as solid white powder. It is to be mentioned that when non-aromatic aldehydes were used, the product was formed in a very minor quantity and that too not stable enough at ambient conditions. Thus the study was restricted to the use of aromatic aldehydes only. The condensation product **3** (1.0 g, 2.4 mmol) was refluxed in ethanol in the presence of hydrazine hydrate (0.24 g, 4.8 mmol) so as to yield the desired pyrazolines. However the products thus obtained were very unstable and they decomposed even at ambient temperature conditions probably because of the inherent instability associated with pyrazolines. The solvent thus used was replaced by acetic acid so as to ensure the formation of N-acetyl pyrazoline **4** (0.99 g, 2.2 mmol, 90%) which was highly stable. The product was precipitated by charging the reaction mass into excessive amounts of ice-cold water. After filtration under suction, the product was obtained in high yields as colourless powder which was later dried in vacuo. The same procedure was followed for the synthesis of all other analogs. The spectral data of various compounds is given as under (most of the peaks due to steroidal skeleton were merged and could not be differentiated. Thus δ values of only those peaks that distinguish the product and could easily be differentiated are reported):

(1) *1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-phenylpyrazol-1-yl) ethanone (**4a**)*: Colourless solid powder. Yield 76%. M.p: 123–125 °C. $[\alpha]_D^{25}$ −16.9 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3384, 2926, 1717, 1646, 1404, 1042, 699. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.63 (s, 3H), 1.06 (s, 3H), 1.82–1.90 (m, 6H), 2.17 (s, 3H), 2.65 (t, 1H, J = 8.8), 2.79 (m, 2H), 3.26 (m, 1H), 3.49 (m, 1H), 5.33 (s, 1H), 5.44 (m, 1H), 7.15 (d, 2H, J = 6.5), 7.22–7.32 (m, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.85, 20.85, 22.40, 23.32, 25.83, 31.13, 33.04, 33.48, 37.99, 38.72, 39.94, 43.68, 45.30, 47.69, 51.53, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 128.84, 130.28, 142.29, 160.63, 164.62. ESI-MS: 483 (M^++Na). Anal. Calcd. For $C_{30}H_{40}N_2O_2$: C, 78.22; H, 8.75; N, 6.08; found C, 78.47; H, 8.83; N, 6.21.

(2) *1-(5-(3-Fluorophenyl)-4,5-dihydro-3-((10R,13S)2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl) pyrazol-1-yl)ethanone (**4b**)*: Colourless solid. Yield 79%. M.p: 118–121 °C. $[\alpha]_D^{25}$ −19.2 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3408, 2936, 1718, 1448, 1021, 756. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.63 (s, 3H), 1.06 (s, 3H), 1.82–1.90 (m, 6H), 2.15 (s, 3H), 2.67 (t, 1H, J = 8.8), 2.77 (m, 2H), 3.26 (m, 1H), 3.35 (m, 1H), 5.30 (s, 1H), 5.39 (m, 1H), 6.80–6.91 (m, 2H), 7.30–7.34 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.24, 18.40, 19.95, 20.82, 23.58, 28.60, 30.59, 31.03, 35.54, 36.27, 37.38, 41.23, 45.07, 50.69, 55.38, 57.66, 70.64, 111.11, 113.16, 120.30, 129.37, 139.86, 143.79, 159.69, 164.58, 167.71. ESI-MS: 479 (M^++H). Anal. Calcd. for $C_{30}H_{39}FN_2O_2$: C, 75.28; H, 8.21; N, 5.85; found C, 75.43; H, 8.03; N, 6.04.

(3) *1-(5-(4-Fluorophenyl)-4,5-dihydro-3-((10R,13S)2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl) pyrazol-1-yl)ethanone (**4c**)*: Colourless solid powder. Yield 82%. M.p: 156–158 °C. $[\alpha]_D^{25}$ −25.0 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3375, 3166, 2928, 1721, 1404, 1042, 756. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.65 (s, 3H), 1.05 (s, 3H), 1.82–1.90 (m, 6H), 2.17 (s, 3H), 2.76 (m, 2H), 3.26 (m, 1H), 3.35 (m, 1H), 5.35 (s, 1H), 5.37 (m, 1H), 7.04 (d, 2H, J = 8.6), 7.14 (d, 2H, J = 8.4). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.34, 19.40, 19.65, 20.82, 23.58, 28.60, 30.59, 31.03, 35.54, 36.27, 38.38, 41.23, 42.91, 45.07, 51.69, 56.38, 57.66, 70.64, 111.11, 113.16, 120.30, 129.37, 139.86, 143.79, 159.15, 162.71, 164.56, 167.71. ESI-MS: 479 (M^++H). Anal. Calcd. For $C_{30}H_{39}FN_2O_2$: C, 75.28; H, 8.21; N, 5.85; found C, 75.51; H, 8.05; N, 6.09.

(4) *1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-p-tolylpyrazol-1-yl)ethanone (**4d**)*: Greyish powder. Yield 79%. M.p: 130–133 °C. $[\alpha]_D^{25}$ −33.3 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3416, 2936, 1719, 1642, 1455, 1087, 756. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.61 (s, 3H), 1.08 (s, 3H), 1.82–1.92 (m, 6H), 2.05 (s, 3H), 2.22 (s, 3H), 2.67 (t, 1H, J = 8.8), 2.77 (m, 2H), 3.40 (m, 1H), 3.48 (m, 1H), 5.36 (m, 2H), 7.80 (dd, 4H, J = 6.3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.87, 20.54, 22.46, 23.38, 25.83, 31.13, 33.04, 33.19, 33.48, 37.99, 38.72, 39.94, 43.68, 45.30, 47.38, 47.69, 51.53, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 128.84, 130.28, 142.29, 161.65, 162.53. ESI-MS: 497 (M^++Na). Anal. Calcd. For $C_{31}H_{42}N_2O_2$: C, 78.44; H, 8.92; N, 5.90; found C, 78.67; H, 8.73; N, 6.13.

(5) *1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-o-tolylpyrazol-1-yl) ethanone (**4e**)*: Colourless solid. Yield 83%. M.p: 124–126 °C. $[\alpha]_D^{25}$ −23.4 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3406, 2941, 1729, 1642, 1455, 1077, 753. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.61(s, 3H), 1.09 (s, 3H), 1.84–1.93 (m, 6H), 2.10 (s, 3H), 2.66 (t, 1H, J = 8.8), 2.74 (m, 2H), 3.40 (m, 1H), 3.44 (m, 1H), 5.22 (s, 1H), 5.29 (m, 1H), 6.84 (m, 1H), 7.06 (m, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 11.76, 14.85, 20.85, 22.40, 23.32, 25.83, 31.13, 33.04, 33.19, 33.48, 37.99, 36.72, 39.84, 43.68, 45.80, 47.35, 47.69, 51.53, 53.18, 57.94, 60.74, 73.11, 123.80, 126.78, 127.84, 130.28, 145.29, 161.63, 166.62, ESI-MS: 497 (M^++Na). Anal. Calcd. For $C_{31}H_{42}N_2O_2$: C, 78.44; H, 8.92; N, 5.90; found C, 78.27; H, 8.85; N, 6.09.

(6) *1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-m-tolylpyrazol-1-yl) ethanone (**4f**)*: Colourless solid powder. Yield 74%. M.p: 120–122 °C. $[\alpha]_D^{25}$ −25.5 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3374, 2939, 1719, 1638, 1404, 1041, 756, 702. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 1.03 (s, 3H), 1.81–1.92 (m, 6H), 2.19 (s, 3H), 2.20–2.23 (m, 3H), 3.40 (m, 1H), 3.47 (m, 1H), 5.35 (m, 2H), 7.03 (d, 1H, J = 7.19), 7.17 (d, 1H, J = 7.19). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.35, 20.85, 22.40, 23.32, 25.83, 31.13, 33.04, 33.19, 33.48, 37.99, 39.72, 39.94, 43.68, 45.30, 46.38, 47.69, 51.53, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 128.84, 131.35, 143.23, 161.63, 167.62. ESI-MS: 475 (M^++H). Anal. Calcd. For $C_{31}H_{42}N_2O_2$: C, 78.44; H, 8.92; N, 5.90; found C, 78.24; H, 8.84; N, 6.11.

(7) *1-(5-(Furan-2-yl)-4,5-dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)pyrazol-1-yl)ethanone (**4g**)*: Colourless powder. Yield 82%. M.p: 127–129 °C. $[\alpha]_D^{25}$ −25.8 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3386, 2936, 1727, 1647, 1451, 1043, 754. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 1.02 (s, 3H), 1.81–1.90 (m, 6H), 2.17 (s, 3H), 3.10–3.15 (m, 3H), 3.52 (m, 1H), 5.30 (s, 1H), 5.37 (s, 1H), 5.51(m, 1H), 6.28 (m, 2H), 7.30 (s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.85, 20.85, 22.90, 22.32, 25.84, 31.13, 33.04, 33.19, 33.48, 37.99, 38.82, 39.94, 44.68, 45.30, 47.38, 48.69, 51.78, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 128.84, 130.28, 142.29, 160.61, 164.62. ESI-MS: 473 (M^++Na). Anal. Calcd. For $C_{28}H_{38}N_2O_3$: C, 74.63; H, 8.50; N, 6.22; found C, 74.47; H, 8.71; N, 6.47.

(8) *1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-(4-methoxyphenyl) pyrazol-1-yl)ethanone (**4h**)*: Colourless solid. Yield 84%. M.p: 103–105 °C. $[\alpha]_D^{25}$ −28.4 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3406, 2930, 2871, 1719, 1642, 1419, 1041, 757. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 1.01 (s, 3H), 1.82–1.88 (m, 6H), 2.03 (s, 3H), 2.53–2.73 (m, 2H), 3.20 (m, 1H), 3.29 (m, 1H), 3.76 (s, 3H), 5.34 (m, 2H), 6.82 (d, 2H, J = 8.1), 7.05 (d, 2H, J = 8.1). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.38, 20.85, 22.44, 23.32, 25.83, 31.13, 33.04, 33.19, 34.48, 37.99, 38.72, 39.94, 43.68, 45.30, 47.38, 47.69, 51.55, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 127.84, 130.28, 142.29, 160.63, 165.62. ESI-MS: 491 (M^++H). Anal. Calcd. For $C_{31}H_{42}N_2O_3$: C, 75.88; H, 8.63; N, 5.71; found C, 75.63; H, 8.81; N, 5.87.

(9) *1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-(2-methoxyphenyl) pyrazol-1-yl)ethanone (**4i**)*: Colourless solid. Yield 80%. M.p: 130–132 °C. $[\alpha]_D^{25}$ −33.1 (*ca.* 0.20, $CHCl_3$). IR (KBr, cm^{-1}): 3399, 2936, 2871, 1719, 1642, 1419, 1039, 757. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.66 (s, 3H), 1.01 (s, 3H), 1.81–1.88 (m, 6H), 2.04 (s, 3H), 2.54–2.73 (m, 2H), 3.20 (m, 1H), 3.29 (m, 1H), 3.83 (s, 3H), 5.35 (m, 2H), 6.85–6.93 (m, 2H), 7.24–7.33 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 13.67, 20.85, 22.40, 23.32, 25.83, 31.13, 31.04, 33.19, 33.48, 37.99, 38.72, 39.94, 43.68, 45.30, 47.38, 43.69, 51.53, 53.18, 57.94, 60.56, 73.11, 122.80, 126.78, 128.84, 130.28, 142.29, 160.73, 164.62. ESI-MS: 491 (M^++H). Anal. Calcd. For $C_{31}H_{42}N_2O_3$: C, 75.88; H, 8.63; N, 5.71; found C, 75.99; H, 8.41; N, 5.65.

(10) *1-(5-(2-Chlorophenyl)-4,5-dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl) pyrazol-1-yl)ethanone (**4j**)*: Colourless white powder. Yield 79%. M.p: 128–130 °C. $[\alpha]_D^{25}$ −25.6 (*ca.* 0.20, $CHCl_3$). IR ($CHCl_3$, cm^{-1}): 3386, 2944, 1720, 1640, 1492, 1407, 1090, 962, 756. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 1.08 (s, 3H), 1.81–1.88 (m, 6H), 2.04 (s, 3H), 2.57–2.65 (m, 2H), 3.20 (m, 1H), 3.50 (m, 1H), 5.34–5.42 (m, 2H), 7.09 (d, 2H, J = 8.4), 7.37 (d, 2H, J = 8.4). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.27, 12.46, 18.40, 19.61, 20.80, 23.36, 23.55, 30.53, 30.72, 31.01, 35.53, 36.25, 37.51, 41.17, 45.11, 49.23, 50.68, 55.46, 57.52, 70.66, 120.33, 125.82, 127.98, 132.14, 139.80, 158.60, 166.63, 167.71, 173.88. ESI-MS: 517.4 (M^++Na). Anal. Calcd. For $C_{30}H_{39}ClN_2O_2$: C, 72.78; H, 7.94, Cl, 7.16,; N, 5.66; found C, 72.96; H, 8.21; Cl, 7.01; N, 5.79.

2.3. Biology

The human cancer cell lines used for the test were HT-29, HCT-15 (Colon), 502713 (Colon), HOP-62, A-549 (Lung), MCF-7 (Breast) and SF-295 (CNS) and were obtained from National Cancer Institute (NCI), biological testing branch, Federick Research and Development Centre, USA. Cellular viability in the presence and absence of experimental agents was determined using the standard Sulforhodamine B assay. Briefly, cells in their log phase of growth were harvested, counted and seeded (10^4 cells/well in 100 μL medium) in 96-well microtitre plates. After 24 h of incubation at 37 °C and 5% CO_2 to allow cell attachment, cultures were treated with varying concentrations (0.1–100 μM) of test samples made with 1:10 serial dilutions. Four replicate wells were set up for each experimental condition. Test samples were left in contact with the cells for 48 h under same conditions. Thereafter, cells were fixed with 50% chilled trichloroacetic acid (TCA) and kept at 4 °C for 1 h, washed and air-dried. Cells were stained with Sulforhodamine B dye. The adsorbed dye was dissolved in Tris-Buffer and plates were gently shaken for 10 min on a mechanical shaker. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was calculated by subtracting mean OD value of respective blank from the mean OD value of experimental set. Percent growth in presence of test material was calculated considering the growth in absence of any test material as 100% and in turn percent growth inhibition in presence of test material was calculated. Finally the IC_{50} values (Table 1) were calculated using Microsoft Office Excel. The different steroidal derivatives (test material) were dissolved in a mixture of DMSO:water (1:1) and then introduced into the medium containing the cancer cell lines.

3. Results and discussion

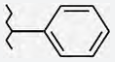
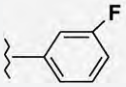
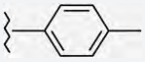
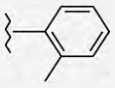
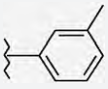
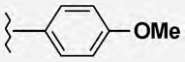
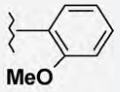
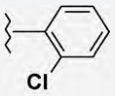
Though the importance of different steroidal D-ring heterocycles is now well validated, only few efforts have been reported for their efficient synthesis, to the best our knowledge. This specially refers to the pyrazoline based heterocycles at the D-ring of steroids including the very important class of pregnanes. Though there are reports for the synthesis of other such heterocycles, the same is not true for the pyrazoline derivatives at the D-ring. Taking inspiration from the number of reported biological activities associated with structurally related analogs, we, in continuation of our efforts towards the synthesis of novel D-ring heterocycles, herein report a novel efficient and simple synthesis of D-ring pyrazoline derivatives of 20-keto pregnenanes and their evaluation as potential anticancer agents. The preparation of the latter involves the following synthetic approach (Scheme 1).

3.1. Biology

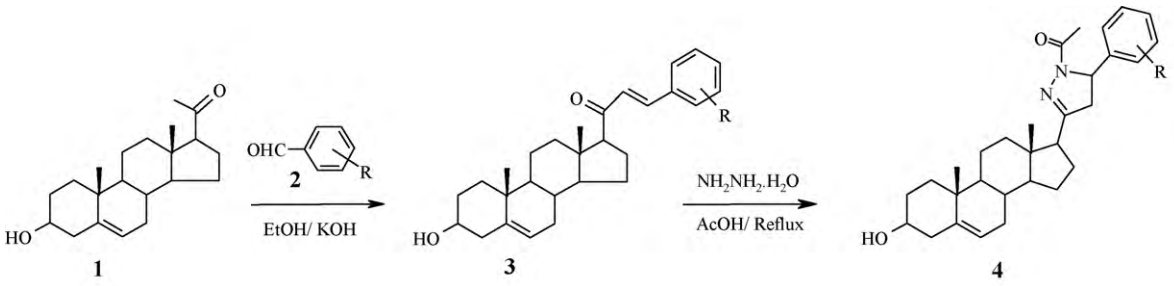
The following table gives the cancer cell inhibitory data obtained after treating different cancer cell lines with test doses of the different steroidal pyrazoline derivatives and the values are reported in terms of IC_{50}.

A number of correlations can be made from the data given in the table above. It is clear from the IC_{50} values, that the compounds **4b**, **4c**, **4e**, **4f** and **4h** showed significant cytotoxic activity especially against HT-29, HCT-15, 502713 cell lines. Moreover the cytotoxicity of the compounds for certain cancer cell lines increases more than hundred times when just the substitutions are changed over the aromatic ring (IC_{50} values of compounds **4i** and **4j** may be compared for *HT-29* and *HCT-15* cancer cell lines). It is also evident from the data that even the change in the position of same substituent on the aromatic ring influences the relative toxicity. This can be attributed to their differences in either polarity which changes their lipophilicity or the conformation which alters the target protein binding properties present within the cell or on the cell membrane (for comparison see the IC_{50} values of **4b** and **4c**). Com-

Table 1
IC_{50} values (μM) of 17-pyrazolinyl derivatives of pregnenolone against a panel of human cancer cell lines.

Compound	HT-29	HCT-15	502713	HOP-62	A-545	MCF-7	SF-295	R
4a	6.35	2.31	0.56	1.46	11.0	0.43	0.63	
4b	0.44	0.53	0.32	0.50	1.42	1.60	3.79	
4c	0.53	1.46	0.72	4.18	1.67	1.84	8.86	
4d	6.25	5.11	20.8	ND	8.11	4.84	3.65	
4e	0.34	0.62	0.41	0.69	0.64	0.79	1.0	
4f	0.35	0.31	0.43	0.57	0.56	1.60	1.67	
4g	11.8	23.4	44.2	50.0	48.1	ND	50.6	
4h	2.37	0.65	0.46	0.81	0.7	1.91	5.44	
4i	25.7	21.8	28.5	32.9	29.2	23.1	49.5	
4j	0.24	0.25	37.0	50.0	32.2	17.5	30.2	

ND, not determined. Cell lines: HT-29, HCT-15 (Colon), 502713 (Colon), HOP-62 (Lung), A549 (Lung), MCF-7 (Breast) and SF-295 (CNS).



Scheme 1. Synthesis of D-ring substituted steroidal pyrazolines.

pound **4j**, which contained an *o*-chloro substitution on the aromatic ring, showed significant cytotoxicity, selective against HT-29 and HCT-15 cell lines.

4. Conclusion

A series of novel 17-pyrazolinyl derivatives of pregnenolones were synthesized and screened for anticancer activity against a panel of seven human cancer cell lines. From the data it was found that all the compounds are having promising anticancer activity and the compound **4j** was found to be the most active especially against *HT-29* and *HCT-15* cancer cell lines indicating the high degree of selectivity of this *o*-chloro phenyl compound against the said cell lines.

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