Journal of Saudi Chemical Society (2016) **20**, 7–12

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Synthesis, characterization, antimicrobial and anticancer studies of new steroidal pyrazolines

Shamsuzzaman [a,*], **Hena Khanam** [a], **Ayaz Mahmood Dar** [a], **Nazish Siddiqui** [b], **Sumbul Rehman** [b]

[a] *Department of Chemistry, Aligarh Muslim University, Aligarh 202002, India*
[b] *Department of Illmul Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh 202002, India*

Received 9 March 2012; accepted 12 May 2012
Available online 19 May 2012

KEYWORDS

Cholest-5-en-7-one;
2,4-Dinitrophenylhydrazine;
Pyrazoline;
Antimicrobial;
Anticancer

Abstract A convenient synthesis of 2′-(2″,4″-dinitrophenyl)-5α-cholestano [5,7-*c d*] pyrazolines 4–6 from cholest-5-en-7-one **1**–**3** was performed and structural assignment of the products was confirmed on the basis of IR, ^{1}H NMR, ^{13}C NMR, MS and analytical data. The synthesized compounds were screened for *in vitro* antimicrobial activity against different strains during which compound **6** showed potent antimicrobial behaviour against *Corynebacterium xerosis* and *Staphylococcus epidermidis*. The synthesized compounds were also screened for *in vitro* anticancer activity against human cancer cell lines during which compound **5** exhibited significant anticancer activity.

1. Introduction

In the last few decades there has been an extensive focus of research towards the rational modification of steroid molecules. This is due to the fact that such type of compounds are less toxic, less vulnerable to multi-drug resistance (MDR) and highly bioavailable because of being capable of penetrating the cell wall. Recent studies reveal that incorporation of heteroatom (N/O/S) enhances the biological activities of steroid molecules. This is proved by various activities shown by these systems like antimicrobial, anti-inflammatory, hypotensive, hypocholesterolemic and diuretic activities (Manson et al., 1963; Hirschmann et al., 1963, 1964; Wang et al., 1993; Gupta et al., 1996). 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. Among these heterocycles, pyrazolines occupy a unique place in the realm of natural and synthetic organic chemistry (Jung et al., 2005).

Pyrazoline derivatives are synthetic targets of utmost importance for the researchers, since such type of compounds have a wide range of biological and pharmaceutical properties such as analgesic, antipyretic and antiandrogenic activities (Jung et al., 2005; Amr et al., 2005). Pyrazolines also possess antidepressant, anti-inflammatory and antirheumatic activities (Palaska et al., 2001; Bansal et al., 2001). Besides this pyrazolines are also used as potent antidiabetic agents (Villhauer et al., 2002; Ahn et al., 2004). Recently, pyrazolines were reported as a DP-IV inhibitors and antitumor agents (Amr, 2000; Hammam et al., 2000; 2003).

* Corresponding author. Tel.: +91 9411003465.
E-mail address: shamsuzzaman9@gmail.com (Shamsuzzaman).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1319-6103
http://dx.doi.org/10.1016/j.jscs.2012.05.004

In the view of reports of synthesis of pyrazolines (Desai et al., 2012) and in continuation of our search for biologically active steroidal pyrazoline derivatives (Shamsuzzaman et al., 2009), we have developed an efficient synthetic strategy for the generation of steroidal N-substituted 2-pyrazoline derivatives. These scaffolds are being subjected to biological screenings like antimicrobial and anticancer activities.

2. Experimental protocol

Melting points were determined on a Kofler apparatus and are uncorrected. The IR spectra were recorded on KBr pellets with Pye Unicam SP3-100 spectrophotometer and values are given in cm^{-1}. ^1H and ^{13}C NMR spectra were run in $CDCl_3$ on a JEOL Eclipse (400 and 100 MHz) instrument with tetramethylsilane (TMS) as internal standard and values are given in parts per million (ppm) (δ). Mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapours to check the homogeneity as well as the progress of reaction. Sodium sulphate (anhydrous) was used as a drying agent.

*2.1. General procedure for the syntheses of the steroidal pyrazoline derivatives (**4**–**6**)*

To a solution of steroidal α,β-unsaturated ketone **1–3** (1 mmol) in DMSO (10 ml), 2,4-dinitrophenylhydrazine (1 mmol) and few drops of acetic acid were added. The reaction mixture was refluxed for 21–35 h. The progress as well as completion of reaction was monitored by TLC. After completion, the reaction mixture was cooled to room temperature and left overnight. The precipitate formed was filtered, washed with water and taken in ether. The ethereal layer was further washed with water and dried over anhydrous sodium sulphate. Removal of solvents gave the crude product which was recrystallized from methanol to furnish corresponding 2′-(2″,4″-dinitrophenyl)-5α-cholestano [5,7-*c d*] pyrazolines **4–6**.

*2.2. 3β-Acetoxy 2′-(2″,4″-dinitrophenyl)-5α-cholestano [5,7-c d] pyrazoline (**4**)*

Yield (80%); mp: 180 °C; Anal. Calcd for $C_{35}H_{50}N_4O_6$: C, 67.50, H, 8.09, N, 9.0. found: C, 67.48, H, 8.11, N, 8.98; IR (KBr) υ cm^{-1}: 1734 ($OCOCH_3$), 1365 (N═O), 1242 (C-O), 1314 (C–N), 1630 (C═N), 1593, 1464, 3130 (aromatic); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.5 (s, 2H, C_6H), 4.6 (m, 1H, C_3 α-*H*, *W½* = 15 Hz, A/B *trans*) (Bhacca and Williams, 1964), 2.01 (s, 3H, $OCOCH_3$), δ 9.09 (s, 1H, $C_3''H$), 8.2 (d, 1H, $C_5''H$), 7.8 (d, 1H, $C_6''H$), 1.12 (s, 3H, C_{10}-CH_3), 0.71 (s, 3H, C_{13}-CH_3), 0.92 & 0.85 (other methyl protons). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170, 156, 136, 130, 127, 126, 124, 123, 116, 72, 54, 50, 49, 49, 46, 45,43, 39, 38, 37, 36, 36, 35, 32, 28, 28, 27, 26, 23, 22, 21, 21, 18,17, 12. ESI MS: *m/z* 622 [M^+].

*2.3. 3β-Chloro 2′-(2″,4″-dinitrophenyl)-5α-cholestano [5,7-c d] pyrazoline (**5**)*

Yield (76%); mp: 170 °C; Anal. Calcd for $C_{33}H_{47}N_4O_4Cl$: C, 66.15, H, 7.91, N, 9.35. found: C, 66.17, H, 7.88, N, 9.37; IR (KBr) υ cm^{-1}: 1365 (N═O), 743(C-Cl), 1325 (C–N), 1635 (C═N), 1590, 1467, 3129 (aromatic); ^1H NMR ($CDCl_3$, 400 MHz): 1.6 (s, 2H, C_6H), 3.9 (m, 1H, C_3 α-*H*, *W½* = 17 Hz, A/B *trans*) (Bhacca and Williams, 1964), δ 9.1 (d, 1H, aromatic), 8.1 (dd, 1H, aromatic), 7.8 (d, 1H, aromatic), 1.12 (s, 3H,C_{10}-CH_3), 0.71 (s, 3H, C_{13}-CH_3), 0.92 & 0.85 (other methyl protons). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158, 137, 130, 129, 128, 127, 123, 116, 59, 54, 50, 49, 43, 42, 39, 39, 39, 38, 36, 35, 32, 28, 28, 27, 26, 23, 22, 22, 21, 20, 18, 18,12. ESI MS: *m/z* 600/598 [M^+].

*2.4. 2′-(2″,4″-Dinitrophenyl)-5α-cholestano [5,7-c d] pyrazoline (**6**)*

Yield (70%); mp: 185 °C; Anal. Calcd for $C_{33}H_{48}N_4O_4$: C, 70.18, H, 8.57, N, 9.92. found: C, 70.20, H, 8.55, N, 9.94; IR (KBr) υ cm^{-1}: 1360 (N═O), 1333 (C–N), 1640 (C═N), 1590, 1460, 3095 (aromatic); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.7 (s, 2H, C_6H), δ 9.1 (s, 1H, $C_3''H$), 8.3 (d, 1H, $C_5''H$), 7.8 (d, 1H, $C_6''H$), 1.12 (s, 3H,C_{10}-CH_3), 0.71 (s, 3H, C_{13}-CH_3), 0.92 & 0.85 (other methyl protons). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155, 137, 130, 129, 128, 127, 123, 116, 59, 54, 50, 50, 42, 40, 39, 38, 36, 35, 33, 29, 28, 28, 27, 27, 23, 22, 22, 22, 20, 20, 18, 18, 12. ESI MS: *m/z* 564 [M^+].

2.5. Organism culture and in vitro screening (antibacterial activity)

The *in vitro* antimicrobial activities of corresponding 2′-(2″,4″-dinitrophenyl)-5α-cholestano [5,7-*c d*] pyrazoline **4–6** were screened for their antibacterial activity against the bacterial cultures of *Corynebacterium xerosis* (ATCC-373), *Staphylococcus epidermidis* (ATCC-29887) and *Escherichia coli* (ATCC-8739) by disc diffusion method (Cruickshank et al., 1975; Collins, 1976). Standard inoculums (1×10^7–2×10^7) c.f.u. ml^{-1} (0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. 1 mg of every trial compound was dissolved in 100 µl DMSO to prepare stock solution and from stock solutions diverse concentrations 10, 20, 25, 50, and 100 µg/µl of every trial compound were prepared. After that the compounds of diverse concentrations were poured over disk plate onto it. The discs measuring 6 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were also kept. Gentamicin was used as positive control. While the disk poured in DMSO was used as negative control. The plates were inverted and incubated for 24 h at 37 °C. The susceptibility was assessed on the basis of diameter of zone of inhibition against different strains of bacteria. Inhibition zones were measured and compared with standard drug. The bacterial zones of inhibition values are given in Table 1.

Minimum inhibitory concentrations (MIC) were determined by broth dilution technique. The nutrient broth which contained logarithmic serially two fold diluted amount of test compound and controls, were inoculated with approximately 5×10^5 c.f.u. ml^{-1} of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 °C and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory con-