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ORIGINAL ARTICLE

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

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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).

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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-

Table 1 Zones of Inhibition of compounds **4–6**, standard (Gentamicin) and negative control (DMSO) with different bacterial strains.

Compound	Zone of Inhibition (mm)		
	E. coli	*C. xerosis*	*S. epidermidis*
4	17 ± 0.3	13 ± 0.2	15 ± 0.2
5	16 ± 0.1	14 ± 0.3	15 ± 0.3
6	15 ± 0.4	27 ± 0.3	30 ± 0.2
Gentamicin	24 ± 0.2	27 ± 0.1	28 ± 0.3
DMSO	–	–	–

centrations (MIC). The MIC's and MBC's of compounds **4–6** are given in Table 3.

2.6. Organism culture and in vitro screening (antifungal activity)

For assaying antifungal activity, different fungal strains like *Mucor azygosporus* (MTCC-414), *Claviceps purpurea* (MTCC-1479) and *Aspergillus niger* (MTCC-281) were taken and antifungal activity was done by agar diffusion method (Khan, 1997; Verma et al., 1998). Sabourand agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. 20 ml of agar media was poured into each Petri dish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h. using an agar punch, wells were made and each well was labelled. 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. A control was also prepared in triplicate and maintained at 37 °C for 3–4 days. The antifungal activity of each compound was compared with Ketoconazole as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2. The nutrient broth which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6×10^4–6×10^6 c.f.u. ml^{-1}. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). The MIC's and MFC's of compound **4–6** are given in Table 4.

Table 2 Zones of Inhibition of compounds **4–6**, standard (Ketoconazole) and negative control (DMSO) with different fungal strains.

Compound	Zone of Inhibition (mm)		
	M. azygosporus	*C. purpurea*	*A. niger*
4	07 ± 0.1	15 ± 0.4	16 ± 0.1
5	12 ± 0.3	16 ± 0.1	18 ± 0.3
6	17 ± 0.2	15 ± 0.2	18 ± 0.4
Ketoconazole	25 ± 0.2	24 ± 0.3	23 ± 0.2
DMSO	–	–	–

2.7. In vitro anticancer activity

2.7.1. Cell lines and culture conditions

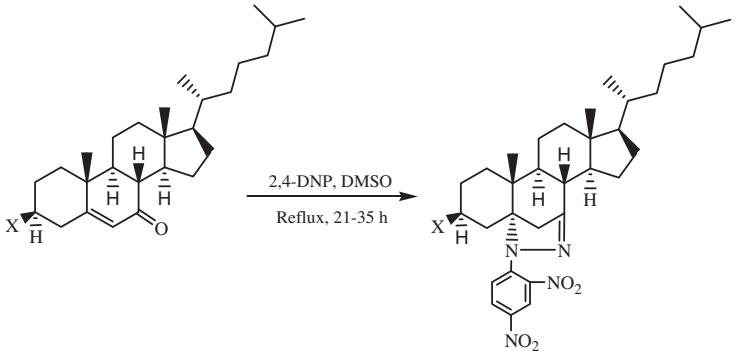
Human cancer cell lines SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), HepG2 (human hepatic carcinoma cells), HL-60 (human Leukaemia) were taken for the study. SW480, A549, HL-60 and HepG2 cells were grown in RPMI 1640 supplemented with 10% foetal bovine serum (FBS), 10U penicillin and 100 µg/ml streptomycin at 37 °C with 5% CO_2 in a humidified atmosphere. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplanted with FCS and antibiotics as described above for RPMI 1640. Fresh medium was given every second day and on the day before the experiments were done. Cells were passaged at preconfluent densities, using a solution containing 0.05% trypsin and 0.5 mM EDTA.

2.7.2. Cell viability assay (MTT)

The anticancer activity *in vitro* was measured using the MTT assay. The assay was carried out according to known protocol (Slater et al., 1963; Mosmann, 1983). Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1×10^4 cells/well. After 24 h incubation at 37 °C under a humidified 5% CO_2 to allow cell attachment, the cells in the wells were, respectively, treated with target compounds at various concentrations for 48 h. The concentration of DMSO was always kept below 1.25%, which was found to be non-toxic to the cells. A solution of 3-(4,5-dimethylthizao1-2-y1)-2,5-diphenyltetrazolium bromide (MTT), was prepared at 5 mg/ml in phosphate buffered saline (PBS; 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4). 20 µl of this solution were added to each well. After incubation for 4 h at 37 °C in a humidified incubator with 5% CO_2, the medium/MTT mixtures were removed, and the formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in 100 µl of DMSO per well. The absorbance of the wells was read with a microplate reader (Bio-Rad Instruments) at 570 nm. Effects of the drug cell viability were calculated using cell treated with DMSO as control.

2.8. Data analysis

Cell survival was calculated using the formula: Survival (%) = [(absorbance of treated cells−absorbance of culture medium)/(absorbance of untreated cells−absorbance of culture medium)] × 100 (Woerdenbag et al., 1993; Saxena et al., 2007). The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated from a dose response curve. IC_{50} is the concentration in 'µM' required for 50% inhibition of cell growth as compared to that of untreated control. IC_{50} values were determined from the linear portion of the curve by calculating the concentration of agent that reduced absorbance in treated cells, compared to control cells, by 50%. Evaluation is based on mean values from three independent experiments, each comprising at least six microcultures per concentration level.



X	X
OAc (**1**)	OAc (**4**)
Cl (**2**)	Cl (**5**)
H (**3**)	H (**6**)

Scheme 1 Formation of Steroidal pyrazolines **4–6**.

3. Results and discussion

3.1. Chemistry

3β-Acetoxy cholest-5-en-7-one **1**, 3β-chloro cholest-5-en-7-one **2** and cholest-5-en-7-one **3** were used as starting materials which have been synthesized by literature methods (Dauben and Takemura, 1953). The yield of 2′-(2″,4″-dinitrophenyl)-5α-cholestano [5,7-*c d*] pyrazolines **4–6** were in the range of 70–80%. All the three compounds (Scheme 1.) were prepared by refluxing of compounds **1–3** with 2,4-dinitrophenylhydrazine in dimethyl sulfoxide (DMSO). The structures of the compounds were established by means of their IR, ^{1}H NMR, ^{13}C NMR, MS and analytical data. The selected diagnostic bands of IR spectra of synthesized products provide useful information for determining structures of the pyrazoline derivatives. The absorption bands at 1630–1640 cm^{-1} (C═N) and1314–1333 (C–N) confirmed the formation of pyrazoline ring in all compounds. The absorption bands at 1593, 1464, 3130 cm^{-1} are attributed to the aromatic ring in the products. The absorption bands at 1337–1511 cm^{-1} show the presence of NO_2 in the compounds.

The formation of steroidal pyrazolines was further confirmed with the ^{1}H NMR spectra. Assignments of the signals are based on the chemical shift and intensity pattern. The ^{1}H NMR spectra of the compounds exhibited singlet for two protons of (C_6-*H*) at δ 1.5–1.7. The presence of singlet (C_3″-*H*, aromatic) at *δ* 9.06, doublet (C_5″-*H*, aromatic) at *δ* 8.2, doublet (C_6″-*H*, aromatic) at *δ* 7.8 ppm confirm the presence of aromatic ring in the compound. ^{13}C NMR signals are in good agreement with proposed structures of synthesized compounds. The compounds exhibited signal at *δ* 155–158 which shows the presence of C═N. The signals obtained at *δ* 130, 127, 126, 124, 123,116 confirm the presence of aromatic carbons.

The distinctive signals were observed in the mass spectra of compounds **4–6** which followed the similar fragmentation pattern. The molecular ion peaks (M^+) for compound **4–6** were observed at *m*/*z* 622, 598/600 and 564, respectively.

The stereochemical assignation of C_5-N bond has been established on the basis of half band width ($W_{1/2}$) values of

Table 3 MIC's and MBC's of compounds **4–6** on different bacterial strains.

Compound	*E. coli*		*C. xerosis*		*S.epidermidis*	
	MIC	MBC	MIC	MBC	MIC	MBC
4	3.125	1.250	1.250	2.250	3.125	1.250
5	3.125	1.250	1.250	2.50	1.562	0.625
6	3.125	1.250	0.781	0.312	0.195	0.781

Table 4 MIC's and MFC's of compounds **4–6** with different fungal strains.

Compound	*M. azygosporus*		*C. purpurea*		*A. niger*	
	MIC	MFC	MIC	MFC	MIC	MFC
4	> 5.00	–	3.125	2.50	3.125	1.250
5	0.25	2.50	3.155	1.250	1.562	0.625
6	3.125	1.20	6.250	2.50	1.562	0.625

Table 5 Showing IC_{50} values of compound **4–6** with different cancer cell lines.

Compound	IC_{50} (μmol l^{-1})				
	SW480	A549	HepG2	HeLa	HL60
4	> 50	31.71	26.53	> 50	27.63
5	27.62	18.31	23.52	33.61	15.39
6	> 50	> 50	42.16	38.91	> 50

C_3-axial proton in the ^{1}H NMR spectra of compounds **4** and **5** which clearly suggested that A/B ring junction is *trans* (Bhacca and Williams, 1964) and also on the basis of the fact that during the reaction, the attack of N of the reagent should be from that side (α) which is less hindered, not from that side (*β*) which is more hindered due to the presence of C_{10} methyl group, hence the C_5-N bond should be axial (α) and *trans* to C_{10} methyl.

3.2. Pharmacology

3.2.1. Antibacterial activity

The *in vitro* antibacterial activities of steroidal pyrazolines **4–6** were screened for their antibacterial activity against the bacterial cultures of *C. xerosis*, *S. epidermidis* and *E. coli* by disc diffusion method. Standard inoculums (1×10^7–2×10^7 c.f.u ml^{-1} 0.5 McFarland standards) were introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculums. 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, Gentamicin. The bacterial zones of inhibition values are given in Table 1.

Minimum inhibitory concentrations (MICs) 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 bacterial 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 concentration (MIC). The minimum inhibitory concentrations of compound **4–6** are given in Table 3. The compound **6** was found to be more potent than the reference drug, Gentamicin, in case of *S. epidermidis* by showing larger zone of inhibition than reference drug. The compound **6** was also equally potent as the reference drug, Gentamicin, in case of *C. xerosis* by showing zone of inhibition of same diameter as that of reference drug.

3.2.2. Antifungal activity

For assaying antifungal activity, different fungal strains like *M. azygosporus, C. purpurea* and *A. niger* were taken and antifungal activity was done by agar diffusion method. The antifungal activity of each compound was compared with Ketoconazole as standard drug. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 2. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6×10^4–6×10^6 c.f.u. ml^{-1}. The cultures were incubated for 48 h at 35 °C and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (MIC). The minimum inhibitory concentrations of compound **4–6** are given in Table 4.

The antifungal screening data showed moderate to good fungal inhibition. Among the screened compounds, **5** and **6** were found to have good zones of inhibition. The compound **5** and **6** showed moderate to good inhibition against *A. niger* strain. The compound **6** also showed moderate inhibition against *M. azygosporus* strain in comparison with the reference drug, Ketoconazole.

3.2.3. Anticancer activity

The *in vitro* anticancer screening of 2′-(2″,4″-dinitrophenyl)-5α-cholestano [5,7-*c d*] pyrazoline **4–6** was done using Human cancer cell lines SW480, HeLa, A549, HepG2, HL-60. SW480, A549, HepG2 and HL-60 cells were grown in RPMI 1640 while as HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM). After 24 h incubation at 37 °C under a humidified 5% CO_2 to allow cell attachment, the cancer cells in the wells were, respectively, treated with target compounds at various concentrations for 48 h. The experiment was done in triplicate and the inhibitory concentration (IC) values were calculated from a dose response curve. IC_{50} is the concentration in 'μM' required for 50% inhibition of cell growth as compared to that of untreated control. The growth inhibitory effect of compound **4–6** towards the cancer cells was measured by means of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay in which the cell viability was measured with the purple formazan that was metabolized from MTT mitochondrial dehydrogenase, which is active only in live cells. The data reported in Table 5 indicates that compound **4–6** showed different levels of anticancer inhibition. The compound **5** showed minimum IC_{50} = 15.39 (HL-60), 18.31 (A549), 23.52 (HepG2). While compound **4** showed minimum IC_{50} = 26.53 (HepG2), 27.63 (HL-60), 31.71 (A549). The compound **6** is not showing effective IC_{50} values (Table 5). Thus compound **5** can be considered better anticancer agent [IC_{50} = 15.39 (HL-60)] among compounds **4–6**.

4. Conclusion

In summary, the successfully developed, convenient and simple reaction for the synthesis of steroidal pyrazolines involves the reaction of steroidal α,β-unsaturated ketones and 2,4-dinitrophenylhydrazine in DMSO. The reaction completed in 21–36 h and on completion better yields were obtained. This strategy offered a very straight forward and efficient method for access to steroidal pyrazolines. During the antibacterial screening of newly synthesized steroidal pyrazolines, the compound **6** showed impressive behaviour by being more potent than reference drug in case of *S. epidermidis* strain and almost equally potent in case of *C. xerosis* strain. Thus, compound **6** was found to be the most active compound in case of antibacterial activity. During the antifungal screening the compound **6** was also active by showing moderate to good inhibition on different fungal strains in comparison with the reference drug. During anticancer assay compound **5** showed minimum IC_{50} values against HL60 cell line.

Thus the overall idea of appending the pyrazoline moiety to steroidal nucleus so as to combine the beneficial effects in a

single structure by expecting some biological activities like antibacterial, antifungal and anticancer activity proved to be successful. In conclusion, the present study showed that the synthesized compounds can be used as template for future development through modification and derivatization to design more potent and selective antimicrobial as well as anticancer agents.

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