IN VITRO CYTOTOXICITY AND GENOTOXICITY EVALUATION OF NEWLY SYNTHESIZED STEROIDAL THIAZOLES

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A preparation of new series of 2'-hydrazinocholest-6-eno[4,5-d]thiazoles **4-6** from 5α-cholestan-6-ones **1-3** are herein reported. After characterization by IR, ^{1}H NMR, ^{13}C NMR, MS and analytical data, the synthesized compounds **4-6** were tested for anticancer activity *in vitro* against the human cancer cell lines A549, HepG2, HeLa, SW480 and HL-60 by MTT assay during which compounds **4-6** showed significant anticancer behaviour. The gel electrophoresis pattern demonstrated that the compound **4** alone or in presence of Cu(II) causes the nicking of super coiled pBR322. Further the compound **4** is also able to generate reactive oxygen species (hydroxyl radical) in a dose dependent manner, which correlates its ability to cause DNA breakage in cancer cells. The genotoxicity of the compounds was studied by comet assay involving potential apoptotic degradation of DNA and was analyzed by agarose gel electrophoresis and visualized by ethidium bromide staining.

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Introduction

Steroids have always attracted considerable attention because of being a fundamental class of biologically signalling molecules. In addition to their profound physiological and clinical importance[1], they have the potential to be developed as drugs for the treatment of a large number of diseases including cardiovascular, autoimmune, brain tumours, breast cancer, prostate cancer and osteoarthritis.[2-4] Most of the steroid based pharmaceuticals are semi-synthetic compounds prepared by connecting a special functionality to the core structure of a steroid.[5] Most important of such functionalities are the heterocyclic systems because of their potent receptor binding properties. The advantage of employing hydrophobic steroid units is their ability to interact with cell membranes and thus pave the way for biological activity of such hybrid molecules.[4]

Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities. They have been used for the treatment of allergies,[6] hypertension,[7] inflammation,[8] schizophrenia,[9] bacterial infections,[10] HIV infections,[11] hypnotics[12] and more recently for the treatment of pain,[13] as fibrinogen receptor antagonists with antithrombotic activity[14] and as new inhibitors of bacterial DNA gyrase B.[15] The substituted thiazoles have number of other characteristic pharmacological features such as relative stability and ease of starting materials built in biocidal unit, enhanced lipid solubility with hydrophilicity and easy metabolism of compounds.[16]

DNA cleaving agents have attracted extensive attention in the field of molecular biology due to their potential applications.[17] Under uncatalyzed physiological conditions, the phosphodiester bonds of DNA are extremely stable and the half life of DNA hydrolysis is estimated to be around 200 million years.[18] Some of the metal complexes have been widely investigated as efficient cleaving agents of nucleic acids[19] but the serious issues over their lability and toxicity restricted the practical usage of these compounds in pharmacy.[20] To overcome these limitations, Gobel and co-workers[21] put forward the concept of 'metal free cleaving agents' which are being applied to active phosphodiesters like 'nucleic acid mimic' and RNA. In view of the pharmacological importance of thiazoles, our aim here is to synthesize the new steroid derivatives with a substituted thiazole ring attached at ring B of tertracyclic core and to study the *in vitro* anticancer activity.

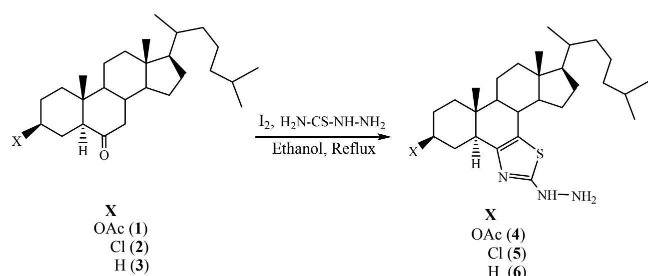
Experimental

Materials and instruments

All the reagents and solvents were obtained from best known commercial sources and were freshly distilled. 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}. ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ on a JEOL Eclipse (400 MHz) instrument with TMS as internal standard and values are given in 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 progress of reaction. Sodium sulphate (anhydrous) was used as a drying agent. Super coiled pBR322 DNA was purchased from GeNei (India) and used for the agarose gel experiment without further purification. Double-stranded calf thymus DNA, purchased from Sigma, was dissolved in a 0.1M Tris-buffer.

General procedure for the synthesis of steroidal thiazoles (4-6)

To a solution of steroidal ketones **1-3** (1 mmol) in absolute ethanol (15 mL) was added thiosemicarbazide (1 mmol) and iodine (2 mmol) in the same solvent (25 mL) and the reaction mixture was refluxed for about 13-17 h. The progress of the reaction was monitored by TLC. After completion of reaction, the excess solvent was removed to three fourths of the original volume under reduced pressure. The reaction mixture was cooled to room temperature, diluted with $Na_2S_2O_7$ solution and subsequently with water. The mixture was taken in ether, washed with water and dried over anhydrous Na_2SO_4. Evaporation of solvents and recrystallization from methanol afforded respective products **4-6**.



Scheme 1. Showing the synthesis of fused steroidal thiazoles

3β-Acetoxy-2'-hydrazinocholest-6-eno[4, 5-d]thiazole (4)

Yield 82 %, m.p.163-164 °C, IR (KBr, cm^{-1}): 3395, 3310 (NH, NH_2), 1730 (OAc), 1625 (C=C), 1555 (C=N), 1320 (C-N), 645 (C-S). ^1H NMR (400 MHz, $CDCl_3$): δ 6.8 (brs, 2H, N*H₂*, exchangeable with D_2O), 4.7 (m, 1H, $C_3\alpha$-*H*, *W½* =15 Hz), 4.4 (s, 1H, N*H*, exchangeable with D_2O), 2.7 (dd, 1H, $C_5\alpha$-*H*, *J* =15 Hz, 5 Hz), 2.03 (s, 3H, $OCOCH_3$), 1.18 (s, 3H, C_{10}-CH_3), 0.70 (s, 3H, C_{13}-CH_3), 0.97 & 0.83 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2 ($OCOCH_3$), 163 (*C*=N), 132 (C_6), 120 (C_7), 70.2 (C_3), 46 (C_{14}), 44 (C_{13}), 42 (C_4), 39 (C_{10}), 35 (C_5), 26 (C_{19}), 24 (C_{11}), 22 (C_{18}), 20 (C_{15}), 17 (C_{16}). Anal. Calcd for $C_{30}H_{49}N_3O_2S$: C, 69.84, H, 9.39, N, 8.11 % found: C, 69.90, H, 9.51, N, 8.15 %. ESI MS: *m/z* 515 [$M^{+\cdot}$].

3β-Chloro-2'-hydrazinocholest-6-eno [4, 5-d] thiazole (5)

Yield 76 %, m.p.143-144 °C, IR (KBr, cm^{-1}): 3370, 3320 (NH, NH_2), 1622 (C=C), 1560 (C=N), 1323 (C-N), 745 (C-Cl), 635 (C-S). ^1H NMR (400 MHz, $CDCl_3$): δ 6.63 (brs, 2H, N*H₂*, exchangeable with D_2O), 4.45 (s, 1H, N*H*, exchangeable with D_2O), 3.9 (m, 1H, $C_3\alpha$-*H*, *W½* = 17 Hz), 2.8 (dd, 1H, $C_5\alpha$-*H*, *J* =17.05 Hz, 5.3 Hz), 1.18 (s, 3H, C_{10}-CH_3), 0.70 (s, 3H, C_{13}-CH_3), 0.97 & 0.83 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162 (*C*=N), 134 (C_6), 120 (C_7), 57.7 (C_3), 46 (C_{14}), 45 (C_{13}), 42.6 (C_4), 39 (C_{10}), 35 (C_5), 26 (C_{19}), 24 (C_{11}), 22 (C_{18}), 20 (C_{15}), 17 (C_{16}). Anal. Calcd for $C_{28}H_{46}ClN_3S$: C, 68.37, H, 9.29, N, 8.49 % found: C, 68.43, H, 9.36, N, 8.54%. ESI MS: *m/z* 491/489 [$M^{+\cdot}$].

2'-Hydrazinocholest-6-eno[4, 5-d]thiazole (6)

Yield 73 %, m.p.129-130 °C, IR (KBr, cm^{-1}): 3376, 3328 (NH, NH_2), 1617 (C=C), 1557 (C=N), 1328 (C-N), 634 (C-S). ^1H NMR (400 MHz, $CDCl_3$): δ 6.2 (brs, 2H, N*H₂*, exchangeable with D_2O), 3.8 (s, 1H, N*H*, exchangeable with D_2O), 2.74 (dd, 1H, $C_5\alpha$-*H*, *J*=16.9 Hz, 5.5 Hz), 1.18 (s, 3H, C_{10}-CH_3), 0.70 (s, 3H, C_{13}-CH_3), 0.97 & 0.83 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163 (*C*=N), 130 (C_6), 120 (C_7), 46 (C_{14}), 42.2 (C_4), 39 (C_{10}), 35 (C_5), 26 (C_{19}), 24 (C_{11}), 22 (C_{18}), 20 (C_{15}), 17 (C_{16}). Anal. Calcd for $C_{28}H_{47}N_3S$: C, 73.47, H, 10.19, N, 9.13 % found: C, 73.52, H, 10.28, N, 9.19%. ESI MS: *m/z* 457 [$M^{+\cdot}$].

***In vitro* anticancer activity (MTT assay)**

Cell culture and conditions: Human cancer cell lines SW480 (colon adenocarcinoma cells)/ATCC (CCL-228), HeLa (cervical cancer cells)/ATCC (CCL-2), A549 (lung carcinoma cells)/ATCC (CCL-185), HepG2 (hepatic carcinoma cells)/ATCC (CRL-8065) and HL-60 (Leukaemia cells)/ATCC (CCL-240) 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^{-1} 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.

Cell viability assay (MTT): The anticancer activity *in vitro* was measured using the MTT assay. The assay was carried out according to known protocol.[22,23] 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 and Cisplatin 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-dimethylthiazo1-2-y1)-2,5-diphenyltetrazolium bromide (MTT), was prepared at 5 mg mL^{-1} 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 was 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 at 570 nm. Effects of the drug cell viability were calculated using cell treated with DMSO as control.

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.[24,25] 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 cisplatin as the values is shown in Table 1. 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.

Treatment of supercoiled plasmid pBR322 DNA with compound 4

To investigate the mechanism of anticancer activity by studying the effect of compound **4** on supercoiled plasmid pBR322 DNA, an experiment was done in which the reaction mixture containing 10 mM Tris HCl (pH 7.5), 0.5 μg of pBR322 plasmid DNA, 100 μM copper, varying with concentrations of compound **4** was taken. Incubation at room temperature was performed for specified time periods. After incubation, 10 μL of a solution containing 40 mM EDTA, 0.05 % Bromophenol blue tracking dye and 50 % glycerol was added and the solution was subjected to electrophoresis in submarine 1 % agarose gel. The gel was stained with ethidium bromide (0.5 mg mL^{-1}), viewed and photographed on a transilluminator.

Detection of hydroxyl radicals (OH)

The detection of hydroxyl radicals was investigated by the method studied by Quinlan and Gutteridge.[26] The reaction mixture (0.5 mL) containing Tris HCl (10 mM, pH 7.5), Calf thymus DNA (200 μg), increasing concentrations of compound **4** (12.5 μM, 25 μM, 50 μM, 75 μM, 100 μM, 200 μM, 400 μM, 600 μM), Cu(II) (100 μM) and volume is made up to 1mL by distilled water and incubated for 60 minutes at 37 ºC. Reaction is stopped using 0.5 ml of TCA (28 %) and 0.5 mL of 1% TBA is added and boiled for 15 minutes and cooled to room temperature. The intensity was read at 532 nm.

Molecular docking

The rigid molecular docking studies were performed using HEX 6.1 software.[27] The initial structure of the steroidal thiazoles was generated by Discovery Studio 3.5. The molecules of compound were optimized for use in the following docking study. The crystal structure of the B-DNA dodecamer d(CGCAAATTTCGC)2 (PDB ID: 1BNA) were downloaded from the protein data bank. All calculations were carried out on an Intel CORE i5, 2.6 GHz based machine running MS Windows 7 as the operating system. Visualization of the docked pose have been done using PyMol molecular graphics program.[28]

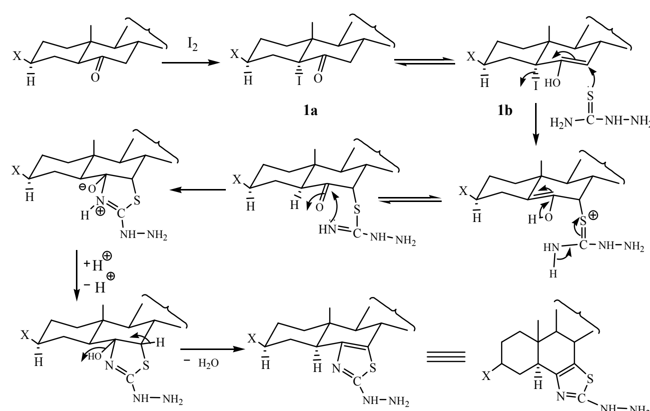
Comet assay

To assess the genotoxic effect of the steroidal thiazoles (**4-6**), comet assay[29] was performed in SW480 cells. SW480 (1×10^6) cells were treated with three different concentrations, 20 μg mL^{-1} of steroidal thiazoles (**4-6**) and cisplatin (20 μg mL^{-1}) for 24 h. The cells were then washed and 200 μL of cell suspension in low melting agarose (LMA) was layered on to the labelled slides precoated with Agarose (1.5 %). The slides were placed on ice for 10 min and submerged in lysis buffer (2.5 % NaCl, 100 mM EDTA, 10 mM Tris, 10 % DMSO and 1 % Triton X-100) at pH 10 at 4 °C for more than 1 h. The slides were then equilibrated in alkaline buffer (30 mM NaOH, 1 mM EDTA) at pH 13 at 4 °C, electrophoresed at 0.86 V cm^{-1} at 4 °C, neutralized, washed and dried. At the time of image capturing, the slides were stained with ethidium bromide (ETBr, 150 μL 1X) and cover slips were placed over them. For visualization of DNA-damage, ETBr stained slides were observed under 209 objectives of a fluorescent microscope (Olympus BX-51, Japan). The images of 50-100 randomly selected cells were captured per slide using a CCD camera.

Results and discussion

Chemistry

3*β*-Acetoxy-5*α*-cholestan-6-one **1**, 3*β*-chloro-5*α*-cholestan-6-one **2** and 5*α*-cholestan-6-one **3** were prepared according to the literature procedure.[30-32] Steroidal thiazoles 4-6 were conventionally prepared in one pot synthesis by reacting steroidal ketones **1-3** with iodine and thiosemicarbazide in absolute ethanol (Scheme 1). The key intermediates, α-haloketones are important precursors for the synthesis of a variety of heterocyclic compounds. Literature reveals about the synthesis of thiazoles via a Hantzsch protocol which also makes the reaction of α-haloketones with thiosemicarbazide mechanistically analogous.[33,34] The important feature of this reaction is the formation of α-haloketone intermediate which may be obtained separately by the treatment of ketones with halogens. The advantage of this synthesis is to evade the α-haloketones as a starting material. In spite of this modification, the method still remains cumbersome (13-17 h reflux).



Scheme 2. Allylic displacement of iodine by the attack of sulphur atom of reagent

The formation of products **4-6** can be explained by considering that during the reaction the α-iodoketone 1a formed *in situ* undergoes allylic displacement of iodine via enolization and the subsequent attack of sulphur

Table 1. Showing the IC_{50} values of compounds **4-6** against human cancer cell lines

Compound	**IC_{50}, µmol L^{-1}**				
	SW480	**A549**	**HepG2**	**HeLa**	**HL-60**
4	13.04±0.6	11.32±0.2	9.71±1.1	13.17±0.4	14.71±0.3
5	21.66±0.4	15.44±1.3	>50	11.74±0.7	26.27±0.5
6	14.03±0.2	13.22±0.7	17.37±1.5	16.62±0.4	>50
Cisplatin	3.52±0.3	10.51 ±0.2	9.8±0.9	9.43±0.5	7.8±1.5

of thiosemicarbazide followed by cyclization leads to the formation of products **4-6** as shown in Scheme 2. An enol tautomeric form **1b** might be the driving force to accelerate the reaction towards product formation.[35]

In vitro anticancer activity

The growth inhibitory effect of compounds **4-6** towards the human cancer cells was measured by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial lactate dehydrogenase of living cells has been used to develop an assay system for measurement of cell proliferation.[22,23] The results are expressed as IC_{50} values (Table 1) which indicate that compounds **4-6** showed different levels of anticancer activities. The compound **4** showed minimum IC_{50}= 9.71±1.1 (HepG2), 11.32±0.2 (A549), 13.04±0.6 (SW480) and 13.17±0.4 µmol L^{-1} (HeLa). While compound **5** showed minimum IC_{50}=11.74±0.7 (HeLa) and 15.44±1.3 µmol L^{-1} (A549). The minimum inhibitions shown by compound **6** were 13.22±0.7 (A549), 14.03±0.2 (SW480) and 16.62 ±0.4 µmol L^{-1} (HeLa).

From these results it is clear that the IC_{50} for compound **4** against A549 cell line is 11.32 ± 0.2 which is very close to the IC_{50} of cisplatin (10.51 ± 0.2) against the same cell line. IC_{50} for compound **5** against HeLa cell line is 11.74 ± 0.7 which is also close to the IC_{50} of cisplatin (9.43 ± 0.5) against the same cell line. Similarly IC_{50} for compound **4** against HepG2 is 9.71 ± 1.1 which is also near to the IC_{50} of cisplatin (9.6 ± 0.3) against the same cell line. It can be concluded that Compound **4** and **5** are showing potential anticancer activity against A549, HepG2, HeLa cell lines by showing IC_{50} close to that of standard drug, Cisplatin thus can be considered as potential cytotoxic agents. The graphical representation of IC_{50} values in MTT assay is shown in Fig. 1.

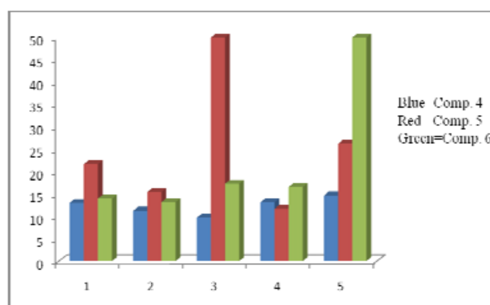


Figure 1. Graphical representation of IC_{50} values shown by compound **4-6** against SW480, A549, HepG2, HeLa and HL-60 by MTT assay

Treatment of supercoiled plasmid pBR322 DNA with compound 4 and detection of hydroxyl radicals (OH)

Anticancer activity mechanism was also confirmed by studying the treatment of supercoiled plasmid pBR322 DNA with different concentrations of compound **4** and 100 µM copper. Our nucleolytic experiments suggest that cell death may be due to cleavage or fragmentation of DNA of these cancer cells and that the active species responsible for this are ROS (hydroxyl radical) which resulted from the *in vitro* reaction of different concentrations of compound **4** with copper in presence of thiobarbituric acid. We observe from gel electrophoresis that after adding copper (100 µM) the concentration of radicals increase which in presence of different concentrations of compound **4** show the nicking of plasmid pBR322 DNA from its supercoiled form (form I) to open circular form (form II). Fig. 2 reveals that in lane 6, 7 and 8, the nicking is quite obvious by the disappearance of form I and appearance of form II and with the increase in concentration of compound **4** (lane 8) the band intensity (form II) became maximum, depicting the more pronounced cleavage at high concentration.

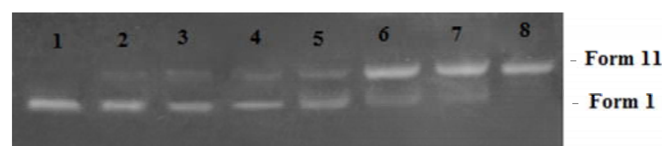


Figure 2. Fragmentation pattern of supercoiled plasmid pBR322, Lane 1 contains DNA only, lane 2 contains DNA and copper, lane 3, 4 and 5 contain DNA and compound **4** (100, 200 and 300 µM respectively) and lane 7, 8 and 9 contain DNA and compound **4** (100, 200, 300 µM respectively) plus 100 µM copper added to it.

In the DNA cleavage reactions mediated by various antioxidants in the presence of Cu(II), it has been established that Cu(II) is reduced to Cu(I) by the antioxidants and that Cu(I) is an essential intermediate in the DNA cleavage reactions.[36,37] It is also generally understood that DNA cleavage by various antioxidants and Cu(II) is the result of the generation of hydroxyl radicals. As mentioned in literature also, Cu(II) is reduced to Cu(I) and the re-oxidation of Cu(I) to Cu(II) by molecular oxygen gives rise to superoxide anion which in turn leads to the formation of H_2O_2.[38] Presumably Cu(I) is oxidized to Cu(II) by H_2O_2 in a Fenton type reaction giving rise to hydroxyl radicals. To determine the hydroxyl radical production and the role of copper ions in DNA cleavage, an experiment was performed where progressively increasing concentrations of compound **4** and cisplatin (12.5-600 µM) were tested on thiobarbituric acid induced DNA breakage (Fig. 3) and from these results we may conclude that the DNA cleavage by thiobarbituric acid involves endogenous copper ions and also that Cu(I) is an intermediate in the pathway that leads to DNA cleavage.

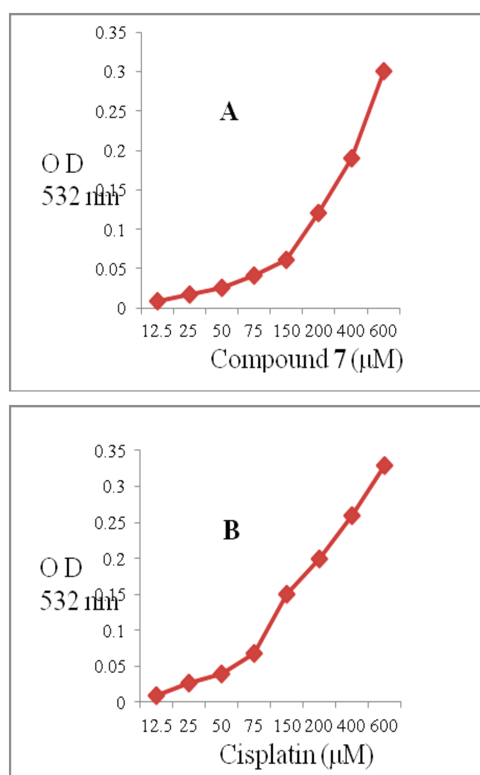


Figure 3. Showing comparative determination of hydroxyl radical production by compund **4** (A) and cisplatin (B) by the assay of thiobarbituric acid

The compound **4**–Cu(II) (Fig. 3A) and cisplatin–Cu(II) (Fig. 3B) are shown to generate the hydroxyl radicals that react with CT DNA, result in strand breaks. The assay is based on the fact that degredation of DNA by hydroxyl radical results in the release of TBA reactive material, which forms a coloured adduct readable at 532 nm.[39] Increasing concentrations of compound **4** or Cisplatin in presence of Cu(II) showed a corresponding increase in the generation of hydroxyl radicals. However, the generation of hydroxyl radical being more in case of cisplatin as shown in Fig. 3B. The results in Fig. 3 confirmed the relatively higher rate of formation of hydroxyl radicals and correlated with the rate of DNA degredation by the compound **4** as well as cisplatin.

Comet assay

In the comet assay, the images of SW480 cells treated with compounds (**4-6**) showed the formation of comets. Compound **4** presented maximum apoptotic DNA damage followed by compound **6** and **5**, which is in accordance with its maximum cytotoxicity as seen in MTT assay. None of the steroidal thiazoles exhibited apoptotic DNA damage to the extent of Cisplatin. The quantified increase in DNA damage suggested that all three thiazole derivatives induced dose dependent fragmentation of chromosomal DNA leading to apoptosis. The images of comet assay for control, cells treated with Cisplatin (20 µg mL^{-1}), **4** (20 µg mL^{-1}), **5** (20 µg mL^{-1}), and **6** (20 µg mL^{-1}) are shown in Fig. 4. Slides were analyzed for parameter like tail length (TL), using image analyzer CASP software version 1.2.2. The results of the assay for tail length are shown in graph given in Fig. 4.

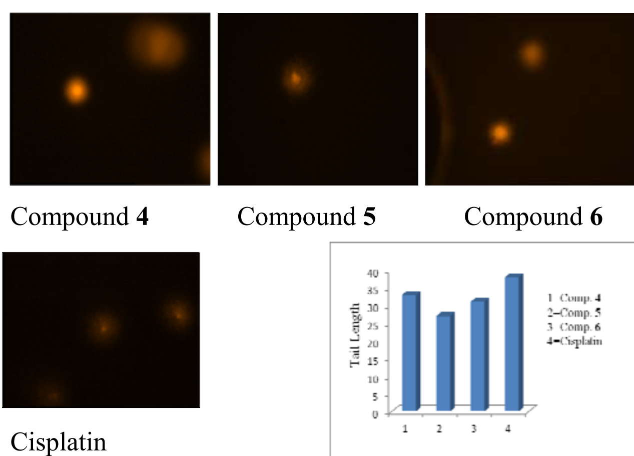


Figure 4. Detection of DNA damage in SW480 cells. Treated cells (24 h) were layered over agarose gel, lysed, electrophoresed in alkaline buffer and stained with propidium iodide. Control cells were treated with DMSO alone. The DNA fragmentation resulting in a comet-like appearance in cells treated with cisplatin and compounds **4-6**.

Molecular docking studies with DNA

In our experiment, molecular docking studies of steroidal thiazoles with DNA duplex of sequence $d(CGCGAATTCGCG)_2$ dodecamer (PDB ID: 1BNA) were performed in order to predict the chosen binding site along with preferred orientation of the molecules inside the DNA groove. The resulted docked model (Fig. 5) depicted that all the three compounds recognized minor groove interaction leading to van der Waals and hydrophobic interaction with DNA functional groups which stabilizes the groove and leads to the stablity of the complex. The compound **4** showed electrostatic interaction in the form of hydrogen bonding with NH of 7th Thiamine at a distance of 2.88 Å by the acetate group at 3β-position of steroidal molecule. The compound **5** showed the groove fit behaviour and arranged in a perpendicular manner with respect to the minor groove walls of the DNA helix while as compound **6** showed electrostatic interaction in the form of hydrogen bonding with NH of 11th Thiamine at a distance of 3.21 Å. The resulting relative binding energies of docked steroidal thiazole (**4-6**)–DNA complexes were found to be -308, -319 and -314 kJ mol^{-1}, respectively depicting the decrease in the energy after forming complexes with DNA.

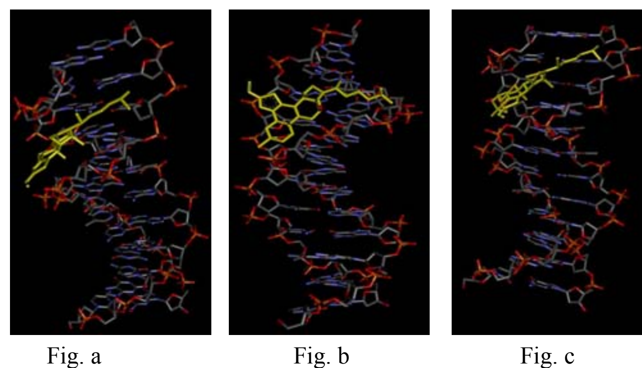


Figure 5. Cartoon representation of DNA molecules with the bound compounds. The steroidal thiazole derivatives are shown as yellow colour. Figure (a), (b), and (c) shows minimum energy poses of DNA–Steroidal thiazole (**4-6**) complexes, respectively.

Conclusion

In summary, we have developed a facile and convenient approach for the preparation of new steroidal thiazole derivatives in one-pot synthesis. All the newly synthesized compounds were evaluated for the anticancer activity *in vitro* against five cancer cell lines. The preliminary results showed that compounds **4-6** were found active during anticancer as well as genotoxic screening but compounds **4** and **6** were found to be potential anticancer agents. These compounds were also found to catalyze the oxidative degradation of isolated DNA either alone or in the presence of transition metal ions such as copper. However, in presence of copper the oxidative cleavage was enhanced. As mentioned earlier cancer cells being rich in transition metal ion like copper[40] we conclude that compound **4** in presence of endogenous copper may give rise to hydroxyl radical this may lead to the oxidative DNA cleavage in cancerous cells. Hence this protocol provides a convenient strategy to annelate steroid nucleus with widespread bioactive thiazoles there by extending the categories of heterosteroids. This strategy may also provide valuable information for the further design and development of more active anticancer agents through various modifications and derivatizations.

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