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Synthesis of steroidal imidazolidinthiones as potential apoptotic agents: Investigation by theoretical and experimental studies

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ABSTRACT

New steroidal imidazolidinthione derivatives (**4–6**) were synthesized from steroidal thiosemicarbazones and dichloroethane. The synthesized compounds were characterized using spectral data analysis. Theoretical DFT involving B3LYP/6-31G** level of theory was employed to gain insights into the molecular structure of the target compounds. MEPS and FMO analysis were carried out. HOMO-LUMO energy gap was determined which helped to evaluate various global descriptors like hardness, chemical potential, electronegativity, nucleophilicity and electrophilicity index, etc. The calculated properties established that the synthesized products are more or less similar in their reactivity behaviour. To explore their biological potential, interaction studies of compounds (**4–6**) with DNA were carried out using various biophysical techniques. The compounds bind DNA preferentially through electrostatic and hydrophobic interactions with K_b of $3.21 \times 10^3\ M^{-1}$, $2.79 \times 10^3\ M^{-1}$ and $2.26 \times 10^3\ M^{-1}$, respectively indicating the higher binding affinity of compound **4** towards DNA. Gel electrophoresis of compound **4** demonstrated strong interaction during the concentration dependent cleavage activity with pBR322 DNA. It was observed that these steroidal imidazolidinthiones are minor groove binders of DNA which was validated using molecular docking studies. An *in vitro* cytotoxicity screening using MTT assay revealed that the compounds (**4–6**) exhibit potential toxicity against different human cancer cells. Highest antiproliferative effect was observed on HeLa cells by compound **4**. The results suggested that compounds **4–6** cause apoptotic cell death by cleaving apoptotic protein caspase-3 and suppress anti-apoptotic protein Bcl-2 in HeLa cancer cells.

1. Introduction

Heterocyclic chemistry with nitrogen heteroatom is emerging as one of the interesting topics in the synthetic organic chemistry arena due to its wide presence in various bioactive natural compounds, synthetic intermediates and pharmaceuticals [1]. The imidazole core is found mainly in the structure of some well-known components of human organisms, i.e. the histidine, vitamin B_{12}, purines, histamine and biotin [2–4]. Imidazole derivatives have been reported to possess various biological properties viz. antiprotozoal [5], hepatitis B inhibitors [6], antimicrobial [7], anti-inflammatory [8,9], anticancer [8], anticonvulsant [10], antiallergy [9], FTase and p38 MAP kinase inhibitory activities [11]. There are various drugs with imidazole nucleus such as Omeprazole, Losartan, Etomidate and Ketoconazole currently in clinical use. The excellent therapeutic properties of imidazole-related drugs have encouraged medicinal chemists to synthesize a large number of novel chemotherapeutic agents [12].

DFT methods along with their complementary experimental techniques play crucial role in studying structural and spectral properties as well as various structure based molecular properties of organic molecules. The hybrid functional, B3LYP [13,14] is most widely used for the organic compounds as it offers low computational cost than Post Hartree-Fock methods and commonly yields comparable results. Nowadays, the dispersion corrected functional B3LYP-D3 is preferred for molecules having inter- or intramolecular interactions where Grimme's D3 correction is employed for long range dispersion interactions [15].

In pharmacology, the DNA cleaving agents have attracted attention due to their potential applications [16]. The DNA phosphodiester bonds

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are much stable and under uncatalyzed conditions, the half life of DNA hydrolysis is estimated to be around 200 million years [17]. Literature reveals that the organic compounds being no doubt mild cleaving agents but have less labile and toxic nature [18] associated with them unlike those of metal complexes which are strong cleaving agents [19] but have complex issues of lability and toxicity [20]. Hence there came the concept of 'metal-free cleaving agents' which are being applied to active phosphodiesters like 'nucleic acid mimic' and RNA.

In continuation of our previous work [21–23], herein, we report the synthesis of new steroidal imidazolidinthione as DNA binding agents. The new compounds were characterized by elemental analysis and spectral techniques. Density functional theory (DFT) (B3LYP/6-31G**) calculations were carried out for these compounds using Gaussian program package to gain insights into their electronic structure. UV–vis absorption, fluorescence, gel electrophoresis and molecular docking studies were employed to study the binding pattern and interaction of these compounds with DNA. MTT Assay and confocal microscopy was employed to study the cytotoxicity of the these compounds and morphological changes in cancer cells, respectively, however immunoblotting and other *in vitro* assays were employed to investigate the intervention of the signalling involved in cancer cells by these synthesized imidazolidinthione compounds.

2. Experimental

2.1. Materials and 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 (400 MHz) in $CDCl_3$ with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Super coiled pBR322 DNA was purchased from GeNei (India) while as double-stranded calf thymus DNA, purchased from Sigma, was dissolved in a 0.1 M Tris-buffer. The purity of DNA was verified by monitoring the ratio of absorbance at 260 nm to that at 280 nm, which was in the range 1.8–1.9. The concentration of the DNA was determined spectrophotometrically using $\varepsilon_{260} = 6600\ M^{-1}\ cm^{-1}$ [24]. The human cancer cell lines used for the cytotoxicity experiment were MCF-7 HeLa, HL-60, SW480 and HepG2 which were obtained from National Cancer Institute (NCI), biological testing branch, Frederick Research and Development Centre, USA. Antibodies such as β-Actin (A5441) were from Sigma Aldrich, Caspase-3 (SC-65497), PARP1 [poly (ADP-ribose) polymerase 1; SC-7150], were procured from Santa Cruz Biotechnology, Bcl-xL (#2762) and Bax (#2772) antibodies were obtained from Cell Signalling Technology. The treated and control cancer cells were viewed with a FluoView FV1000 (Olympus, Tokyo, Japan) confocal laser scanning microscope (CLSM) equipped with argon and HeNe lasers.

2.2. General synthesis of new steroidal derivatives (4–6)

To a solution of steroidal thiosemicarbazone (**1–3**) (1 mmol) in absolute ethanol (20 mL), [25] an equimolar amount of ethyl chloroacetate and fused sodium acetate was added. The reaction mixture was refluxed for 3 h. The progress and completion of reaction was monitored by thin layer chromatography. After completion of reaction, the reaction mixture was concentrated under reduced pressure. It was cooled and then poured into cold water. The obtained solid was extracted with ether and ethereal layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvents and recrystallization from methanol afforded respective product (**4–6**).

2.3. (3S,10R,13R,E)-10,13-dimethyl-17-octyl-6-((2-thioxoimidazolidin-1-yl)imino)hexadecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate (4)

Yellow solid (70%). m.p: 144 °C; IR (KBr, cm^{-1}): 3330 (NH), 1734 ($OCOCH_3$), 1560 (C═N), 1230 (C═S), 1370 (C−N), 1080 (C−O). ^1H NMR (400 MHz, $CDCl_3$): δ 7.7 (s, 1H, N*H*, exchangeable with D_2O), 4.7 (m, 1H, C_3α-H, J = 15 Hz), 3.21 (t, 2H, CH_2 (C_5'), 3.04 (t, 2H, CH_2 (C_4'), 2.04 (s, 3H, $OCOCH_3$), 1.20 (s, 3H, C_{10}-CH_3), 0.78 (s, 3H, C_{13}-CH_3), 0.96 and 0.99 (other methyl protons); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181, 171, 157, 70, 56.5, 53, 46, 43, 42, 39.7, 36, 35, 32, 30, 29, 28, 27.5, 27, 26.5, 26.2, 26, 24, 23, 22.5, 22, 21.4, 21.3, 21, 20.6, 20, 19, 18. Anal. Calcd. for $C_{32}H_{53}N_3O_2S$: C, 70.67; H, 9.82; N, 7.73. Found C, 70.64; H, 9.78; N, 7.70; ESI-MS: m/z 543 [M^+].

2.4. 1-((E)-((3S,10R,13R)-3-chloro-10,13-dimethyl-17-octyltetradecahydro-1H-cyclopenta[a]phe-nanthren-6(10H)-ylidene) amino)imidazolidine-2-thione (5)

White solid (75%). m.p: 139 °C; IR (KBr, cm^{-1}): 3328 (NH), 1570 (C═N), 1255 (C═S), 1372 (C−N), 741 (C−Cl); ^1H NMR (400 MHz, $CDCl_3$): δ 7.4 (s, 1*H*, NH, exchangeable with D_2O), 3.24 (t, 2H, CH_2 (C_5'), 3.16 (t, 2H, CH_2 (C_4'), 3.9 (m, 1H, C_3α-H, J = 17 Hz), 1.22 (s, 3H, C_{10}-CH_3), 0.74 (s, 3H, C_{13}-CH_3), 0.97 and 0.98 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 183, 159, 52.5, 52, 46, 43, 42, 39.7, 36, 35, 32, 30, 29, 28, 27.5, 27, 26.5, 26.2, 26, 24, 23, 22.5, 22, 21.4, 21.3, 21, 20.6, 20, 19, 18. Anal. calcd. for $C_{30}H_{50}N_3ClS$: C, 69.26; H, 9.69; N, 8.08. Found C, 69.21; H, 9.64; N, 8.05; ESI-MS: m/z 519/521 [M^+].

2.5. 1-((E)-((10R,13R)-10,13-dimethyl-17-octyltetradecahydro-1H-cyclopenta[a]phenanthren-6(10H)-ylidene)amino)imidazolidine-2-thione (6)

White solid (73%). m.p: 135 °C. IR (KBr, cm^{-1}): 3325 (NH), 1573 (C═N), 1252 (C═S), 1374 (C−N). ^1H NMR (400 MHz, $CDCl_3$): δ 7.6 (s, 1H, N*H*, exchangeable with D_2O), 3.19 (t, 2H, CH_2 (C_5'), 3.13 (t, 2H, CH_2 (C_4'), 1.22 (s, 3H, C_{10}-CH_3), 0.74 (s, 3H, C_{13}-CH_3), 0.97 and 0.98 (other methyl protons). ^{13}C NMR (100 MHz, $CDCl_3$): δ 182, 154, 52.5, 52, 46, 43, 42, 39.7, 36, 35, 32, 30, 29, 28, 27.5, 27, 26.5, 26.2, 26, 24, 23, 22.5, 22, 21.4, 21.3, 21, 20.6, 20, 19, 18; Anal. calcd. for $C_{30}H_{51}N_3S$: C, 74.17; H, 10.58; N, 8.65. Found C, 74.13; H, 10.53; N, 8.62; ESI-MS: m/z 485 [M^+].

2.6. Computational details

All the DFT calculations were performed using the Gaussian 09 code [26]. B3LYP functional was employed for the optimization of all the structures [27]. The B3LYP functional is believed to yield the correct structures [28]. The geometry optimization was carried out using a 6-31G** basis set for all the atoms. All structures studied in this work were fully optimized in gas-phase without any restriction.

2.7. DNA binding studies

2.7.1. Absorption and emission spectroscopy

The DNA binding experiments were carried out by using absorption titration and emission spectroscopy as per the practices reported in literature [29]. The UV–vis spectra for DNA-steroidal imidazolidinthione interactions were obtained using an Agilent 8453 spectrophotometer while as fluorescence measurements were carried out with a JASCO spectrofluorimeter (FP 6200). Solutions of DNA and steroidal imidazolidinthione were scanned in a 1 cm quartz cuvette. To eliminate the absorbance of the DNA while measuring the absorption spectra, an equal amount of DNA was added to both the compound solution and the reference solution.

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