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Synthesis, characterization, biological evaluation and molecular docking of steroidal spirothiazolidinones

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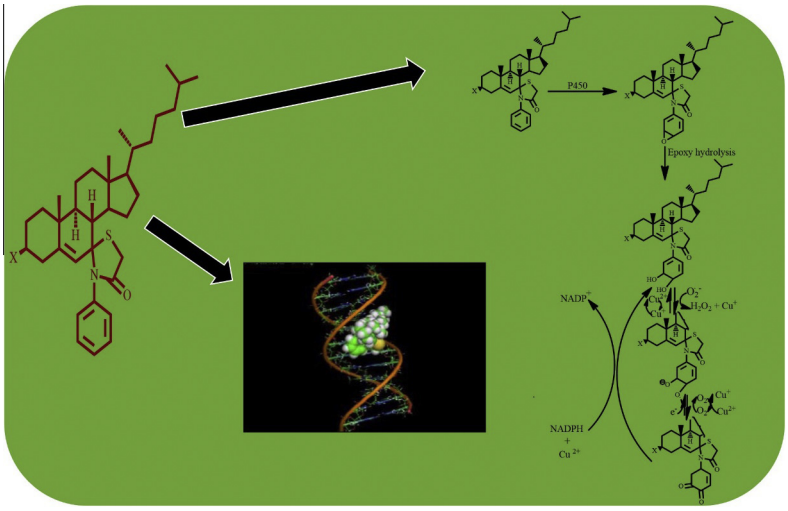
HIGHLIGHTS

- The synthesis of a new series of steroidal spirothiazolidinones has been performed.
- The interaction of products with DNA has been studied.
- Apoptosis and nonenzymatic degradation of DNA have also been investigated.
- The new compounds were tested for in vitro cytotoxicity against Jurkat and PBMCs.
- The AChE inhibitor activities were evaluated by Ellman's method.

GRAPHICAL ABSTRACT

A convenient synthesis of new series of steroidal spirothiazolidinones has been performed. After characterization, the interaction of the synthesized compounds with DNA was evaluated by UV–vis, fluorescence spectroscopy and docking studies. MTT assay has been performed to check the *in vitro* cytotoxicity of new compounds.



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ABSTRACT

The present work describes a convenient synthesis of steroidal spirothiazolidinone derivatives (**3**, **10–12**) in a two-step process. All the newly synthesized compounds have been characterized by means of elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and MS. Lipinski's 'Rule of Five' analysis and biological score predicted higher intrinsic quality of the synthesized compounds and revealed that these compounds have good passive oral absorption. The DNA binding studies of the synthesized compounds with CT-DNA were carried out by UV–vis and fluorescence spectroscopy. The molecular docking study suggested electrostatic interaction between synthesized compounds and nucleotide base pairs. The antitumor activity was tested *in vitro* against human leukemia cancer cell (Jurkat) and blood peripheral mononuclear normal cell (PBMCs) lines by MTT method. In addition, apoptosis and nonenzymatic degradation of DNA have been

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Anti-tumor
Apoptosis

investigated. The acetylcholinesterase (AChE) inhibitor activities of the derivatives were also evaluated using Ellman's method. The present study has shown that steroidal spirothiazolidinone derivatives (**3**, **10–12**) can be used as template to design more potent and selective cytotoxic and AChE inhibition agents through modification and derivatization.

Introduction

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Cancer is caused by both external and internal factors and these factors may act together or in sequence to initiate or pro mote the development of cancer [1]. Many of these cancers are very difficult to treat, while some cancers rapidly become resistant to approved chemotherapeutic drugs. As a consequence, new therapies, especially those with novel modes of action, are urgently required [2]. The International Agency for Research on Cancer (IARC) has estimated that 7.6 million people worldwide die every year because of cancer and 4 million of these people are between 30 and 69 years [3]. Cancer cells are formed as a result of genetic transformation of normal cells. Because of these alterations, an abnormal, cancer specific microenvironment is created in the tumor tissues. These differences can be exploited to design cancer-specific drugs [4]. The increased ROS (reactive oxygen species) level is an especially attractive target for anticancer therapy because it seems to be a general feature of cancer [5]. Drugs targeting this feature can potentially exhibit activity against all cancer types. Several anticancer drugs have been described that act by increasing the ROS concentration in cancer cells beyond the apoptotic level and thereby inducing their death [6]. Steroids play an important biological role and have occupied a prominent position in medicinal chemistry field [7]. Steroids have always attracted considerable interest because they act as biological signaling molecules. Many representatives of this group are widely used as anticancer agents [8]. Thiazolidinone, is a very attractive target for combinatorial library synthesis because of their structure activity relationship [9] and is an important class of N and S containing heterocycles, which are widely used as key building blocks in the field of drugs and pharmaceutical agents [10]. Thiazolidinone systems are present in many pharmacologically important compounds (Fig. 1).

Numerous reports have appeared in the literature describing a broad spectrum of pharmacological properties of thiazolidinones due to their synthetic and biological versatility. The diversity of their biological functions has stimulated efforts in the expedient development of their synthesis [11]. Due to the importance of thiazolidinones, various research groups incorporated this heterocyclic moiety to the steroidal skeleton [12]. As a part of our intensive research program focused on the design and synthesis of structurally modified steroidal derivatives [13,14], herein we describe the synthesis of steroidal spirothiazolidinones (**3**, **10–12**) derived from steroidal ketones (**1**, **4–6**) in a two-step process. In order to explore the bioavailability of the synthesized derivatives, we have calculated the compliance of compounds to the Lipinski's rule-of-five and biological score and studied their DNA binding and *in vitro* antitumor activity against human leukemia cancer cell (Jurkat) and blood peripheral mononuclear normal cell lines (PBMCs) by MTT method. Moreover, apoptotic and nonenzymatic degradation of DNA in the presence of steroidal spirothiazolidinone derivatives (**3**, **10–12**) have also been studied.

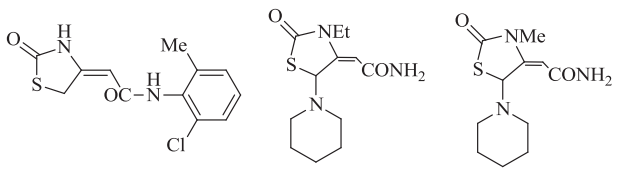

Fig. 1. Thiazolidinone derivatives registered as drugs.

Experimental

Chemistry

All glass apparatus were oven-dried prior to use. Chemicals were purchased from Merck and Sigma–Aldrich as 'synthesis grade' and solvents were purified prior to use. Melting points were determined on a Kofler apparatus in degree Celsius. The IR spectra were recorded on KBr pellets with Interspec 2020 FT-IR Spectrometer Spectro Lab 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). Splitting patterns are described as singlet (s), doublet (d) and multiplet (m) and coupling constants (J) are given in Hertz. Elemental analyses for C, H, and N were within ±0.4% of the theoretical values. Mass spectra were recorded on a JEOL D-300 mass spectrometer. Thin layer chromatography (TLC) plates were coated with silica gel G and exposed to iodine vapors to check the homogeneity as well as the progress of reaction. Sodium sulfate (anhydrous) was used as a drying agent. We used the definitive rules for the nomenclature of steroids published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC for the nomenclature of the synthesized compounds. The purity of the synthesized compounds used in the biological tests was determined by C, H and N analysis. According to these data, the purity was greater than 95%. Calf thymus DNA was purchased from Sigma. The purity of DNA was verified by monitoring its absorbance ratio at 260–280 nm, which was in the range 1.8–1.9. DNA concentration was determined spectrophotometrically using ε_{260} = 6600 M^{-1} cm^{-1}.

*General method for the synthesis of steroidal iminophenyls (**2**, **7–9**)*

Steroidal ketones (**1**, **4–6**) (1 mmol) was fused with aniline (1 mmol) for 30 min, then 25 mL of absolute ethanol was added to the reaction mixture. The reaction mixture was refluxed for 6–8 h and then cooled to room temperature. The separated solid was filtered, washed with water and recrystallized from benzene to give steroidal iminophenyls (**2**, **7–9**).

*3-Iminophenylcholest-5-ene (**2**)*

White powder (71%), Mp: 202–204 °C; IR (KBr, ν, cm^{-1}): 3083, 1569, 1397 (C—H, aromatic), 1623 (C=N), 1605 (C=C); ^1H NMR (400 MHz, $CDCl_3$): δ 7.52–7.9 (m, 5H, aromatic), 5.82 (dd, 1H, C_6—H, J = 5.6, 8.4 Hz), 2.22 (s, 2H, C_4—H_2), 1.22 (s, 3H, C_{10}—CH_3), 0.74 (s, 3H, C_{13}—CH_3), 0.97 & 0.98 (other methyl protons); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.2, 141.2, 138.2, 129.4, 128.1, 127.2, 125.4, 121.1, 119.4, 56.6, 54.1, 50.2, 46.6, 42.7, 39.3, 38.5, 37.3, 35.2, 33.2, 32.4, 31.5, 28.4, 27.4, 26.3, 25.2, 24.4, 23.6, 22.5, 21.2, 20.4, 19.4, 16.4, 11.4; Anal. Calcd for $C_{33}H_{49}N$; C, 86.21; H, 10.74; N, 3.05 found; C, 86.37; H, 10.55; N, 3.08; MS (EI): m/z 459 [$M^{+\cdot}$].