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Microwave-assisted one pot synthesis, characterization, biological evaluation and molecular docking studies of steroidal thiazoles

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ABSTRACT

In the present manuscript, a series of steroidal thiazole derivatives (**4**–**6**, **8**) have been synthesized in efficient manner by one step reaction methodology employing microwave irradiation. The synthesis involves the reaction of steroidal ketones (**1**–**3**, **7**) with thiosemicarbazide and phenacyl bromide. 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 antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. In addition, the products **4**–**6**, **8** were also tested for pBR322 DNA cleavage activity, genotoxicity, reactive oxygen species (ROS) production and RBC hemolysis. Molecular docking analysis was carried out to validate the specific binding mode of the newly synthesized compounds into the active site of DNA. Docking showed formation of more stable complexes of compounds **4** and **8** with the free binding energies −8.1 and −8 kcal/mol, respectively. Hence, it could be suggested that the steroidal compounds bearing a core thiazole scaffold would be a potent biological agent.

1. Introduction

The design and development of synthetic functional molecules with desirable properties are of considerable importance in chemistry due to their applications in different fields [1]. The conventional methods for the synthesis of these molecules involve large operational time, multistep synthetic routes that resulting in overall inefficiency and the generation of huge amounts of waste. Multi-component reactions (MCRs) are useful for the creation of chemical libraries of drug-like compounds with variety of molecular diversity [2]. Steroids are an important group of natural compounds occurring in the living organisms with broad array of biological activities [3]. Such compounds are the fundamental class of biologically signalling molecules, less toxic and highly bio-available in the living system. During the course of last few decades the major effort of chemists was directed towards the modification in structure of steroids in order to enhance their valuable biological activities. These structural modifications have not only furnished new steroidal derivatives but have also led to the introduction of more specific and potent therapeutic agents [4]. The discovery of several biologically active steroids with wide applications in therapy led to the preparation of various modified steroids with new facet of chemistry. Thiazole is an important structural scaffold present in a variety of natural products [5]. Their derivatives have gained continuous attention among the researchers and hold a prominent position in drug designing as they exhibit broad range of biological and medicinal properties. They showed antimicrobial [6], antimalarial [7], antischizophrenic [8], anti-HIV [9], antiallergic activity [10] and more recently used for the treatment of pain [11]. Keeping in view of high degree of biological activities possessed by thiazoles derivatives several methods of their synthesis have been reported [12–13]. However, many of these reported methods suffer from draw backs such as harsh reaction conditions, wastage of solvents, long reaction time and unsatisfactory yields. Microwave assisted organic reactions attracted much interest because of the simplicity in operation, greater selectivity and rapid synthesis of a variety of organic compounds. This method also eliminates the use of noxious and expensive solvents, and as such can be more eco-friendly [14]. Motivated by the aforementioned facts and in continuation of our previous work in the field of synthetic transformation of steroids [15] herein, from chemistry point of view, we wish to report a more simple protocol for rapid and convenient synthesis of steroidal thiazole derivatives in excellent yields through a one-pot three component condensation reaction involving steroidal ketones, thiosemicarbazide and phenacyl bromide. The newly synthesized steroidal thiazole derivatives (**4**–**6**, **8**) have been characterized on the basis of IR, ^{1}H NMR, ^{13}C NMR, MS and analytical data. These compounds were evaluated for their DNA cleavage affinity using plasmid pBR322DNA as a target molecule by agarose gel electrophoresis method. In addition, physicochemical calculations, ROS production and in vitro antioxidant activity were also evaluated. The viscosity as a transport property was studied for their entire composition range. RBC hemolysis was carried out to assess intrinsic toxicity of the compounds. The specific binding mode of the newly synthesized compounds with DNA was revealed through molecular docking analysis.

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2. Experimental

2.1. Materials and Methods

Chemicals and solvents used in this study were of ACS grade and used directly without additional steps of purification. Melting points were determined on a Biogen digital auto melting point apparatus. Microwave reaction was performed on Anton paar monowave 300 oven. The IR spectra were recorded on KBr pellets with Perkin Elmer FT-IR spectrometer spectrum Two and values were given in cm^{-1}. ^1H and ^{13}C NMR spectra were run on a Bruker Avance II 400 NMR Spectrometer (operating at 400 MHz for ^1H and at 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard and values are given in parts per million (ppm) (δ). Mass spectra were recorded on a JEOL D-300 mass spectrometer. Elemental analyses were recorded on Perkin Elmer 2400 CHN. 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. Agarose, low melting point agarose (LMPA), RPMI 1640, Triton X-100, Trypan blue, Histopaque1077 and phosphate buffered saline (PBS) free from Ca^{+2} and Mg^{+2}were purchased from Sigma (St. Louis, MO). Heparinized blood samples (2 mL) from a single healthy donor was obtained by vein puncture and diluted suitably in Ca^{2+} and Mg^{2+} free PBS. Lymphocytes were isolated from blood using Histopaque 1077 (Sigma) and the cells were finally suspended in RPMI 1640. A single donor donated blood for all experiments. The lymphocytes were checked for their viability before the start and after the end of the reaction using Trypan Blue Exclusion Test [16]. Compounds **4**–**6**, **8** were dissolved in 2% DMSO to prepare a stock of 1 mM solution. Upon addition to reaction mixtures, in the presence of buffers mentioned and at concentrations used, all the compounds used remained in solution. The volume of stock solution added did not lead to any appreciable change in the pH of reaction mixtures.

2.2. General Procedure for the Synthesis of Steroidal Thiazoles (*4*–*6*, *8*)

In a sealed tube, a stirred solution of steroidal ketones **1**–**3**, **7** (1.0 mmol), thiosemicarbazide (1.0 mmol) and phenacyl bromide (1.0 mmol) in ethanol (15 mL) were mixed and subjected to microwave heating at 60 °C for 35–45 min. After the completion of reaction, solvent was evaporated to dryness and was taken in diethyl ether, washed with water and dried over sodium sulfate (anhydrous). The crude product obtained was purified over silica gel column (petroleum ether-ethylacetate; 4:1). Recrystallization from methanol afforded respective products (**4**–**6**, **8**).

2.2.1. 3β-acetoxy-5α-cholestane-6-ylidine-(4′-phenyl)-thiazole-2′-yl-hydrazone (*4*)

Yield (80%), m.p. 166–167 °C; IR (KBr, ν_{max} cm^{-1}): 3280 (NH), 1730 ($OCOCH_3$),1650 (C═N), 1622 (C═C), 673 (C—S), 3135, 1560, 1385 (aromatic ring). ^1H NMR (400 MHz, $CDCl_3$): δ: 6.7 (s, 1H, NH, exchangeable with D_2O), 7.7–7.82 (m, 5H, aromatic), 7.01 (s, 1H, $H_{thiazole}$), 4.7 (m, 1H, $C_3\alpha$-*H*, *W*½ = 15 Hz), 2.7 (dd, 1H, $C_5\alpha$-*H*, *J* = 12 Hz, 4 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 and 0.83 (other methyl protons); ^{13}C NMR ($CDCl_3$, 100 MHz):171.2, 166.5, 154.1, 152.9, 133.47, 130.05, 129.75, 128.83, 128.32, 126.45, 102.9, 74, 60, 58, 57, 45, 43.5, 42.5, 40.2, 39.3, 38.2, 37.8, 36.1, 35.0, 32.3, 30.1, 29.4, 28.9, 27.5, 25.7, 24.6, 23.9, 22.7, 21.2, 20.3, 19.4, 16.0, 14.7. Anal. Calcd for $C_{38}H_{55}N_3O_2S$; C, 73.86, H, 8.97, N, 6.80; found; C, 73.83, H, 8.95, N, 6.77; ESI MS: *m*/*z* 617 [$M^{+\cdot}$].

2.2.2. 3β-Chloro-5α-cholestane-6-ylidine-(4′-phenyl)-thiazole-2′-yl-hydrazone (*5*)

Yield (85%), m.p. 153–154 °C; IR (KBr, ν_{max} cm^{-1}): 3287 (NH), 1655 (C═N), 1618 (C═C), 740 (C—Cl), 667 (C—S), 3122, 1562, 1387 (aromatic ring). ^1H NMR ($CDCl_3$, 400 MHz,): δ: 6.8 (s, 1H, N*H*, exchangeable with D_2O), 6.92 (s, 1H, $H_{thiazole}$), 7.7–7.8 (m, 5H, aromatic), 4.7 (m, 1H, $C_3\alpha$-*H*, *W*½ = 15 Hz), 2.7 (dd, 1H, $C_5\alpha$-*H*, *J* = 12 Hz, 4 Hz), 1.20 (s, 3H, C_{10}–CH_3), 0.70 (s, 3H, C_{13}–CH_3), 0.97 and 0.85 (other methyl protons). ^{13}C NMR ($CDCl_3$, 100 MHz): 167.3, 155.5, 152.3, 133.5, 130.1, 129.75, 129.9, 128.35, 126.5, 102.3, 63, 62, 61.5, 59, 46.5, 44.5, 42.7, 41.3, 40.5, 39.7, 38.7, 37.2, 36.8, 34.1, 31.9, 30.3, 29.1, 28.3, 26.5, 25.1, 24.4, 22.5, 21, 20, 16,14.3. Anal. Calcd for $C_{36}H_{52}ClN_3S$: C, 72.75, H, 8.82. N, 7.07: found: C, 72.73, H, 8.85, N, 7.05.ESI MS: *m*/*z* 593/595 [$M^{+\cdot}$].

2.2.3. 5α-Cholestane-6-ylidine-(4′-phenyl)-thiazole-2′-yl-hydrazone (*6*)

Yield (83%), m.p. 173–174 °C; IR (KBr, ν_{max} cm^{-1}): 3290 (NH), 1657 (C═N), 1620 (C═ C), 681 (C—S), 3129, 1565, 1403 (aromatic ring). ^1H NMR (400 MHz, $CDCl_3$): δ: 6.8 (s, 1H, N*H*, exchangeable with D_2O), 6.98 (s, 1H, $H_{thiazole}$),7.7–7.82 (m, 5H, aromatic), 2.4 (dd, 1H,$C_5\alpha$-*H*, *J* = 12.01 Hz, 4.2 Hz), 1.18 (s, 3H, C_{10}–CH_3), 0.70 (s, 3H, C_{13}–CH_3), 0.97 and 0.83 (other methyl protons). ^{13}C NMR ($CDCl_3$, 100 MHz); 166.3, 155.8, 152, 133.1, 130.5, 129.6, 128.5, 128.3, 125, 102, 62.3, 61.1, 60, 47.5, 44.6, 43.1, 41.1, 40.7, 39.4, 38.8, 38.1 37.7, 35.1, 33, 32.1, 30.5, 29.5, 28.7, 27.5, 26.1, 25.5, 23.1, 21.5, 19, 16, 15.1. Anal. Calcd for $C_{36}H_{53}N_3S$; C, 77.23, H, 9.54, N, 7.51; found; C, 77.26, H, 9.52, N, 7.53; ESI MS: m/z559 [$M^{+\cdot}$].

2.2.4. 3β-Acetoxy-pregn-5-ene-20-ylidiene-(4′-phenyl)-thiazole-2′-yl-hydrazone (*8*)

Yield (80%), m.p. 146–147 °C; IR (KBr, ν_{max} cm^{-1}): 3309 (NH), 1728 ($OCOCH_3$), 1661 (C═N), 1615 (C═C), 673 (C—S), 3138, 1567, 1409 (aromatic ring). ^1H NMR (400 MHz, $CDCl_3$): δ6.75 (s, 1H, N*H*, exchangeable with D_2O), 6.95 (s, 1H, $H_{thiazole}$), 7.75–7.85 (m, 5H, aromatic), 2.07 (s, 3H, $OCOCH_3$)·^{13}CNMR ($CDCl_3$, 100 MHz); 171.1, 168.8, 156.7, 151.9, 138.8, 134.2, 133.5, 130.1, 129.6, 128.4, 126.5, 120.8, 102.4, 76.1, 55.1, 48.5, 44.7, 42.5, 41.2, 40.2, 39.5, 38.5, 36.7, 34.3, 33.1, 30.3, 29.2, 24.1, 23.0, 22.4, 21.5, 16.3. Anal. Calcd for $C_{32}H_{41}N_3O_2S$; C, 72.28, H, 7.77, N, 7.90; found; C, 72.30, H, 7.80, N, 7.87. ESI MS: *m*/*z* 531 [$M^{+\cdot}$].

2.3. Biological Evaluation

2.3.1. Molecular Properties Predictionand Drug-likeness

The physicochemical parameters including MiLogP (octonal partition coefficient), TPSA (topological surface area), HBA (hydrogen bond acceptor), HBD (hydrogen bond donar), Mw (molecular weight), rotatable bonds, molar volume and bioactivity scores (GPCR, G protein coupled receptor) ligand, Ion channel modulator, Kinase inhibitor, Protease inhibitor, Nuclear receptor ligand, Enzymes inhibition were calculated using online database (www.molinspiration.com). The maps of molecular lipophilicity potential (MLP) were viewed in Molinspiration Galaxy 3D Structure Generator. The hidden biological activity of the synthesized molecules was predicted by using PASS server [17].

2.3.2. Free Radical Scavenging Activity (Antioxidant Assay)

In present study, the free radical scavenging activity of synthesized steroidal thiazole derivatives was measured by means of the DPPH assay [18]. DPPH radical is a stable free radical having maximum absorbance at 517 nm. DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. It is considered as convincing and easy assay to appraise radical-scavenging activity of antioxidants [19]. Stock solution of DPPH (1 mg/ml) in methanol was prepared. The various concentrations of compounds (25, 50, 75 & 100 mg/ml) were prepared. All sample solutions 1 mL each is diluted to 3 mL and 100μL of stock solution of DPPH was added. Then the absorbance was determined at 517 nm after incubation for 30 min in the darkness at room temperature. The DPPH scavenging activity was calculated using the following equation.

$$\text{DPPH scavenging effect}(\%) = A_{control} - A_{sample}/A_{control} \times 100 \quad (1)$$