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Synthesis, chemical characterization, DNA binding and antioxidant studies of ferrocene incorporated selenoure

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HIGHLIGHTS

- We reported the synthesis of possible antitumor agent MOT.
- Its interaction with DNA has been evaluated.
- Computational and experimental results have been compared.

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ABSTRACT

In this article we have reported synthesis, chemical characterization (with single crystal XRD, elemental analysis, FTIR and multinuclear NMR spectroscopy), DNA binding (with cyclic voltammetry, UV–vis spectroscopy, molecular docking and viscometry) and antioxidant activities (1,1-diphenyl-2-picrylhydrazyl scavenging) of 1-(2-methylbenzoyl)-3-(3-ferrocenylphenyl)selenourea (MOT). We found that this compound interacts electrostatically with DNA and has a binding constant value of $1.703 \times 10^4\ M^{-1}$. Lower value of diffusion coefficient for MOT-DNA adduct ($1.35 \times 10^6\ cm^2\ s^{-1}$) relative to free MOT ($1.66 \times 10^6$ - $cm^2\ s^{-1}$) in cyclic voltammetry (CV) indicated the binding of the compound with DNA. Smaller value of binding site size (0.88 base pairs) in CV, hyperchromism in UV–vis spectroscopy and decrease of relative specific viscosity of DNA in viscometry favored electrostatic interactions. Binding energy of experimental (-5.77 kcal mol^{-1}) and simulated (-5.86 kcal mol^{-1}) work are in close agreement with each other. IC_{50} value of MOT for 1,1-diphenyl-2-picrylhydrazyl scavenging was found to be 27 μM.

1. Introduction

Acylselenoureas were synthesized way back in 1937 by Douglass [1] but early developments in their synthetic chemistry and biological applications were slow because selenium was considered to be an absolute biological poison those days [2]. After the findings that organoselenium derivatives are far less toxic than inorganic selenium species and presence of selenium in bacterial enzymes (formate dehydrogenase and glycine reductase) broke this cyst and developments started in bioorganic chemistry of selenium [3]. As a result efforts have been made for the application of selenium derivatives as photochemotherapeutic agents, antitumor agents, anti-infective drugs (antifungal, antibacterial and antiviral), cytokine inducers/immunomodulators, antioxidant defense enzymes (reduction of hydroperoxides-GPx mimics, reduction of peroxinitriles, and lipid peroxidation), enzyme inhibition (nitric oxide synthase inhibitors, ionosine monophosphate dehydrogenase inhibitors, lipoxygenase inhibitors, urdpase and thymidylate synthase inhibitors, and tyrosine kinase and iodothyronine deiodinase inhibitors), antihypertensive and carditonic agents [2]. But the interaction of selenoureas with DNA has not been studied in details [4]. Selenoureas particularly are good as free radical scavengers, enzyme inhibitors and anticancer agents [5–7].

Meanwhile ferrocene derivatives find their applications as anticancer, antimalarial and antibiotic materials [8]. Moreover some ferrocene derivatives have also been evaluated for their DNA binding studies and have shown good values of DNA binding constants [4]. Introduction of ferrocene in a compound not only improves the electrochemical and spectroscopic behavior but also enhances the possible applications of the compound in which it is incorporated. In this article we have combined ferrocene and selenourea moieties in ferrocene incorporated selenourea (MOT) and evaluated it for its DNA binding and antioxidant activities [9–11]. Magnitude of DNA binding constant and mode of interaction with DNA of a certain possible drug provides preliminary information about the possibility of its use as antitumor agent. It is believed that if uncon-

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trolled growth of the tumor cell is stopped or slowed by interacting its DNA with the drug, then cancer can be controlled. In this article we have used cyclic voltammetry (CV) for the determination of binding behavior of DNA with our synthesized compound 1-(2-methylbenzoyl)-3-(3-ferrocenylphenyl)selenourea (MOT). The results obtained from CV have been verified with UV–vis spectroscopy, molecular docking and viscometry. Due to the suspected side effects of antioxidants during cancer therapy they are generally forbidden for the patients, although it is not yet very clear whether to use them or not [12]. Therefore, we have also carried out antioxidant studies of MOT with 1,1-diphenyl-2-picrylhydrazyl using ascorbic acid as a reference. The idea was to use the same compound as anticancer and antioxidant if it passes the criteria.

2. Experimental, materials and methods

Melting point was determined in a capillary tube using Gallenkamp (UK) electrothermal melting point apparatus. Infrared spectrum was taken on Thermoscientific NICOLET 6700 FTIR between 4000 and 400 cm^{-1}. ^{1}H and ^{13}C spectra were recorded between 0–13 ppm and 0–210 ppm respectively on Jeol JNM-LA 500 FT-NMR. $Si(CH_3)_4$ was used as internal reference. The elemental analysis was performed using a LECO-932 CHNS analyzer while the Fe concentrations were determined on an Atomic Absorption Spectrophotometer Perkin–Elmer 2380.

Commercial salmon DNA was solubilized in doubly distilled water to prepare a stock solution of 5.9×10^{-4} M from which working concentrations of DNA were prepared. Concentration of stock solution was measured by UV absorbance at 260 nm using an epsilon value of 6600 M^{-1} cm^{-1}. This DNA was protein free because $A_{260}/A_{280} > 1.8$. Working solutions for DNA binding studies were prepared by our previously reported method [4].

Cyclic voltammetry was performed on Biologic SP-300 cyclic voltammeter running with EC-Lab Express V 5.40 software, Japan. Before every reading working electrode was polished with alumina powder and rinsed with distilled water. Analytical grade TBAP (tertiary butyl ammonium perchlorate) was used as supporting electrolyte and nitrogen gas (99.9%) was purged through the mixture to avoid interference of oxygen.

Absorption spectra were recorded on Shimadzu 1800 spectrophotometer. First the spectra of the pure MOT solution were recorded between 220 and 800 nm in the absence of DNA and then in the presence of different concentration of DNA. Equal amount of DNA was added to both reference and sample cells in order to avoid the appearance of its peak at 260 nm.

The Ubbelohde viscometer was used for viscosity measurements at room temperature (25 ± 1 °C). Flow time was measured with a digital stopwatch. Flow time measurements were made in triplicate for the measurement of average flow time. Data were presented as relative specific viscosity (η/η_o), vs. binding ratio ([MOT]/[DNA]) where η is the viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone.

Docking studies were carried out using Autodock (Version 4.2) docking software [13]. Structure of B-DNA dodecamer $d(CGCGAATTCGCG)_2$ (1BNA) was taken from protein data bank (PDB) [14] while selenium atoms were replaced by sulfur [15] from crystallographic information file of MOT for performing docking. Essential hydrogen atoms and Gasteiger charges were added with the aid of Auto-Dock tools (ADT). The grid size was set to 64, 66 and 126 along the *X*, *Y* and *Z*-axis, respectively. The center of the grid was set to 14.980, 20.976 and 8.807. After DNA was enclosed in the grid, defined with 0.375 Å spacing, the grid map was calculated using the AutoGrid program. Docking to macromolecule was performed using an empirical free energy function and Lamarckian Genetic Algorithm, with an initial population of 50 randomly placed individuals. A maximum number of 100,000 energy evaluations at a mutation rate of 0.02, and a crossover rate of 0.80 were performed. MOT was allowed to move within a specified region to achieve the lowest energy conformation while B-DNA dodecamer was kept rigid during docking.

Ferrocene, metanitro aniline, sodium nitrite, diethyl ether, acetone, DMSO, Pd-charcoal, carboxylic acid chloride and hydrazine were purchased from Sigma–Aldrich. Meta ferrocenyl aniline was synthesized by a procedure reported by our group previously [16].

2.1. Synthesis of 1-(2-methylbenzoyl)-3-(3-ferrocenylphenyl)-selenourea (MOT)

MOT was synthesized in two necked round bottom flask by the reaction of 0.30 g (0.00208 mol) of potassium selenocyanate with 0.27 mL (0.00208 mol) of 2-methylbenzoyl chloride in acetone under constant stirring. Resulting yellow colored product with a suspension of potassium chloride was allowed to stir for a further 3 h for the completion of reaction and then 0.576 g (0.00208 mol) of metaferrocenyl aniline was mixed to it. Completion of reaction was monitored with the help of thin layer chromatography (TLC). After 6 h reaction mixture was mixed with cold water and left overnight for precipitation. Settled solid product was then filtered, washed with n-hexane and recrystallized in toluene (Scheme 1). Yield 75%. M.p 145-146 °C (decomposed). δ_H (ppm) (Acetone) 13.27 (s, 1H), 10.47 (s, 1H) 8.20-7.34 (m, 8H), 4.81 (t, *J* 1.8, 2H), 4.37 (t, *J* 1.8, 2H), 4.11 (s, 5H), 2.46 (s, 3H). δ_C (ppm) (Acetone) 180.4, 167.6, 144.5, 140.4, 139.2, 129.4, 128.6, 124.3, 122.3, 121.8, 84.3, 69.5, 69.0, 66.5, 20.7. υ_{max}/cm^{-1} 3364-3229 (b), 3100, 2956, 1644, 1605, 1514, 1495, 1450, 1375, 1258, 1153, 1065. Anal. Calc. for $C_{25}H_{22}N_2SeFeO$: C 59.88, N 5.58, H 4.39, Fe 11.17. Found: C 59.84, N 5.56, 4.31, Fe 11.15%.

3. Results and discussion

MOT is solid, crystalline and orange in color. It is very easily purified from possible impurities (amide, potassium chloride, unreacted meta ferrocenyl aniline and carboxylic acid) on the basis of its different solubilities in water and common organic solvents. ^{1}H and ^{13}C NMR data of MOT were recorded in deuterated acetone. There are four different types of protons in MOT i.e. protons of —NH, aromatic protons, protons of ferrocene and methyl protons. In ^{1}H NMR, the NH proton which is between the C=O and C=Se is maximum deshielded and provides a signal at 13.27 ppm whereas the other NH proton between C=Se and phenyl moiety is comparatively less deshielded and appears at 10.47 ppm as a singlet [4,17]. Two aromatic rings provide their signals as multiplet between 7.34 and 8.20 ppm. Five protons of unsubstituted Cp ring of ferrocene yielded an intense singlet at 4.11 ppm and substituted Cp ring provided two triplets downfield from the singlet of unsubstituted Cp ring at 4.81 and 4.37 with a coupling constant value of 1.8 Hz. In ^{13}C NMR, carbon attached with selenium provides a weak signal at 180.4 ppm and carbonyl carbon appeared at 166.7 ppm. Carbons of the unsubstituted Cp ring yield one signal at 69.5 and substituted Cp ring gives three signals, two of them are between 60 and 70 ppm, and one signal for the substituted carbon of Cp ring is visible at 84.3 ppm. Aromatic carbons are visible in a region between 120 and 140 ppm [4,17].

In FTIR, —NH of MOT gave a broad band above 3200 cm^{-1} owing to the intramolecular hydrogen bonding between the oxygen of the carbonyl and —NH. Just above 3000 cm^{-1} Ar—H stretch was evident and carbonyl group appeared as an intense band at 1644 cm^{-1}. C=Se was available between 1050 and 1250 cm^{-1} [4,17].