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Orginal article

Nuclear blebbing of biologically active organoselenium compound towards human cervical cancer cell (HeLa): *In vitro* DNA/HSA binding, cleavage and cell imaging studies

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ABSTRACT

New pharmacophore organoselenium compound (**1**) was designed, synthesized and characterized by various spectroscopic methods (IR, ESI–MS, ^{1}H, ^{13}C and ^{77}Se NMR) and further confirmed by X–ray crystallography. Compound **1** consists of two 3,5–bis(trifluoromethyl)phenyl units which are connected to the selenium atom *via* the organometallic C–Se bond. *In vitro* DNA binding studies of **1** was investigated by absorption and emission titration methods which revealed that **1** recognizes the minor groove of DNA in accordance with molecular docking studies with the DNA duplex. Gel electrophoretic assay demonstrates the ability of **1** to cleave pBR322 DNA through hydrolytic process which was further validated by T4 religation assay. To understand the drug–protein interaction of which ultimate molecular target was DNA, the affinity of **1** towards HSA was also investigated by the spectroscopic and molecular modeling techniques which showed hydrophobic interaction in the subdomain IIA of HSA. Furthermore, the intracellular localization of **1** was evidenced by cell imaging studies using HeLa cells.

1. Introduction

Medicinal inorganic chemistry is an interdisciplinary thrust area of chemical research; is currently much more known for its many applications in catalysis and also has enormous potential to act as therapeutic and diagnostic agents. Small molecules capable of binding to deoxyribonucleic acid (DNA) has received immense influence in the development of new therapeutic modalities for cancer chemotherapy owing to the fact that many present treatment regimes (Platinum based drugs) in chemotherapy have failed or fall short either in terms of efficiency or toxicity problems [1–3]. Therefore, other non–platinum complexes are being evaluated in cancer chemotherapy, and in particular organometallic compounds have been extensively investigated for their medicinal properties [4–6]. Koepf and Koepf–Maier started to explore the anticancer activity of titanocene dichloride, in the 1970s with several clinical trials conducted (trials on actual patients with various forms of cancer that have not responded to existing treatments, including surgery, chemotherapy, and radiotherapy), although finally the compound was not approved for use [7]. Nevertheless, the early and highly promising research on $Ti(\eta^5–C_5H_5)_2Cl_2$ inspired other researchers to study the anticancer properties of organometallic compounds, which brought rational design and many new ideas for the development of cancer chemotherapeutic drugs. Among the non–platinum complexes for metal based chemotherapy, organoselenium (Se) molecules are emerging as new chemotherapeutic agents due to their ability to modulate multiple physiological effects on a wide range of cancer cell types as well as reverse the

Abbreviations: UV–vis, UV–visible; CT DNA, Calf thymus DNA; Tris, Tris(hydroxymethyl)aminomethane; EB, Ethidium bromide.

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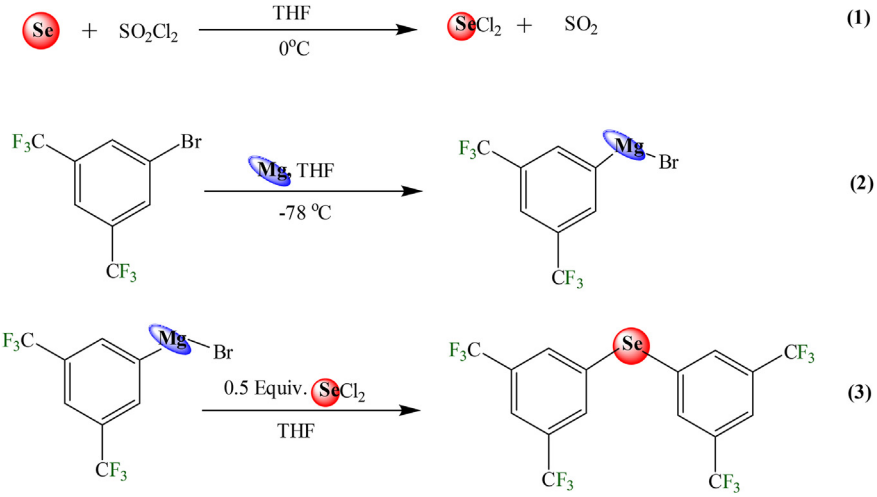
activity of drug resistance mechanisms to potentiate chemotherapy/radiotherapy efficacy [8]. The trace element selenium (Se), at very high doses is potential toxic and fatal for the human body, but the low doses of Se supplementation (nutritional intake of 50–350 μg per day) seems to be beneficial not only for cancer prevention, but also influence other functions in organism by reducing inflammations, heart diseases and regulating the blood pressure. Moreover, selenium is essential for cell metabolism as a constituent of several seleno–proteins, including glutathione peroxidase, thioredoxin reductase etc which are primarily involved in antioxidant functions and maintenance of cellular redox state [9]. Since the discovery of the first GPx (Glutathione Peroxidase) mimics organoselenium compound, 2–phenyl–1,2–benzoisoselenazol–3–(2H)–one (ebselen), considerable efforts have been made for the design and development of several organoselenium compounds as GPx mimics for the treatment of oxidative stress initiated by excess reactive oxygen species (ROS), and thereby preventing the cellular damage [10].

DNA is the essential carrier of genetic information which is concerned with most cancers resulting from DNA damage, and thus it is considered as a main target for anticancer drugs development. Interaction between small molecules and DNA is of great significance in the elucidation of the mechanisms of anticancer drugs and the screening of DNA targeted drugs [11].

In view of the aforementioned fact, the present work embodies the spectroscopic and single crystal X–ray diffraction of the newly synthesized bis(3,5–bis(trifluoromethyl)phenyl)selane. It would be pertinent to mention here that synthesis of compound **1** was based on our understanding of a series of diselenides synthesized by our group, wherein we found that the molecule having bistrifluoromethyl phenyl moiety displayed most potent anticancer activity [12]. With this rationale, *in vitro* DNA binding profile of organoselenium compound **1** with CT–DNA and cleavage studies of **1** with pBR322 plasmid DNA by agarose gel electrophoresis were evaluated. The role of compound **1** was not only merely antioxidative (by detoxification of reactive oxygen species as part of glutathione peroxidases), but rather acting as a DNA binding and cleaving agent. Furthermore, affinity of **1** towards human serum albumin (HSA) was also investigated since the drug–albumin interaction plays an important role in drug distribution and pharmacokinetics which influences the solubility of the prospective drug, extend its *in vivo* half–life, slow down or prevent its passive extravasations to the target tissues [13]. These studies provide an important rationale for the design of new lead anticancer drugs and their specific delivery at the active site of action, besides providing the pharmacological profile *in vitro*.

2. Results and discussion

We report the synthesis of new organoselenium bis(3,5–bis(triflouromethyl)phenyl)selane compound **1** and the detailed procedure was described in experimental section (Scheme 1) [14]. The product was isolated as a yellowish red solid, and suitable crystals for X–ray crystallography was grown by slow evaporation of its solution in $CHCl_3$/MeOH (8:2 v/v), over a period of 1 week at room temperature. The resulting compound **1** was stable towards air and moisture and readily soluble in DMSO and MeOH. The single–crystal X–ray analysis reveals that the compound **1** crystallizes in a triclinic crystal system with *P*–1 space group. The unit cell parameters were found to be $a = 8.7402(5)$ Å, $b = 8.9544(5)$ Å, $c = 12.4403(6)$ Å, $\alpha = 107.674(4)^\circ$, $\beta = 102.497(4)^\circ$, $\gamma = 99.079(5)^\circ$ with unit cell volume, $V = 879.16(9)$ Å^3. A perspective view of the ORTEP diagram and molecular structure of compound **1** was shown in Fig. 1. From the crystal structure it has been observed that two 3,5–bis(trifluoromethyl)phenyl moieties are attached to the Selenium atom. The C–Se–C bond angle is 100.9(2)°, as expected for the divalent sp^3 Se atom with lone pair–lone pair repulsions. Selected crystallographic data for the compound is summarized in Table S1. The crystal packing of compound **1** displayed the common feature of layers of weakly interacting stacked phenyl groups of one molecule with the other. Besides, 3D supramolecular architecture of compound **1** was generated which shows two types of secondary interactions, hydrogen and weak halogen bonding between the identical moieties of the same molecules (Fig. 2). The distance between the hydrogen atoms of one molecule with the fluorine atom of another molecule is 2.658(2) Å (H–F), and fall in the range of moderate hydrogen bonding distance. Interestingly, the halogen (F–F) distance in compound **1** was 2.713(3) Å, which was quite favorable for halogen interactions thereby helping the molecules to interact and stay closer. Density functional theory (DFT) is a popular computational method of medicinal chemists widely used today for screening of biologically relevant molecular systems in a time and cost effective manner [15,16]. The DFT studies were performed using Turbomole 6.0 program with B3LYP functional and three different basis sets {6-31G(d,P), LanL2MB, TZVP} were used in



Scheme 1. Synthesis of bis(3,5–bis(trifluoromethyl)phenyl)selane.