Journal of Molecular Structure 1099 (2015) 135–141

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Designing, syntheses, characterization, computational study and biological activities of silver-phenothiazine metal complex

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ARTICLE INFO

Article history:
Received 29 March 2015
Received in revised form
17 June 2015
Accepted 18 June 2015
Available online 20 June 2015

Keywords:
Phenothiazines
Chemical reactivity
Protein binding
Biological activities

ABSTRACT

A noble biologically active compound Ag(I)-PTZ metal complex (**1**) with spherical morphology was synthesized first time. Entire characterization tool (spectral, elemental, mass and thermal analysis) was supported a distorted tetrahedral structure, where two water compounds were coordinated with Ag(I) including one phenothiazine and one nitrate group. For the better insight, obtained spectral/structural results were supported by 3D molecular modeling.

Compound **1** had shown excellent activities against the *Salmonella typhimurium* and *Aspergillus fumigatus* with minimum inhibitory concentration (MIC) value 20 mg/L and 25 mg/L. The observed antioxidant radical scavenging activity (in %) of compound **1** (62.74%) was more than control ascorbic acid (28.58%). The observed protein (BSA) binding constant of **1** was 8.86×10^4 M^{-1}, which is similar to binding constant of salicylic acid with BSA protein. Initial studies have revealed that synthesized compound **1** may act as multipurpose drug analogue in future.

1. Introduction

Phenothiazine (PTZ) and its derivatives are heterocyclic compounds with versatile applications in pharmaceutical, textile, lubrication and rubber industry [1–3]. PTZs belong to the oldest, synthetic antipsychotic drugs, which do not have their precursor in the world of natural compounds [3]. More than hundred derivatives of PTZs exhibited promising antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, antimalarial, antifilarial, trypanocidal, anticonvulsant, analgesic, immunosuppressive, neuroleptic and multidrug resistance reversal properties [4,5].

As per HASB principle the structure of PTZ suggests its ability to act like unidentate or bi-dentate ligand through nitrogen and/or sulfur depending on variation of pH. Although, it is typical to coordinate PTZ with metal ions due to its non planer and steric nature [6,7], but we found our special interest in its complexation with Ag due to versatile applications of silver and its complexes in medicines, water purification, agriculture etc.

After performing reactions of PTZ in 1:1 and 2:1 with fifteen metal ions (details are mentioned in supplementary) at pH ~7, first time we have reported the synthesis, characterization and biological activities of Ag(I) complex with PTZ or **1** in 1:1 ratio. The biological activities like antimicrobial activities, antioxidant activities, and binding ability with BSA protein of the complex **1** have been analyzed and reported.

2. Material and methods

2.1. Material and reagents

Phenothiazine (AR grade), TLC plates were purchased from Sigma Aldrich, DMSO, ammonia, ethanol, hexane, chloroform, hexane, ethyl acetate, $AgNO_3$ and BSA protein were of AR grade purchased from Loba Chemie. All the pathogenic strains were purchased from the National Chemical Laboratory with unique code number, NCIM, Pune India.

2.2. Instruments and conditions

UV–Visible spectra of free ligand and silver complex were recorded in DMSO with concentration (1.0×10^{-3} M) using a Shimadzu-1800 s at Lovely Professional University. Infrared spectra were collected on a Shimadzu-8400s in a working range of

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http://dx.doi.org/10.1016/j.molstruc.2015.06.055
0022-2860/

4000–400 cm^{-1} in dry KBr pellets at Lovely Professional University. Mass (Waters, Q-TOF Micromass) and NMR spectra (in DMSO-d_6 and TMS as internal reference) were recorded on Bruker Avance III, 400 MHz FT-NMR spectrometers at the SAIF Chandigarh, Chandigarh University. SEM (JEOL Model JSM - 6390LV) and TGA (Perkin Elmer STA 6000) were performed under nitrogen atmosphere with heating rate 10 °C/min at STIC University of Coachi (Kerala).

2.3. Synthesis of silver complex of PTZ

Reaction between Ag(I) and PTZ was performed in round bottom flask (RBF) in 1:1 ratio by adding 10 mL (0.169 g, 1 mol) aqueous solution of silver nitrate and 10 mL (0.199 g, 1 mol) ethanolic solution of PTZ (Scheme 1). Reaction mixture was stirred at 120 rpm and heated at 45 °C for 4 h. The reaction progress and purity of the compound has been checked by thin layer chromatography (TLC) and melting point analysis. The detailed analysis of compound 1 or Ag(I)-PTZ is given below.

Ag(I)-PTZ or **1** (Color; purple, m.p. 234 ± 2 °C; yield; 61.89%); UV (DMSO) 343 and 417 nm; FTIR (KBr); 3461 (b), 1590 (s), 1581 (s), 1472 & 1442 (vs), 1308 (s), 1126 (m), 1035 (m), 926 (s), 853 (w), 744 (vs), 622 (m) 544 (m) and 426 (w) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) in ppm; δ, 6.96 (t, 1H, J = 7.72 Hz), 6.93 (t, 1H, J = 7.72 Hz), 6.86 (dd, 2H, J = 8.88 Hz), 6.71 (dd, 2H, J = 7.64 Hz), 6.68 (dd, 2H, J = 8.80 Hz) ESI-MS calculated for [Ag(PTZ). $(H_2O)_2$. NO_3]; 403.56, found: 403.91; Elemental Analysis, Calcd. (Obs.), C, 35.66 (34.88); H, 2.99 (2.97); Ag, 26.69; N, 6.93 (6.85); O, 19.79; S, 7.93 [5–7]. Space for Scheme 1.

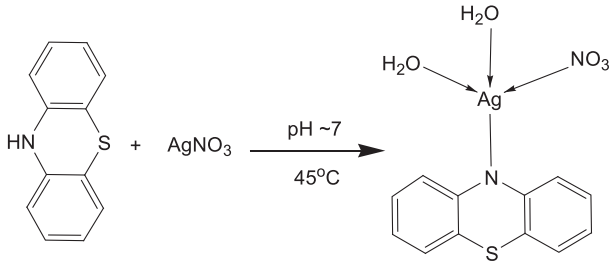
2.4. Computational analysis

Geometric optimization and conformational analysis of compound **1** has been performed by the use of GAMESS ChemBio Office Ultra 13.0 Suite 13.0 [8,9]. Semi empirical method PM3 is then used for optimizing the full geometry of the system using QA algorithm and Unrestricted Hartee–Fock (UHF) is employed keeping 6–311++G(d,p) basis set [8–15].

2.5. Antimicrobial activities

2.5.1. Antibacterial activities

In vitro antibacterial studies were carried out by agar disc diffusion method against test organisms [16,17]. Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 ml) of test bacteria. Sterile paper discs (5 mm) were put into each petriplate. Different concentrations of DMSO dissolved compounds (50, 250, 500 mg/L) were added into the discs by dipping individual disc into solution containing test tubes. In our experiment, DMSO acted as negative control and chloramphenicol as positive control. The plates were incubated at 37 °C for 24 h. After appropriate incubation, diameter of zone of inhibition of each disc was measured.



Scheme 1. Synthesis of silver complex **1** of phenothiazine.

2.5.2. Antifungal activities

In vitro antifungal studies were carried out by potato dextrose agar medium disc diffusion method against test organisms. All fungi were cultured in potato dextrose agar medium. For this purpose, potato dextrose agar medium (prepared from potato 150 g, dextrose 5 g and agar 2 g in 200 mL of distilled water) was poured in the sterilized Petri dishes and allowed to solidify, where dishes were inoculated with a spore suspension of (10^6 spores/mL of medium). Test compound **1** was dissolved in DMSO to a final concentration of 50, 250 and 500 mg/L and soaked in filter paper discs, which were placed on the already seeded plates and incubated at 37 °C for 96 h. After 96 h, inhibition zone appeared around the discs in each plate was measured [16,17]. In this experiment DMSO acted as negative control and ketoconazole as positive control.

2.5.3. MIC determination

The minimum inhibitory concentration (MIC) denotes the lowest drug concentration that prevents the visible growth of tests microorganisms. MIC (mg/L) values were evaluated by using the serial double dilution method in the appropriate medium which is inoculated with a standardized number of microorganisms [16–18].

2.6. Protein binding

PTZ and its silver metal complex were tested for protein binding at five different concentrations of PTZ; 0.55×10^{-3} to 8.88×10^{-3} mM (0.55×10^{-3}, 1.11×10^{-3}, 2.22×10^{-3}, 4.4×10^{-3}, and 5.55×10^{-3} mM) and at five different concentrations of silver complex **1**; 0.55×10^{-4} to 8.88×10^{-4} mM (0.55×10^{-4}, 1.11×10^{-4}, 2.22×10^{-4}, 4.4×10^{-4}, and 5.55×10^{-4} mM) as described by Abdi et al. [19]. An increase in the concentrations resulted in an increase in UV light absorption and shifting of BSA band from 277 to 272 nm that can be related to complex formation. The binding constants of the complexes are determined by using UV–vis spectroscopic method. The double reciprocal plot of $1/(A-A_0)$ versus 1/(ligand concentration) is found linear and the binding constant (k) can be estimated from the ratio of the intercept to the slope.

2.7. Antioxidant assay

DPPH (1, 1-diphenyl-2-picrylhydrazyl (α, α-diphenyl-bpicrylhydrazyl) radical scavenging analysis was performed according to the reported method [21]. 0.01 mM solutions of silver complex of phenothiazine and ascorbic acid were prepared by dissolving these in DMSO and distilled water respectively. 250 μL of each was added to 10.0 ml of 0.01 mM solution of DPPH in 10.0 mL amber color volumetric flasks, which was placed in the spectrophotometer immediately and monitored for 30 min. The absorbance at 517 nm was recorded after every 2 min. Pure DPPH solution was used as a control. The decrease of in absorbance equates the DPPH radical scavenging capacity. The above process was repeated three times for ascorbic acid (control) and Silver complex of phenothiazine. The radical scavenging ability was calculated according to the formula:

$$\text{Radical scavenging activity } \% = (A_0 - A_T/A_0) \times 100.$$

where, A_0 = absorbance of pure DPPH solution, and A_T = absorbance of (DPPH + Ag-phenothiazine) complex solution at 30 min.