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Experimental and theoretical investigation of a pyridine containing Schiff base: Hirshfeld analysis of crystal structure, interaction with biomolecules and cytotoxicity

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ABSTRACT

A pyridine containing Schiff base (E)-2-methoxy-6-(((pyridin-2-ylmethyl)imino)methyl) phenol (L) was isolated in single crystals. The molecular structure of L was studied by FT-IR, NMR, UV–Vis techniques, single crystal XRD analysis and computationally by DFT method. L prefers enol form in the solid state. Electronic spectrum of L was recorded in different organic solvents to investigate the dependence of tautomerism on solvent types. The polar solvents facilitate the proton transfer by decreasing the activation energy needed for transition state. Potential energy curve for the intramolecular proton transfer in the ground state is generated in gas and solution phases. The 3D Hirshfeld surfaces and the associated 2D fingerprint plots were investigated. The percentages of various interactions were analyzed by fingerprint plots of Hirshfeld surface. The interaction of L with CT DNA was investigated under physiological conditions using UV–Vis spectroscopy, fluorescence quenching and molecular docking methods. Molecular docking studies reveal that binding of L to the groove of B-DNA is through hydrogen bonding and hydrophobic interactions. The *in vitro* cytotoxicity of L was carried out in two different human tumor cell lines, MCF 7 and MIA-Pa-Ca-2 exhibits moderate activity.

1. Introduction

Schiff base compounds have received considerable scrutiny from both theoretical and experimental standpoints [1–4]. These compounds are used in diverse fields of chemistry and biochemistry. Schiff bases are acting as ligands to metal ions because of their multiple ligation sites. Pyridine derivatives have occupied a unique position in the field of medicinal chemistry. Many naturally occurring compounds having pyridine moiety exhibit interesting biological and pharmacological activities. Pyridine derivatives have been used as herbicides, for regulation of arterial pressure and cholesterol levels in blood [5,6]. Some of them constitute an important class of antitumor compounds [7]. 2-hydroxy Schiff base compounds received considerable attention mainly due to the presence of strong hydrogen bonds (O–H···N) and (O···H–N) and tautomerism between phenol–imines and keto–amine forms [8–10]. In crystalline materials, intermolecular interactions play crucial role in the packing of molecules [11]. This molecular arrangement leads to physical properties of the compound. These interactions play a vital role in specific biological reactions associated with supramolecular chemistry, in particular, drug–receptor interactions, enzyme inhibition and protein folding [12,13]. They play a role in managing protein and DNA structure and enzyme–substrate binding. Thus, the investigation and understanding of these interactions have become important. Hirshfeld surface [14–16] and associated 2D fingerprint plots [17] are simple visualization tool for the analysis of intermolecular interactions. Density functional theory calculations have been used extensively for calculating a wide variety of molecular properties such as equilibrium structure, charge distribution, FT-IR and NMR spectra, and provided reliable results which are in agreement with experimental data. The charge density data has been used to understand the properties of molecular systems.

In continuation of our interest on Schiff bases [18,19], we report herein a pyridine containing Schiff base, (E)-2-methoxy-6-(((pyridin-2-ylmethyl)imino)methyl)phenol (**L**). The compound was prepared and characterized by UV, FT-IR, NMR and single crystal XRD analysis. Density Functional Theory (DFT) with B3LYP was

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used to perform theoretical calculations on the structure [20,21]. The IR and NMR spectra were computed at this level and compared with the experimental results. This paper describes the tautomeric effect of **L** in different solvents in the UV–visible spectra. Potential energy curve for the intramolecular proton transfer in the ground state is generated in gas phase and methanol solution. Molecular interactions were studied by 3D Hirshfeld surface analysis and the associated 2D fingerprint plots. Electronic absorption and emission spectral studies were used to study the binding of L with CT-DNA. The molecular docking was done to identify the interaction of **L** with B-DNA. *In vitro* anticancer activity against human pancreatic cancer (MIA-Pa-Ca-2) and human breast cancer (MCF-7) cell lines was also evaluated.

2. Experimental

2.1. Materials and instrumentation

Picolylamine and *o*-vanillin were purchased from Sigma, USA and used without further purification. All the solvents used were of UV spectral grade. Doubly distilled deionized water was used throughout the experiments. CT-DNA was purchased from Genei, Bangalore and used without purification. Trizma base was purchased from Sigma Aldrich. Tris–HCl and ethidium bromide were obtained from HiMedia. Elemental analysis was carried out using a Thermo Finnigan Flash EA 1112 series CHN analyzer. FT-IR spectra were recorded on a Shimadzu 8400S spectrophotometer with KBr pellets in the range of 400–4000 cm^{-1}. Electronic absorption spectra were recorded at room temperature using a Shimadzu UV-2450 spectrophotometer. The fluorescence spectra were recorded on a Jasco FP-8300 spectrofluorophotometer. ^{1}H NMR and ^{13}C NMR measurements were performed in $CDCl_3$ on a Bruker Avance III 400 MHz spectrometer. Single crystal X-ray diffraction was recorded in Bruker Kappa Apex II diffractometer.

2.2. Synthesis of (E)-2-methoxy-6-(((pyridin-2-ylmethyl)imino) methyl)phenol (*L*)

The Schiff base (**L**) was reported in literature [22,23]. But, in the present investigation we were able to isolate the single crystals. The L was prepared by stirring a mixture of a solution containing *o*-vanillin (0.5 g, 3 mmol) in 20 mL methanol and picolylamine (0.28 g, 3 mmol) in 20 mL methanol. The reaction mixture was stirred for 2–3 h (Scheme S1). Yellow colored crystal obtained by the slow evaporation of reaction mixture is suitable for X-ray analysis (yield 84%). M.p. 98 °C, Found: C, 69.41; H, 5.82; N, 11.56; O, 13.21; calc. for $C_{14}H_{14}N_2O_2$; C, 69.28; H, 5.78; N, 11.52. O, 13.42. FTIR (KBr): cm^{-1} 3421 (O–H), 1639 $(C{=}N)_{azomethine}$, 1589 $(C{=}N)_{py}$, 1074 (C–O). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 3.90 (s, 3H, $-O-CH_3$), 4.95 (s, 2H, $-CH_2$), 8.56 (s, 1H, =N–CH–), 8.52 (d, 1H, $(CH{=}N)_{py}$), 7.20–6.81 (m, 6H, Arom-H). ^{13}C NMR (400 MHz, $CDCl_3$) 166.87 (C=N), 56.15 ($-O-CH_3$), 101–108(aromatic carbons), 122–158(pyridine ring carbons).

2.3. Crystallography

Single crystal X-ray diffraction experiment was performed on a Bruker Kappa Apex II diffractometer using M_oK_a radiation; k = 0.71073 Å at 296(2) K. A yellow prism of L with dimensions of 0.25 mm × 0.20 mm × 0.20 mm was used. The structure was solved by direct method procedure using SHELXS-97 program [24]. The refinement was carried out using Full Matrix Least Square method on F2, which is in correspondence with 288 parameters. All the non-hydrogen atoms were refined anisotropically. The hydrogen atoms bonded to carbon were inserted at calculated positions using a riding model. Hydrogen atoms bonded to oxygen were located from difference map and allowed to refine with temperature factors riding on those of the carrier atoms. The geometrical parameters were obtained using PARST [25] and SHELXL-97.

2.4. Computational procedures

All the computational calculations were performed with Gaussian 03W program using Density Functional Theory (DFT) with Becke's three-parameter exchange and Lee–Yang–Parr correlation functional (B3LYP) with a combination of 6-311G basis set [26]. Gauss View program has been used for the molecular visualization of computed structures [27]. The harmonic vibrational frequencies of the studied structures were calculated at the same level to characterize the potential energy surface (PES). NMR signals of the studied structure were computed at the corresponding optimized geometry using the same theory level. The minimum energy structures are ensured by the absence of any imaginary frequency. In solution phase, the geometry optimization of the studied structure is performed at the same level with polarizable continuum model (PCM) [28].

2.5. DNA binding measurements

2.5.1. Electronic absorption titration study

Electronic absorption spectral titration was used to study the binding of **L** with CT-DNA. The binding experiments were performed in Tris–HCl/NaCl buffer (50 mM Tris HCl/NaCl buffer, pH-7.2). The concentration of CT-DNA was determined from the absorption intensity at 260 nm with λ_{max} value 6600 M^{-1} cm^{-1}. Stock solution of DNA was stored at 4 °C and used within seven days. Absorption titration experiments were done using fixed concentration of **L** (40 μM) and varying the concentration of CT-DNA (10–50 μM). While measuring the spectra, an equal amount of DNA was added to both the compound and reference solutions to eliminate the absorbance of DNA itself. From the absorption data, the intrinsic binding constant K_b was determined using the equation [29].

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}$$

where ε_a, ε_f and ε_b are the molar extinction coefficients of the apparent, free and bound compounds, respectively. A plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA] gives slope and an intercept. The ratio of the slope and the intercept gives the binding constant (K_b).

2.5.2. Fluorescence titration study

The interaction of **L** with CT-DNA was studied by fluorescence spectral method using EB-bound CT-DNA in Tris–HCl/NaCl buffer solution (pH 7.2). The excitation wavelength was fixed at 420 nm and the emission range was adjusted before the measurements. Changes in the fluorescence intensities at 610 nm of EB (25 μM) bound CT-DNA (10 μM) were measured with respect to different concentrations of **L** (10–100 μM). The magnitude of the binding strength of **L** with CT-DNA can be calculated using the linear Stern–Volmer equation [30],

$$I/I_o = 1 + K_{sv}[Q]$$

where I_0 and I represents the fluorescence intensities of EB-DNA in