Synthesis, Structural characterization and Nucleic acid interaction of (E)-(2-(2-hydroxybenzylidene) hydrazinyl)(pyridin-4-yl)methaniminium

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Abstract: An environmentally benign and efficient reaction was developed for the preparation of (E)-(2-(2-hydroxybenzylidene)hydrazinyl)(pyridin-4-yl)methaniminium in the presence of water acting solvent and also catalyst with very high yield. In which, amine exchange and condensation reaction taken place between N-salicylidene aniline and isonicotinic acid hydrazide yields a novel methaniminium hydrazone at one step and very short time. The compound was structurally characterized by using single crystal X-RD and spectroscopy (FT-IR and NMR) techniques. The single X-RD results indicate that the ligand crystallizes in monoclinic system with P21/n space group. The investigation of DNA binding of the prepared compound was undertaken by electronic absorption titration method. The methaniminium hydrazone was showed hypochromism of 1.96% at 301nm; intrinsic DNA binding constant is 6.3111x106 M-1.

Keywords: Amine exchange reaction, Condensation reaction, Hypochromism, Methaniminium, Spectroscopy.

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I. Introduction

Hydrazone Schiff bases are well known organic compounds containing azomethine group, having lone pair of electrons in SP2 hybridized orbital of nitrogen atom combined with one or more donor atoms close to this group [1]. This is considerable for chemical and biological importance and imparts excellent chelating ability with metal ions to produce complexes[2, 3] and cyclization ability to produce ring containing heteroatoms such as 1,3,4-oxadiazolines[4], 2-Azetidinones[5] and 4-Thiazolidinones[6]. Methaniminium cations are prepared by photoionization of alkyl amine in mass spectrometry [7-10], heating a guanidine with sulfonyl chloride and triethylamine [11] etc. Methaniminium cations are having positively charged nitrogen in sp2 hybridization with general formula –C=NH2+. This cation is isoelectronic structure with ethylene carbon but lost one electron to become positive charge. This is may be mildly acidic in nature. Methaniminium cation containing organic molecules are used in varies fields such as dye chemistry, biological, pharmacological, forensic science etc. Malachite green (MG) is used in fungal infections of farmed fish and aquaculture to control protozoan. This is also used in food, medical and textile industries [12].

In cell biology, DNA is the primary target molecule for most anticancer and antiviral therapies. Investigations on DNA interactions with small molecules have aroused, especially for those containing heteroatoms and aromatic rings. Since, it has potential applications as new therapeutic agents and interesting properties which make them as possible probes of DNA structure and conformation [13, 14]. Interaction of peptides, small organic and inorganic molecules with DNA intervene a number of processes like translation, transcription and replication [15]. By considering above principle various disorders like cancer, cystic fibrosis etc., can be cured by using DNA as targets, which is called DNA drug interaction. Schiff’s bases have been found to be potential to bind DNA through multitude of interactions such as intercalation binding, groove binding and electrostatic binding. The drugs has been developed in this way may be less toxic and more prone to exhibit anti-proliferative activity against tumors [16, 17]. In the present study, methaniminium hydrazone Schiff base was prepared by amine exchange reaction, which has been described by Reddelen and Danilof in 1921 simultaneously taken place condensation reaction between amide carbonyl group of isonicotinic acid hydrazide and amine group of replaced amine at one step. The structure of the compound was established on the basis of
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single crystal X-ray diffraction studies and spectroscopic data's. In addition, the investigation of DNA binding ability of the compound was undertaken by electronic absorption spectroscopy method.

II. Experimental

2.1. Materials

Aniline and salicylaldehyde were purchased from Merck, Bangalore, India. Isonicotinic acid hydrazide was purchased from Tokyo Chemical Industry, Co., Ltd., India. Distilled water was used throughout experiments.

2.2. Physical Measurements

Melting points of the compounds were recorded by using capillaries in Sigma melting point apparatus, Sigma instruments, Chennai, India. Infrared spectra were recorded in the 400–4000 cm⁻¹ region (KBr disc) on a SHIMADZU, FTIR-8400S. 1H-NMR spectrum obtained in d6-DMSO using tetramethylsilane (TMS) as an internal reference on Advanced 200.12MHz NMR spectrometer. 13C-NMR spectrum was obtained in d6-DMSO using Bruker- 300 MHz NMR. Single X-ray diffraction data was recorded on a Bruker Kappa Apex2 CCD diffractometer at 293(2) K and refined at IISC, Bangalore, India.

2.3. Synthesis of (E)-(2-(2-hydroxybenzylidene)hydrazinyl)(pyridin-4-yl) methaniminium

A hot solution of isonicotinic acid hydrazide (0.696g, 5.076 mmol) in water was added slowly to a hot solution of N-Salicylidene aniline [1g, 5.076 mmol] in methanol with constant stirring at room temperature for 15 minutes. The formed precipitate was filtered, washed with cold methanol and dried. Yield: 92 %, M.P: 294-296°C. 1H-NMR (200.12MHz, DMSO-d6, ppm) δ: 2.49 (s, 2H, NH₂+), 3.62 (s, DMSO), 6.87-8.78 (m, Ar), 8.78 (s, 1H, -CH=N), 11.17 (s, 1H, -NH), 12.36 (s, 1H, -OH). 13C NMR (300MHZ, DMSO-d6 and CHCl₃) δ: 161.86 (C-OH), 157.90 (C=N), 150.75 (C=NH₂+), 149.59-132.20 (C, pyridine), 129.74-116.86 (C, phenyl), 40.57-38.91 (C, DMSO), FT-IR (KBr, cm⁻¹) 3452(OH), 3192(NH₂+), 1678(C=N), 1278(Pyridine(C=N)). The synthetic route of the compound was illustrated in scheme-1.

Scheme 1. Synthesis of the methaniminium hydrazone.

2.4. X-ray crystallography

Single crystal of compound was grown by solvent evaporation method using the mixture of 1:1 methanol and tetrahydrofuran at room temperature for four days. A crystal size 0.26 × 0.25 × 0.24 mm³ was taken for the X-ray crystallographic study. Single crystal X-ray diffraction data for the compound was collected at 298(2) K on a Bruker APEX-II CCD diffractometer using graphite monochromater Mo Kα (λ=0.71073Å). The structure was solved using OLEX2 [18] with SHELXS by direct methods [19]. The structure was refined by full-matrix least-squares minimization with SHELXL [19]. The non-hydrogen atom positions were found and refined anisotropically. Whereas, hydrogen atoms were positioned geometrically and treated as riding atoms where C–H = 0.93 Å with Uiso(H) = 1.2 Ueq(C) for aromatic carbon atoms and C–H = 0.96 Å with Uiso(H) = 1.5 Ueq(C) for methyl carbon atoms [20]. The crystallographic data collection and refinement parameters are presented in Table I.

| CCDC No | 1529789 |
| Empirical formula | C₁₃H₁₃N₄O |
| Formula weight | 241.27 |
| Temperature/K | 298(2) |
| Crystal system | monoclinic |
| Space group | P2₁/n |
| a/Å | 8.1906(12) |
| b/Å | 15.625(2) |
| c/Å | 9.5795(13) |
| α/° | 90 |

Table 1. Crystal data and structure refinement of compound.

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### 2.5. DNA Binding

The DNA binding ability of compound was examined by UV–vis spectroscopic studies in 50 mM Tris-HCl/NaCl buffered solution at pH 7.0. The concentration of CT-DNA was calculated from the Molar absorbance coefficient (6600 dm$^3$ mol$^{-1}$ cm$^{-1}$) at 260 nm [21]. The solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm (A$_{260}$/A$_{280}$) of 1.8–1.9 indicating the protein free nature of DNA. Electronic absorption titration experiment was performed by maintaining the concentration of methaniminium hydrazone as constant (7.0×10$^{-5}$ M) but with variable nucleotide concentration from 0 to 7.9×10$^{-7}$ M. Stock solutions were stored at 4°C and were used after no more than 4 days. Distilled water was used to prepare buffer solutions. Solutions were prepared by mixing the methaniminium hydrazone and CT-DNA in DMSO medium. After equilibrium was reached (ca.5 min) the spectra was recorded against an analogous blank solution containing the same concentration of DNA.

The intrinsic binding constant ($K_b$) was calculated from the following equation (3).

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

Where, [DNA] is the concentration of DNA, $\varepsilon_a$, $\varepsilon_b$ and $\varepsilon_f$ correspond to apparent, bound and free compounds extinction coefficients respectively. A plot of $[\text{DNA}]/(\varepsilon_a-\varepsilon_f)$ Vs [DNA] gave slope of $1/ (\varepsilon_b-\varepsilon_f)$ and a Y-intercept equal to $1/K_b(\varepsilon_b-\varepsilon_f)$, $K_b$ is the ratio of the slope to the Y-intercept.

### III. Results and Discussion

#### 3.1. Single crystal X-ray diffraction studies

The numbering scheme, hydrogen bonding interactions and unit cell packing diagram of the compound is given in figure 1, 2 and 3. The selected bond lengths, bond angles and hydrogen bonding data’s are presented in table 2, 3 and 4. The phenyl ring showed C-C distances ranging from 1.381(3) to 1.412(3) Å reveals that delocalized bonding structure. The C13-N1 and C2-N1 bonds distances of 1.346(2) and 1.343(3) Å agree with pyridyl ring [22]. The bond length of hydrazinyl (N7-N6) nitrogen atoms 1.377(2) Å is in agreement with literature reports [23, 24]. Bond length of azomethine group (C8-N7) is 1.285(2) Å as expected for formal double bond [25]. Bond length of C5-N0AA is 1.213(2) Å, may be characteristic of methaniminium group. The bond lengths of N6-C5 and C5-C4 are 1.366(2) and 1.508(3) Å respectively, which are supporting methaniminium group in the molecule. The molecular conformation is stabilized by a strong intra-molecular O2-H2….N7 (1.800 Å) hydrogen bond. Molecule is further stabilized by medium strength inter molecular N6-H6….N1hydrogen bond (1.800 Å). Strength of the hydrogen bond is confirmed by bond length.

#### 3.2. DNA binding studies

The electronic absorption spectrum of methaniminium hydrazone in the absence and presence of increasing amounts of CT-DNA (25µL) was recorded (Fig. 4). The methaniminium hydrazone showed
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hypochromism of 1.96% at 301nm; intrinsic DNA binding constant is $6.3111\times10^6\text{M}^{-1}$. This result suggested that the compound was bound with CT-DNA strand through intercalation. When compound bind into the base pairs of DNA, the $\pi^*$ orbital of the intercalator may couple with the $\pi$ orbital of the base pairs of DNA. Thus, decreasing the $\pi - \pi^*$ transition and hence a hypochromism is observed in the compound [26].

IV. Figures and Tables

Fig. 1. ORTEP diagram of the compound with numbering scheme.

Fig. 2. ORTEP diagram of compound with hydrogen bonds.

Fig. 3. Unit cell packing diagram of the methaniminium hydrazone.
In this work, we have synthesized stable methaniminium hydrazone Schiff base by novel reaction. This reaction has significant advantages such as high conversion, very short reaction time, mild reaction conditions, inexpensive, simple work up and non-toxic. Generally this kind of molecules studied in organic reactions as intermediates. However, this compound may be shown versatile properties used for various fields. Several new drugs with reduced toxicity and high specificity have been developed by understanding the mechanism of DNA binding. Development of medicinal chemistry requires an understanding of the physiological processing of compounds with DNA, to provide a rational basis for the design of new drugs.
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References