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ESIPT reaction of potential bioactive heterocyclic Schiff base: Atomic visualization coupled with *in vitro* spectroscopy

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ABSTRACT

The present study epitomizes the identification of ground state geometry, determination of X-ray structure and solvent dependent excited state intramolecular proton transfer (ESIPT) process of a potential bioactive benzothiazole Schiff base ligand viz (*E*)-2-((6-chlorobenzo[d]thiazol-2-ylimino) methyl)-5-(diethylamino)phenol (CBMDP) using ^{1}H and ^{13}C NMR, FTIR, single crystal XRD, electronic absorption, steady state emission, time resolved and 3D-fluorescence spectroscopic techniques. The experimental observations have further been corroborated with quantum chemical calculations using Density Functional Theory (DFT) and Time Dependent DFT (TDDFT) methods. Single crystal X-ray structure confirms the existence of enol-imine (N) form of CBMDP in the ground state due to strong intramolecular hydrogen bond between phenolic hydrogen and azomethine nitrogen. The photophysical studies reveal that on photoexcitation, the molecule undergoes structural changes via ESIPT reaction. Dual emission have been identified from the excited enol-imine (N*) and tautomeric keto-amine (T*) species in non-polar heptane. The redistribution of the electron density in the photoexcited CBMDP molecule is visualised from DFT and TDDFT calculations. This electronic redistribution enhances the in basic character of azomethine nitrogen which is reflected in the Mulliken partial atomic charges increases from −0.21 to −0.56, which ultimately leads to an intramolecular hydroxyl proton transfer in the excited state. The simultaneous emission from two states are also theoretically supported by the low energy difference (0.66 kcal/mol) between the N* and T* form in the potential energy surface diagram. The consolidated spectroscopic research, described herein, provides enormous information to open up the new avenues in designing and synthesizing potential bioactive benzothiazole Schiff bases for medicinal chemistry research.

1. Introduction

Photoinduced electron transfer and proton transfer are important fundamental processes for many biochemical reactions and find numerous applications in scientific field [1–13]. Amongst all the photoinduced processes, excited state proton transfer are of special interest. When a photoinduced proton transfer occurs from one atom to another atom within a single molecular framework, it is known as excited state intramolecular proton transfer (ESIPT) reaction. If a molecule possesses electron donor and acceptor group in the same skeleton, there is a strong possibility of excited state intramolecular charge transfer (ESICT) process to occur upon photoexcitation [13,14]. As a consequence, ESIPT processes are normally accompanied by intramolecular charge transfer process or vice versa [8]. From the viewpoint of physicochemical properties of a bioinorganic ligand, it seems very interesting to see when ESIPT and ESICT are coupled in a single molecular system [15]. Both these processes can be *"fine tuned"* by introducing proper substituent in the main skeleton [16]. The ESIPT process is very much influenced by its surrounding microenvironment that may include nature of solvent, polarity, pH of the medium, temperature etc. This alteration in the microenvironment around the probe may be reflected in relative intensities and positions of the bands that appear in spectral analysis. Thus detailed study of ESIPT reaction of biologically active molecule can provide important information about the physicochemical nature of its surrounding medium and it can be used as an environment sensitive probe for the studies of

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its interaction with proteins, polymers, lipid membrane, reverse micelle and so on.

The intramolecular electron transfer and proton transfer reactions of Schiff bases containing nitrogen, sulphur and/or oxygen have been extensively investigated over few decades owing to their rich photochemical and photophysical properties which enables them to find wide range of important applications [17–20]. Heterocyclic Schiff base like benzothiazole, benzoxazoles and benzimidazole which are also medicinally important compounds as they comprise bioactive scaffold of many drugs [8]. Hence, considering the immense applicability of Schiff bases and the benzothiazole heterocycles, we have strategically adopted and synthesized an interesting ortho hydroxy benzothiazole based Schiff base having electron donor and acceptor moieties in same skeleton. The compound ((*E*)-2-((6-chlorobenzo[d]thiazol-2-ylimino)methyl)-5-(diethylamino)phenol (CBMDP)) has shown profound multifunctionality and amyloid specificity to address Alzheimer's disease (AD) [21]. AD is mainly associated with misfolding and aggregation of Amyloid Beta (Aβ) protein monomer [22]. Hence understanding the aggregation kinetics and size of aggregates seems to be very essential for complete diagnosis of such lethal disease. Many techniques have been developed to investigate the structural conformation as well as mechanism of aggregation of the polypeptides. Nevertheless, fluorescence based techniques are most popular because of their inherent sensitivity, versatility and availability of wide variety of fluorescence probe. In this scenario, this fluorescent based investigation needs small target specific fluorescent molecule, like CBMDP, having high affinity towards the amyloid protein. Moreover the localised fluorophore that undergoes geometric transformation in the excited state can produce multiple emission bands which may be very sensitive towards its surrounding environment and therefore it can be used as an extremely sensitive and tunable reporter for amyloid assemblies. Ortho hydroxyl benzothiazole based Schiff base derivatives is expected to be very promising in this regard owing to their high affinity towards amyloid protein and presence of intramolecular hydrogen bond which can facilitate the ESIPT.

In this background, we have thoroughly investigated ground and excited state geometry and the influence of solvents on ESIPT reaction of this potential amyloid targeted heterocyclic Schiff base (CBMDP) along with its single crystal XRD analysis and quantum chemical calculations. This study is expected to provide useful information for designing and synthesizing more efficient therapeutic as well as imaging agent in the field of medicinal chemistry research.

2. Results and discussion

The synthesis of small, neutral, lipophilic, potential amyloid targeted heterocyclic Schiff base CBMDP has been executed by condensing benzothiazole and salicyldehyde derivatives (Scheme 1). The adopted design [23], amyloid specificity, metal ion chelation and other related biological activities of this synthesized CBMDP molecule have been communicated recently by our group elsewhere [21]. However the main focus of the present article is to explore the physicochemical behavior of the probe CBMDP, especially in terms of identification of the ground state geometry and the excited state intramolecular proton transfer reaction using ^{1}H, ^{13}C NMR, FTIR, X-ray crystallography, UV–vis absorbance, steady state fluorescence, and quantum chemical calculations to understand its photophysical behavior.

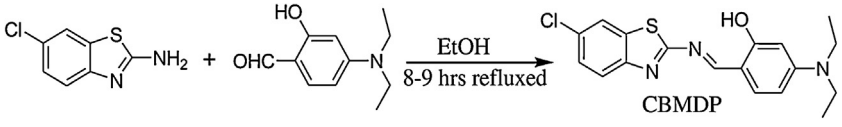
2.1. Identification of ground state geometry and the existence of intramolecular H-bonding

2.1.1. Spectral characterization

The ^{1}H NMR spectrum of CBMDP shows multiplet in the region 6.2–7.7 ppm due the presence of aromatic protons (Fig. S1). One triplet at 1.23 ppm and one quartet at 3.4 ppm are assigned to protons of $—CH_3$ and $—CH_2$ groups respectively present on diethylamine substituent. Singlet peak at 8.9 ppm appear due to presence of azomethine proton. In $CDCl_3$, the appearance of azomethine proton peak and the absence of any N—H peak indicate the sole existence of enol-imine (N) form of the synthesized compound. This is further supported by singlet peak of phenolic proton at 12.63 ppm. The higher chemical shift of phenolic proton may be due to its intramolecular H-bonding with azomethine nitrogen. ^{13}C NMR spectrum exhibits aromatic carbon peaks in the expected range of 100–165 ppm (Fig. S2). The azomethine carbon peak is found at ∼97 ppm while $-CH_3$ and $-CH_2$ group carbon peaks of diethyamine are observed at ∼13 ppm and ∼45 ppm respectively. The absence of any carbonyl carbon peak in ^{13}C NMR spectrum is in good agreement with the existence of the enolic form of the compound in the ground state. The presence of azomethine group is further confirmed by the FTIR peak that observed around ∼1558 cm^{-1} (Fig. S3) whereas the absence of any carbonyl peak (1700 cm^{-1}) logically supports the NMR observations. Moreover the weakening of the −OH peak in FTIR spectrum around the region of 3300–2700 cm^{-1} is consistent with the existence of strong intramolecular H-bonding in the enol-imine form of the compound [24]. Thus ^{1}H, ^{13}C NMR and FTIR studies not only confirm the existence of the enol-imine form of CBMDP in the ground state but also attest a strong intramolecular H-bonding between phenolic hydrogen and azomethine nitrogen in the enolic form of the synthesized molecule.

2.1.2. X-ray crystallography

Single crystal of the compound has been obtained by solvent diffusion method using chloroform and hexane system after repeated trials. The atomic visualisation in the single crystal, authenticates the structure of the synthesized molecule in the ground state. From the Ortep view of CBMDP (Fig. 1), it is clear that the molecule exists as *E* isomer with respect to C8 = N2 double bond. The distance between phenolic hydrogen and azomethine nitrogen has been observed as 1.851 Å, which indicates the presence of a strong hydrogen bond (H-bond) having bond energy lies in the range 15–40 kcal/mol [25]. The six membered ring that is formed by the above mentioned strong intramolecular H-bonding between phenolic hydrogen and azomethine nitrogen (O1-H1A-N2), stabilizes the *E* isomer of the synthesized molecule [26]. Moreover in CBMDP, the relative longer C8=N2 bond length (1.304 Å) as compared to normal C=N bond length (1.279 Å) may be attributed to the extended π-conjugation in the main skeleton. The appreciable H-bonding present in the enol-imine form gives the



Scheme 1. Synthesis of CBMDP.