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Improved solubility and bioactivity of theophylline (a bronchodilator drug) through its new nitrate salt analysed by experimental and theoretical approaches

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ABSTRACT

Synthesis, crystal structure, vibrational spectroscopy, quantum chemical studies and biological activity of the new semi organic compound, Theophyllinium Nitrate $[C_7H_9N_4\ O_2)^+.\ (NO_3)^-]$, are reported here. Crystals of Theophyllinium nitrate (TN) were grown by slow solvent evaporation technique. The crystal packing is dominated by N–H···O intermolecular hydrogen bonds. The cations and anions are aggregated almost parallel leading to a lamellar structure. This molecular aggregation features two alternate hydrogen bonded chain $C_2^2(8)$ and $C_2^1(6)$ motifs. Further, a bifurcated ring $R_1^2(4)$ motifs is also seen. This aggregated molecular sheets are parallel to $(\bar{2}06)$ and $(20\bar{6})$ planes of the crystal. The solubility test is carried out to enhance the physico–chemical activity of the compound. The atomic charge distribution on different atoms of TN has been calculated by Mulliken charge analysis. A detailed interpretation of FT-IR and FT-Raman spectra of TN show that most of the bands are matching between the experimental and theoretical methods. The strong intensity bands and shifting of bands due to intermolecular hydrogen bonds are also investigated. The NBO analysis is carried out to elucidate the stability of the molecule and charge delocalization within the molecule. The HOMO–LUMO analysis reveals molecular stability and chemical reactivity of the present compound. Also, the compound was examined for its antibacterial activity and found to exhibit notable activity against *Pseudomonas aeruginosa*. This shows that the present compound is a good candidate for the antimicrobial agent apart from its inherent Bronchodilator drug property. Hence, the new compound (TN) may be a good alternative for patients with Chronic Obstructive Pulmonary Disease (COPD) and bacterial infections.

1. Introduction

The crystallization of solid state acid–base donor–acceptor systems with more than one component is a commonly used route to tailor the physical and chemical properties of solid organic products and their formulation. Active pharmaceutical ingredients (APIs) are frequently delivered in the solid-state as an approved dosage form to the patient for treatment. Solid state of API or a drug product provides a suitable, convenient, compact and more stable format to store for long period. Studying and controlling the physico–chemical properties of APIs in solid state, both as pure drug and in formulated products, is therefore an important aspect of the drug development process. In crystal engineering studies, Theophylline (TP) is a popular model compound used to explore polymorphism and especially hydrate formation [1–4]. Theophylline has biological importance as it is structurally related to nucleic acids components. It is used as a drug in therapy for respiratory diseases such as Chronic Obstructive Pulmonary Disease (COPD) or asthma under a variety of brand names [5]. It is the standard drug substance for the treatment of bronchial asthma. It was first extracted from tea leaves and it has been in pharmaceutical use since 1902 [6]. Theophylline is known to exist as a crystalline monohydrate and three anhydrous polymorphs (Form I, II III and IV) [7]. From the review of literature, it is observed that more co-crystal works were done on Theophylline to improve its physico–chemical properties [8].

Nitrates are identified to be an effective co-former for API to improve the physiochemical properties of the compound and hence improve its pharmaceutical activity [9,10]. This created an interest to study the nitrate salt of Theophylline drug i.e.,

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Theophyllinium nitrate. Hydrogen bonding is the key to understand how molecules align themselves in solid state. The hydrogen bond interaction between nitric acid and a number of atmospheric bases has been investigated by IR spectroscopy even in low-temperature matrices [11–15]. Further, physicochemical properties of a drug, such as solubility, bioavailability and stability, may be improved by incorporation of an additional component forming salt (ionic) or co-crystal (non-ionic, non-covalent intermolecular interaction). Single crystal X-ray diffraction (XRD) is most commonly used to determine the intermolecular interaction in unambiguous manner often in conjunction with an analysis of structural indicators such as bond angles and bond lengths [16–19]. A primary goal of any drug design strategy is to predict the activity of new compounds to improve efficacy, potency and to minimize or eliminate untoward side effects by analysing it in molecular level. Also, a quantum chemical method has shown significant promise and appears to be adequate for pointing out the changes in electronic structure responsible for pharmacological action in the same scale of level.

In the present work, the structural and electronic properties of new Theophyllinium nitrate (TN) compound have been studied using Density Functional Theory (DFT) and Hartree–Fock (HF) methods. Investigations on the interaction between these two molecules may provide useful information for researchers to improve the efficacy of the drug. The optimized structure, vibrational frequencies, frontier molecular orbital energies and NBO analyses are also done using quantum chemical methods. The vibrational spectra of the compound obtained experimentally have been compared theoretically using DFT/B3LYP method to identify the higher wave number accuracy. TN shows more effective antibacterial activity against *Pseudomonas aeruginosa* than *Escherichia Coli* and *Klebsiella Pneumonia*.

2. Experimental

Anhydrous Theophylline was dissolved in distilled water with few drops of Nitric acid. The resulting solution was stirred for 2 h at room temperature. The colourless solution obtained after filtration was left to evaporate slowly at room temperature. Needle types of single crystals suitable for X-ray diffraction were obtained after three days. The density of the crystals was measured by sink and swim method (flotation technique). Thus, it was observed to be 1.45 (1) $Mg.m^{-3}$. X-ray intensity data of the grown crystals were collected at room temperature with MoK_{α} radiation ($\lambda = 0.71073$ Å) using Bruker AXS KAPPA APEX-2 diffractometer equipped with graphite monochromator. The structure was solved by direct methods and refined by full-matrix least-squares calculations using SHELXL–2014 [20]. Infrared spectrum of the sample was recorded in the region 4000-400 cm^{-1} using IR Tracer-100 Shimadzu Spectrophotometer at a resolution of 0.25 cm^{-1}. The FT-Raman spectrum was recorded in the frequency range of 50–4000 cm^{-1} using a BRUKER RFS 27 FT-Raman Spectrometer module with the resolution of 2 cm^{-1} and the Nd:YAG Laser source was operated at 1064 nm.

2.1. Solubility studies

Solubility analysis was done using water as the solvent and the crystallized TN salt is dissolved in water at room temperature and stirred continuously to make the solution to be saturation. By varying the concentration the process was continued. The amount of salt in the solution was analyzed gravimetrically, after reaching the saturation. The same procedure was repeated in TP with different concentration.

2.2. Antibacterial test

The Muller Hinton medium was seeded with 24hr culture of bacterial strains such as *P. aeruginosa, E. coli and Staphylococcus aureus* in petriplates. Wells of approximately 10 mm was bored using a well cutter and 25 μl, 50 μl and 100 μl of sample was added to the well. The plates were then incubated at 37 °C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

2.3. CIM analysis

Petriplate containing 20 ml Muller Hinton medium were seeded with 3 h grown culture of bacterial strain; *P. aeruginosa*. Wells of approximately 10 mm was bored using a well cutter and samples in 25, 50, 100, 150, 200 and 250 μl concentrations were added. The plates were then incubated at 37 °C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993).

2.4. Computational details

In order to provide information with regard to the structural characteristics and the normal vibrational modes of TN, Hartree–Fock and Density Functional Theory (DFT) calculations have been carried out. The entire calculations were performed using GAUSSIAN 09W software package [21]. Initially, the HF level calculations, adopting the 6–31++G(d,p) basis set were carried out and then the DFT employing the Becke-3–Lee–Yang–Parr (B3LYP) supplemented with the standard 6–31++G(d,p) basis set. The harmonic vibrational wavenumbers have been analytically calculated by taking the second order derivative of energy using the same level of theory [22,23]. A comparison was performed between the theoretically calculated frequencies and the experimentally measured frequencies. Frontier molecular orbital (FMO) analysis based on DFT calculation was another approach to investigate the charge transfer at a molecular level. The charge transfer reactions consisted of a charge movement between the relevant orbitals, in which the interactions between the occupied and unoccupied orbitals played a vital role [24]. The HOMO–LUMO energy gap is calculated and compared with pure TP. The NBO analyses [25] were performed in order to understand various second order interactions between the filled orbitals of one subsystem and vacant orbitals of another subsystem, which quantify the intermolecular delocalization or hyper conjugation.

3. Results and discussion

3.1. Molecular geometry

The molecular structures of the TN elucidated through single crystal XRD and optimized through HF and DFT level calculations are shown in Fig. 1. The present compound crystallized in the monoclinic $P2_1/n$ space group (Table 1). The calculated geometric parameters (bond lengths, bond angles and torsion angles) are listed in Table 2. The protonation on the one of the N atoms (N15 atom) of the cation is confirmed from the elongated C–N bond distances and increased C–N–C angle. The calculated bond lengths slightly differ from experimental values by 0.01–0.05 Å. The difference is due to the fact that in theoretical calculation isolated molecule is considered in gas phase while in experimental measurement the molecules are in condensed phase. The calculated C–N bond length in pyrimidine ring agrees well with the experimental value whereas there is slight deviation in imidazole ring.