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Hydrogen bonding motifs, spectral characterization, theoretical computations and anticancer studies on chloride salt of 6-mercaptopurine: An assembly of corrugated lamina shows enhanced solubility

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ABSTRACT

6-Mercaptopurine (an anti cancer drug), is coming under the class II Biopharmaceutics Classification System (BCS). In order to enhance the solubility with retained physiochemical/pharmaceutical properties, the present work was attempted with its salt form. The single crystals of 6-mercaptopurinium chloride (6MPCl) were successfully grown by slow evaporation technique under ambient temperature. The X-ray diffraction study shows that the crystal packing is dominated by N–H···Cl classical hydrogen bonds leading to corrugated laminar network. The hydrogen bonds present in the lamina can be dismantled as three chain $C_2^1(6)$, $C_2^1(7)$ and $C_2^1(8)$ motifs running along *ab*-diagonal of the unit cell. These primary chain motifs are interlinked to each other forming ring $R_6^3(21)$ motifs. These chain and ring motifs are aggregated like a dendrimer structure leading to the above said corrugated lamina. This low dimensional molecular architecture differs from the ladder like arrays in pure drug though it possess lattice water molecule in lieu of the chloride anion in the present compound. Geometrical optimizations of 6MPCl were done by Density Functional Theory (DFT) using B3LYP function with two different basis sets. The optimized molecular geometries and computed vibrational spectra are compared with their experimental counterparts. The Natural Bond Orbital (NBO) analysis was carried out to interpret hyperconjugative interaction and Intramolecular Charge Transfer (ICT). The chemical hardness, electronegativity, chemical potential and electrophilicity index of 6MPCl were found along with the HOMO–LUMO plot. The lower band gap value obtained from the Frontier Molecular Orbital (FMO) analysis reiterates the pharmaceutical activity of the compound. The anticancer studies show that 6MPCl retains its activity against human cervical cancer cell line (HeLa). Hence, this anticancer efficacy and improved solubility demands 6MPCl towards the further pharmaceutical applications.

1. Introduction

Enhancement of the physicochemical properties of the known active pharmaceutical ingredients (APIs) has received major attention of pharmaceutical scientist around the world for the past few decades [1–3]. In which, the formation of its salts or co-crystals has attracted the field of crystal engineering because of the change in properties of a drug using a generally regarded as safe [4] coformer, especially to get advantageous solubility, bioavailability, stability and good tablet processing qualities [5–8]. Almost 40% of the APIs in the market today face a major problem of poor solubility [9]. Generally, this problem is addressed by making powdered form of drug through solid dispersion or by mixing polymer, additive, inactive substance or inclusion compounds [10,11]. In some cases, it is achieved by structural modification, such as i) replacement of ionizable/or non-ionizable groups; ii) increase of lipophilicity; iii) replacement of polar groups; iv) reduction of hydrogen bonding and polarity by polymorphs; v) reduction of size as nanotechnology approach; vi) addition of a nonpolar side chain [12–14]. However, salt formation is still one of the best approaches to improve the solubility of the API without disturbing the intrinsic properties. By this fact, more than 50% of the marketed drugs are available in salt

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Table 1
Crystallographic data and structure refinement Parameters for the 6MPCl.

Empirical formula	$C_5\ H_5\ N_4\ Cl\ S$
Formula weight	188.64
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, $P2_1/n$
Unit cell dimensions	a = 7.896 (6) Å b = 11.794 (9) Å β = 115.274 (1)° c = 9.034 (7) Å
Volume	760.85 (1) $Å^3$
Z, Calculated density	4, 1.647 Mg/m^3
Absorption coefficient	0.709 mm^{-1}
F(000)	384
Crystal size	0.18 × 0.21 × 0.24 mm
Theta range for data collection	2.88 to 25.00°
Limiting indices	$-9 \le h \le 9, -14 \le k \le 14, -10 \le k \le 10$
Reflections collected/unique	7805/1340 [R(int) = 0.0204]
Completeness to theta = 25.00	100%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1340/0/116
Goodness-of-fit on F^2	1.043
Final R indices [I > 2sigma(I)]	R1 = 0.0248, wR2 = 0.0688
R indices (all data)	R1 = 0.0256, wR2 = 0.0698
Largest diff. peak and hole	0.223 and −0.297 e.$Å^{-3}$
CCDC No	1032004

form of its API. However, salt formation can sometimes lead to lower stabilities and, hence, low solubility [15–17]. These physiochemical properties of any crystalline solids are moulded due to the molecular arrangement and can be improved by altering the intermolecular interactions between the constituent molecules. In this ground, structure–property relationship studies are crucial to understand the nature of the salts or co-crystals of the API [18].

6-Mercaptopurine (6 MP), an API for antineoplastics agent, is also used as an immunosuppressive [19] and anti-inflammatory [20] agent. Specifically, the drug is known for its efficacy against the tumors and leukemia and permeability in biological system [21–23]. Further, the inflammatory bowel problem such as Crohn's disease is also treated with this drug [24]. The chemotherapeutic activity of 6-mercaptopurine in cancer cells is due to its ability of transforming the donor sites into the respective ribosides [25]. 6 MP belongs to the class II drug of Biopharmaceutics Classification System (BCS) with the poor solubility and low bioavailability [26]. The structural and solubility studies of co-crystals of 6 MP with4-hydroxybenzoic acid and 2,4-dihydroxybenzoic acid were already reported [27]. The crystal and molecular structure of anhydrous 6-mercaptopurine [28], 6-mercaptopurine monohydrate [29] and 6-mercaptopurine hydrochloride [30] were also reported. The present work is carried out as a re-determination of the crystal and molecular structure of chloride salt of 6 MP in an elaborate narration about hydrogen bonding network and the intermolecular assembly with improved R-factor. Further, computed structural properties, electronic properties and characteristic harmonic vibrational frequencies were also attempted through quantum chemical approach. Hence, a complete discussion about the molecular assembly, an intact vibrational assignment and molecular orbital analyses of chloride salt of 6 MP are attempted here. The vibrational analyses are crucial for understanding the strength of the intermolecular forces and delocalization of electron density inside the molecule besides the predicted molecular geometry. Anticancer activity of the present compound is also evaluated in human cervical cancer cell line (HeLa) and it was compared with its API (6 MP). The chemical hardness, chemical Potential and electronegativity were calculated by Frontier Molecular Orbitals (FMO) analyses. As the stabilization energy is playing an important role in the biopharmaceutical field, it is calculated using the Natural Bond Orbitals (NBO) analyses.

2. Experimental

2.1. Materials and methods

The single crystals of 6-mercaptopurinium chloride (6MPCl) have been grown by the slow evaporation method under ambient temperature from the equimolar solution of 6-Mercaptopurine monohydrate and hydrochloric acid in Dimethylsulfoxide (DMSO) solvent. The good quality crystals were obtained within two weeks. The density of the crystals were measured by sink and swim method (flotation technique) using a liquid mixture of carbon tetra chloride and Bromoform. The observed density of the crystal was found to be 1.65 (1) Mg m^{-3}.

2.2. Single crystal XRD studies

The preliminary crystallographic calculations, i.e., the unit cell parameters of 6MPCl and full data collection were made from single-crystal X-ray diffraction by Bruker SMART APEX CCD area detector diffractometer (graphite-monochromated, MoK_α = 0.71073 Å) [31]. Crystallographic data, details of the data collection and refinement statistics are given in Table 1. The structure was solved by direct methods using SHELXL 2014 [32]. All the H atoms were positioned geometrically and refined using riding model approximation, with C–H = 0.86–0.93 Å and $U_{iso}(H)$ = 1.2 U_{eq} (parent atom). The R-factor of the structure (2.48%) confirms the convergence of the structure, which is better than the earlier report [30].

2.3. Solubility study

Solubility analysis of 6MPCl was carried out against the DMSO solvent, because it is acting as the medium in pharmaceutical field, such as anti-inflammatory and antioxidant studies. The crystallized

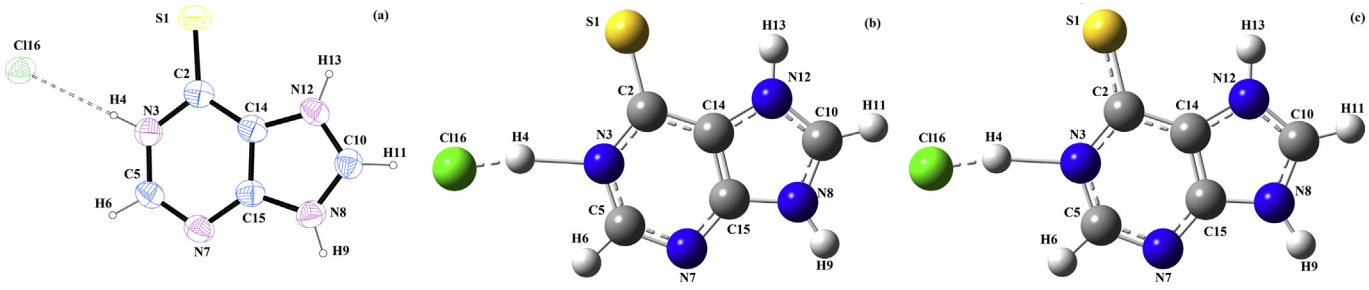

Fig. 1. (a) The molecular structure of the 6-MPCl with atom numbering scheme and 50% probability displacement ellipsoids and (b) Optimized Structure with atomic numbering for DFT/6-311++G(d,p) (c) Optimized Structure with atomic numbering for DFT/6-311++G(2d,p). The double dotted line indicates hydrogen bond interaction.