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Synthesis, characterization and molecular modelling of a novel dipyridamole supramolecule – X-ray structure, quantum mechanics and molecular dynamics study to comprehend the hydrogen bond structure–activity relationship

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ABSTRACT

Hydrochloride salt formation for Active Pharmaceutical Ingredients (APIs) is the primary choice to impart aqueous solubility and to promote dissolution. Dipyridamole (DIP) is a cardiovascular drug which is practically insoluble in water. We discovered a new form of DIP called as dipyridamole hydrochloride trihydrate (DIPHT), which was prepared by an unusual method of reacting the DIP with hydrated hydrochloric acid (HCl) that was liberated *in situ* by the reaction of ferric chloride with water. The liberated HCl was consumed as reagent *in situ* by the scavenger (API) and was converted to a hydrochloride trihydrate. The product was characterized by FTIR, mass spectroscopy, PXRD and DSC. Supramolecular structure of this novel DIPHT was revealed by single crystal XRD. A sustained intramolecular hydrogen bond alliance was found in DIP and the DIPHT. Stability of this hydrogen bond was further evaluated by means of molecular modelling studies. We performed electron calculations using quantum mechanics (QM) on both the base and salt structures to compare their geometry and molecular orbital energy levels. Molecular Dynamics (MD) simulations were also conducted in explicit solvent models to provide more insights into the hydrogen bond strength and conformational preferences of the base and salt structure. Together with QM and MD, we were able to explain the influence of hydrogen bonds on proton uptake activity of DIP and stability of DIP and DIPHT. DIPHT which can dissolve faster than DIP in water may enhance the dissolution and bioavailability of the drug. As the current drug development research is shifting to repurpose the existing drugs in order to subside the untoward risks in new drug development, we believe that DIPHT with its intrinsic aqueous solubility could bring more application for DIP and generate interest within the pharmaceutical industry.

1. Introduction

The Active Pharmaceutical Ingredient (API), Dipyridamole (DIP) is an anti platelet drug used as a coronary vasodilator, in the treatment of thromboembolic disorders. Chemically, DIP is 2-({6-[bis(2-hydroxy ethyl)amino]-4,8-bis(piperidin-1-yl)- [1,3] diazino [5,4-d]pyrimidin-2-yl}(2-hydroxyethyl) amino)ethan-1-ol. DIP is administered alone or with aspirin in the management of myocardial infarctions and strokes [1–3]. The API is available as a anhydrous yellow crystalline powder. DIP has pKa value of 6.4, and is soluble in dilute acid with a pH of 3.3 or lower. DIP is insoluble in water but highly soluble in methanol, ethanol and chloroform [4]. The API melts in the range of 164–167 °C [4]. The water insoluble property of DIP prescribes use of solubilizers as excepients to develop a formulation for the API. Several technologies including microemulsification, solid dispersion, pellets, fluidized bed coating pellets, microspheres and liposomes have been adopted to improve the biopharmaceutical properties for the API [2,3,5–10]. Other than the above mentioned formulation strategies, the obvious choice to enhance solubility for weak base drugs like DIP is salt formation. Hydrochlorides are the most successful salt forms developed so far for the APIs [11]. These are usually prepared by the reaction of aqueous hydrochloric acid with the API solution which is prepared

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by dissolving a water miscible polar solvent such as methanol, ethanol, propanol, butanol, acetone, 2-butanone and acetonitrile etc. [11]. Recently high-throughput screening methods have been applied to prepare potential salt forms for new drug candidates [12,13]. Apart from the traditional salt choice, the modern strategies like polymorphs and cocrystals can also be considered for product enhancement. Surprisingly, no structural data was reported until now on salts, polymorphs or cocrystal of DIP. The single crystal structure of DIP however was reported in 1983 [14]. Hence, we were interested to develop a water soluble form for the DIP which can enhance its biopharmaceutical properties. In this attempt we discovered a new form of DIP called as dipyridamole hydrochloride trihydrate (DIPHT). In this paper we report the synthesis and characterization of the DIPHT and also discuss the supramolecular architecture of the compound as evidenced from X-ray structure and molecular modelling studies.

The atomic structure of DIP from an X-ray diffraction study as reported by Roch et al. [14], contains an intramolecular hydrogen bond between N14 and O4 hydrogen of the ethanol side chain as illustrated in Fig. 1A. Authors reported that the geometry of the structure in a solid state deviated from the expected centrosymmetry due to the intramolecular hydrogen bond and the non-identical arrangement of the piperidine rings with respect to the molecule center. Additionally, it was reported that except from the above mentioned deviations the rest of the molecule is centrosymmetric which includes the pyrimido[5,4-d]pyrimidine system, and all hydroxyethylamino substituents which does not take part in intramolecular hydrogen bonds. The intramolecular hydrogen bonds, other than affecting the geometry of the molecule, could also alter the chemical reactivity of the molecule due to charge transfer [15–18]. Since our attempts to produce the hydrochloride salt of DIP involved proton transfer to the nitrogen atom(s), the quantity of charges on these atoms is highly imperative for explaining the product formation. As the charge on hydrogen bonded nitrogen could differ from that of non-hydrogen bonded ones, and assuming that the intramolecular hydrogen bonds of a solid state conformation will not change in solution, the hydrogen bonded nitrogen should be regarded as chemically nonequivalent with the other. In such a case, the protonation may occur on different nitrogen and the product will maintain intramolecular hydrogen bonds. Although *in vitro*, the chemical reaction occurs in the solution state and the kinetic energy of molecule produces more flexible geometries. In these conditions, the intramolecular hydrogen bonds cannot be sustained at one position, thus all the center ring nitrogen atoms can participate in intramolecular hydrogen bonds at one or more instances. Alternately, the center ring nitrogen atoms of DIP will become chemically equivalent in solution. In order to find the protophilic sites and the influence of intramolecular hydrogen bonds, we performed charge calculations using quantum mechanics (QM) and molecular dynamics (MD) simulations respectively on the DIP structure. The above mentioned molecular modelling techniques collectively can explain the mechanism behind the product formation.

The formation of the salt product DIPHT in our study was generated using an *in situ* ferric chloride reaction to protonate DIP. We believe that this process is unique, and to date has not yet been reported in the literature for such an application. While comparing the molecular structures of DIP and DIPHT, we noticed the presence of the intramolecular hydrogen bonds in a product similar to that of substrate, with the exception of the donor acceptor relationship as shown in Fig. 1B. This sustained intramolecular hydrogen bond has caught our interest and enabled us to perform in-depth study on its strength and stability with the aid of molecular modelling studies. In this context, we report the optimized geometry of DIPHT by performing QM calculations on its X-ray structure. Further, we report the stability of this intramolecular hydrogen bond in solution by employing MD in explicit solvent simulations. Analysis of the intramolecular hydrogen bonds between the stable conformations generated during the MD simulations, we can better explain the strength and stability. Further, we also report our analysis on natural bond orbitals (NBO) of DIPHT to explain the electron delocalization and intramolecular interactions. Finally we conclude the study by mentioning the importance of the intramolecular hydrogen bonds in substrate reactivity and product formation.

2. Materials and methods

2.1. Synthesis and characterization

All reagents and solvents used for the synthesis of DIPHT were of analytical grade; DIP, anhydrous ferric chloride and methanol were procured commercially from Sigma–Aldrich, and were used without further purification.

DIP (5.05 g, 10.0 mmol) was added to a solution of anhydrous ferric chloride (0.81 g, 5.0 mmol) in 50 ml methanol. The solution was stirred at room temperature for 2–3 h. The reaction mixture was then filtered to remove the insoluble brown precipitate of ferric hydroxide. The solvent in the filtrate was evaporated under reduced pressure to obtain the yellow crystalline solid. The solid was then purified by washing twice with chloroform. The product

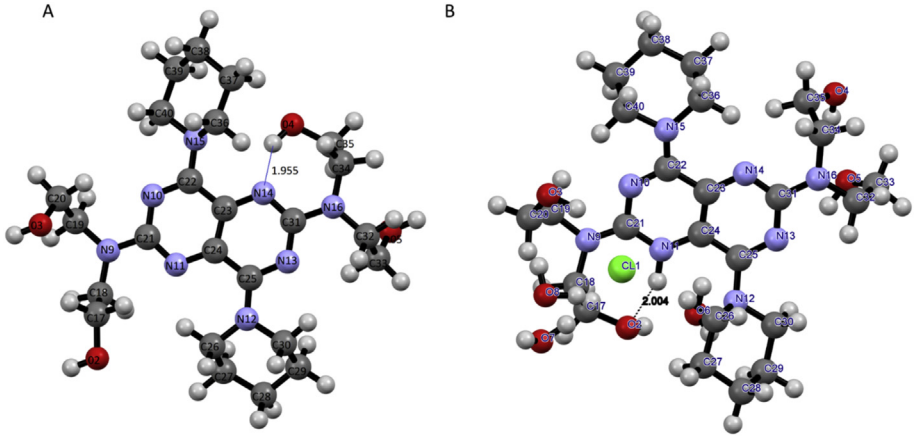

Fig. 1. Molecular structure of DIP (A) and DIPHT (B).