Chapter IV

Hydrogen bonding patterns in crystal structure of trimethoprim o-aminobenzoate

4.1. Introduction

Trimethoprim [2, 4-diamino-5-(3’, 4’, 5’-trimethoxybenzyl) pyrimidine] is a well known antibacterial drug. The drug exerts its activity via the inhibition of dihydrofolate reductase (DHFR) enzyme. As already mentioned in chapter I, tetrahydrofolate is involved in the synthesis of DNA, which is essential for all living organisms. Trimethoprim [TMP] in its protonated form binds 3000 times more strongly to the bacterial DHFR than the human DHFR enzyme. The mode of action of TMP as a drug is similar to that of PMN. It can also act as antimalarial when combined with sulfalene, but they are most commonly used against bacterial infections. An important difference between TMP and PMN is the existence of flexible methylene group between the two aromatic rings which shows greater effectiveness against P. falciparum strains which are resistant to PMN and CYC.

In all the DHFR-TMP complexes, the drug is protonated at N1. It is found that the protonated diaminopyrimidine moiety of the TMP binds deep inside the enzyme cleft through several types of hydrogen bonds. In the bacterial DHFR, the size of the enzyme cavity is somewhat smaller and the trimethoxyphenyl group of TMP is favorably accommodated in the cavity. In the vertebrate enzyme, the DHFR cavity is somewhat larger and the trimethoxyphenyl group is not in a favourable position to interact with the lower part of the cleft. According to Methews et al., this difference in the size of the binding cavities rationalizes the difference in TMP binding in bacterial and vertebral DHFR.

The bound drug (TMP) molecule is protonated and interacts ionically with Asp-27 of E. coli DHFR and likewise with the Glu-30 of the vertebrate enzymes. The 2-amino group hydrogen bonds to a buried water molecule that has been observed in both E. coli and vertebrate DHFR-TMP complexes. In the E. coli enzyme, the 4-amino group donates
hydrogen bonds to two backbone carboxyl units (Ile-5 and Ile-94), whereas in the vertebrate protein complex only one hydrogen bond is observed because of the somewhat different position of the pyrimidine ring. The distance between the amino group nitrogen and the carboxyl oxygen of Val-115 in vertebrate DHFR is 4.1-4.5 Å, too long for an ideal hydrogen bond and could be one of short contacts.

The methoxy groups play an important role in DHFR selectivity of inhibitors\textsuperscript{103}. The trimethoxy group of TMP is found in a hydrophobic region of the \textit{E. coli} DHFR active site, surrounded by the side chains from Met-20, Leu-28, Phe-31, Ile-50 and Leu-54\textsuperscript{101}. Similar non-specific interactions are observed in the vertebrate enzyme complex.

The crystal structure of TMP\textsuperscript{104} and its TMP-carboxylate complexes, such as TMP-monobenzoate\textsuperscript{105}, TMP-acetate\textsuperscript{106}, TMP-monobenzoate benzoic acid (1:1) complex\textsuperscript{107} and TMP-sulphadimidine (1:1)\textsuperscript{108} have been reported in the literature. From our laboratory, crystal structures of TMP-formate\textsuperscript{79}, TMP-nitrate\textsuperscript{109}, TMP-maleate\textsuperscript{93}, TMP-hydrogen glutarate\textsuperscript{110}, TMP-sulfate trihydrate\textsuperscript{111}, TMP-perchlorate\textsuperscript{112}, TMP-salicylate methanol solvate\textsuperscript{81}, TMP-trifluoroacetate\textsuperscript{73}, TMP-p-toluenesulphonate, TMP benzene sulfonate monohydrate, trimethoprim-sulfanilate monohydrate, trimethoprim-3-carboxy-4-hydroxybenzene sulfonate dihydrate\textsuperscript{113}, trimethoprim sorbate dihydrate, trimethoprim \textit{o}-nitrobenzoate\textsuperscript{64}, trimethoprim \textit{m}-chlorobenzoate, trimethoprim \textit{m}-chlorobenzoate dihydrate\textsuperscript{75}, trimethoprim hydrogen phthalate, trimethoprim hydrogen adipate\textsuperscript{69}, TMP \textit{m}-nitrobenzoate, TMP \textit{p}-nitrobenzaoate\textsuperscript{45}, TMP terephthalate-terephthalic acid(2/1/1)\textsuperscript{114}, TMP hydrogen malonate(1/1)\textsuperscript{115}, bis(trimethoprim) dipicolinate pentahydrate\textsuperscript{74}, TMP barbiturate monohydrate\textsuperscript{116}, TMP tetrafluoroborate\textsuperscript{117}, TMP picolinate\textsuperscript{94} and Trimethoprimium 3,5-dinitrosalicylate\textsuperscript{118} have been reported. In this chapter, the crystal structure of trimethoprim \textit{o}-aminobenzoate (TMPOAB) is discussed. The conformation and their hydrogen bonding patterns have been analyzed.
4.2. Experimental section

4.2.1. Preparation

TMPOAB was prepared by mixing hot aqueous solutions of trimethoprim (145 mg, obtained as a gift from Shilpa Antibiotic Ltd) with methanolic solution of o-aminobenzoic acid (34 mg, Loba Chemie) in 1:1 molar ratio. The resultant solution was warmed in a water bath for 30 minutes. The solution was allowed to cool at room temperature. After a few days slab-like light brown crystals were obtained.

4.2.2 X-ray data collection

X-ray data for the compound were collected using Bruker-Nonius 95mm CCD camera on \( \kappa \)-goniostat \(^{90,91} \) provided with a graphite monochromated MoK\( \alpha \) radiation at 120K. The recorded data (\( \theta \) range= 3.2\(^{\circ}\)-27.6\(^{\circ}\)) were corrected for polarization and Lorentz effects. The absorption correction was performed by multi-scan method using SADABS \(^{92} \).

4.2.3. Structure solution and refinement

In the reflections of the type hkl, there are no systematic absences. This indicates that the cell is primitive.

The data set for TMPOAB contains the following systematic absences:
(i) 0k0 type reflections with k odd absent showing 2\( _1 \) screw parallel to b-axis.
(ii) h0l type reflections, with (h+l) odd absent, revealing n-glide perpendicular to b-axis.

Hence the space group P2\(_1\)/n was assigned unambiguously. It was later confirmed by successful structure solution and refinement. The structure was solved by SHELXS97 and refined by SHELXL97 \(^{59} \) program. The non-hydrogen atoms were located from difference Fourier map and refined anisotropically. The hydrogen atoms were fixed geometrically and refined using a riding model. The final R value is 0.0444 for 3231 I>2\( \sigma \)(I). The geometric calculations were performed by PLATON \(^{60} \). The crystal data and details of structural
determination are listed in Table 4.1 for TMPOAB. The fractional atomic coordinates for all the non-hydrogen atoms with the equivalent isotropic temperature factors are given in Table 4.2.

4.3. Results and Discussion

An ORTEP view of the asymmetric unit with atom-labelling scheme is shown in Figure 4.1. In compound TMPOAB, the TMP moiety is protonated at N1, as evident from the increase in the ring angle at N1 from 115.5(5)° in neutral trimethoprim\textsuperscript{104} to 119.38(14)°. This type of enhancement of the internal angle at the protonation site has been observed in many trimethoprim-carboxylate salts\textsuperscript{109-118}. The dihedral angles between the plane of the pyrimidine and phenyl ring is 82.73(8)° which is close to the reported range\textsuperscript{75} of 69.96(8)° - 89.5(2)°. The conformation of trimethoprim cation is described by the two torsion angles $\tau_1$ (C4-C5-C7-C8) = 68.0(2)° & $\tau_2$ (C5-C7-C8-C9) = -152.70(16)°. The conformation plays an important role in DHFR selectivity\textsuperscript{119}. The C—O—C (aromatic) angles at the methoxy groups differ significantly. This difference is also observed in the crystal structure of neutral trimethoprim\textsuperscript{104} and can be attributed to the close approaches involving the atoms of the three methoxy groups. The bond distances and angles of the non-hydrogen atoms in TMPOAB are listed in Table 4.3.

4.3.1. Hydrogen bonding

The protonated N1 cation and 2-amino group of TMP interact with the carboxylate oxygen atoms (O4 and O5) to form $R_2^2(8)$ ring motif\textsuperscript{10,65} via parallel N-H…O hydrogen bonds. This ring motif is observed in a number of TMP salts reported\textsuperscript{109-118}. This motif is a well known supramolecular synthon in aminopyrimidine-carboxylate salts and is one of the 24 most frequently observed bimolecular cyclic hydrogen bonded motifs in organic crystal structures\textsuperscript{20}. The two inversely related $R_2^2(8)$ ring motifs self assemble to form a complementary DDAA (Figure 4.2) array of quadruple hydrogen bonded network with graph set notation $R_2^2(8)$ $R_4^2(8)$ $R_2^2(8)$. These types of arrays have been reported in the crystal structures of trimethoprim formate\textsuperscript{79} and trimethoprim $m$-chlorobenzoate\textsuperscript{75}. The 4- amino group of TMP cation acts as a bifurcated donor to the methoxy oxygen (O1 and O2) atoms of the neighbouring TMP cations via N-H…O hydrogen bonds to form a $R_1^2(5)$ ring motif. Also the methoxy oxygen (O3) atom
of another TMP cation acts as an acceptor to the 4-amino group via N-H...O hydrogen bonds. The interactions are shown in Figure 4.3. The o-aminobenzoate anion forms a typical intramolecular hydrogen bonds involving the amino and carboxylate groups [S6]. Also the amino groups of the anions form a supramolecular chain with the neighboring carboxylate anions via N-H...O hydrogen bonds (Figure 4.4). The geometries of the hydrogen bonding interactions are given in Table 4.4.

4.4. Supplementary Materials

The atomic coordinates and the isotropic displacement parameters for all the hydrogen atoms, the anisotropic displacement parameters for all the non-hydrogen atoms, the bond distances and bond angles involving the hydrogen atom and the torsion angles in TMPOAB are given in Table A 1.4.1(Appendix 1), Table A 2.4.1(Appendix 2), Table A. 3.4.1(Appendix 3) and Table A 4.4.1 (Appendix 4). The least squares plane calculations (Appendix 5. txt & Table A 5.4.1) and Fo-Fc Table [TMPOAB.FCF] are given in the CD attached at the end of the thesis.