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Azithromycin Salt/Cocrystal with Glycine and Acetamide for Superior Drug Performance: Experimental, Characterization and DFT Study

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Abstract

Azithromycin (AZT) is a widely used macrolide antibiotic; however, its clinical performance is often compromised by poor aqueous solubility, low dissolution rate. Moreover, antimicrobial drug resistance emergence significantly decreases their therapeutic efficacy. Although, several strategies have been explored to address these limitations, little attention has been given to

multicomponent solid systems such as salts and cocrystals. In the present study, two solid forms of AZT were successfully developed: an AZT-glycine (Gly) salt and an AZT-acetamide (ACT) cocrystals prepared in a 1:1 molar ratio using a simple solvent evaporation technique. The as-prepared materials were characterized by various techniques including FTIR spectroscopy, Raman spectroscopy, solid state NMR spectroscopy, solid state Uv-vis spectroscopy, PXRD, DSC and TGA. FTIR and solid-state NMR spectroscopies confirmed proton transfer from glycine to AZT, indicating salt formation, whereas the interaction between AZT and ACT was non-covalent, consistent with cocrystal formation. PXRD and DSC results revealed the formation of new crystalline phases distinct from the parent drug. TGA analysis showed that the AZT-Gly salt was hygroscopic, while the AZT-ACT cocrystal remained anhydrous. Solid state Uv-vis, near FTIR and Raman spectroscopies also supported the formation AZT-Gly salt and AZT-ACT cocrystal. Furthermore, Density functional theory calculations also supported strong ionic interaction and weak Vander Waal interactions between AZT-Gly salt and AZT-ACT cocrystal, respectively. The solubility, dissolution rate and antimicrobial activity studies demonstrated improved performance for both forms relative to pure AZT. Hence, these findings revealed salt/cocrystal development is an effective tactic to improve physicochemical properties and antimicrobial efficacy of AZT.

Keywords: Antibiotics, Pharmaceutical salts, Cocrystals, Dissolution rate, Solubility, Characterization of multicomponent

1. Introduction

Azithromycin (AZT), a macrolide antibiotic structurally derived from erythromycin A, is available in several solid-state forms, including monohydrate, dihydrate, semi-hydrate solvates[1-3], and other pseudo-polymorphic forms[4]. Among these, the monohydrate and dihydrate forms exhibit

the highest thermodynamic stability[4, 5], with the dihydrate form being preferred for pharmaceutical applications due to its lower hygroscopicity, which facilitates improved storage and handling[4]. AZT is classified as a Biopharmaceutical Classification System (BCS) Class II compound, characterized by low aqueous solubility but high membrane permeability[6]. It is widely prescribed for both paediatric and adult peoples to treat a variety of bacterial infections[7-9], including upper and lower respiratory tract infections (e.g., bronchitis, laryngitis, pneumonia), otitis media[10], tonsillitis, skin infections, and certain sexually transmitted diseases[11]. Despite its broad-spectrum efficacy, AZT clinical performance is constrained by its poor solubility in water, which limits its oral bioavailability[12]. Once solubilized, AZT exhibits rapid absorption and favourable pharmacokinetic properties. Therefore, enhancing its solubility remains a critical objective in formulation development[6]. In addition to solubility challenges, AZT possesses a pronounced bitter taste[13] and is susceptible to photodegradation [14]. To address these limitations, a variety of formulation strategies have been investigated, including the use of nanoparticles [15, 16], nanosuspensions [17], microemulsions [18], solid dispersions[19], co-spray-dried powders[20], and metal complexation [21]. Taste-masking approaches such as the incorporation of microparticles [22] and nanoparticles [23] have demonstrated efficacy in improving palatability without compromising dissolution behaviour.

Super molecular chemistry and crystal engineering are extensively used to tailor solid-state properties [24, 25]. For many years, attempts have been made to manipulate the physicochemical properties of solid-state active pharmaceutical ingredients (APIs). The marketed APIs are presented in various forms; for example, containing a single (salts and polymorphs) or a multicomponent (solvates and hydrates) solid [26]. In recent past research on multicomponent solid cocrystals has drawn great attention from researchers²⁷ and several pharmaceutical properties

of APIs have been improved, such as solubility, dissolution rate, hygroscopicity, and photostability [27-30]. Transfer of proton and intermolecular interaction is the key parameter for the synthesis of molecular salts and cocrystals of APIs, respectively [31-37]. The APIs and coformers, which are hydrogen bond donor and acceptor sites, are used for the synthesis of molecular salts and cocrystals [32, 38, 39].

AZT has several hydrogen bond donor and acceptor sites, which may potentially interact with other molecules [13, 40]. Currently, hydrogen bond interaction and transfer of proton from excipient to AZT have been reported in literature [13, 40, 41]. The substances that are active sites can interact with AZT. Therefore, glycine (Gly) and acetamide (ACT) are potential candidates for interaction. Additionally, molecular salt and cocrystal of API with Gly and ACT [42, 43] have also been prepared previously. Glycine (2-aminoethanoic acid) is the simplest amino acid. It is one of the most important, nonessential amino acids in humans and animals. It is well-known that the glycine molecule could occur in cationic, anionic, and zwitterionic forms. Therefore, it could non-covalently interact with anionic, cationic, and neutral molecules, forming molecular complexes. This makes it both an interesting counterion and a good coformer [44].

In present study attempt have been made to prepared AZT salt/cocrystals with Gly and ACT in 1:1 mole ratio through solvent evaporation method. The DFT study was performed to evaluate the possible non-covalent interactions. The characterization is one the most important aspect in preparation of salt/cocrystal, therefore, the prepared salt/cocrystal were characterized by various techniques including Fourier transform infrared (FTIR) spectroscopy, Near infrared (NIR) spectroscopy, Raman spectroscopy, Solid State UV-visible spectroscopy, Solid state nuclear magnetic resonance (SSNMR) spectroscopy, X-ray diffraction (XRD), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). After successful characterization,

several physicochemical properties, including solubility, dissolution, and antimicrobial activity, were measured.

2. Experimental section

2.1. Materials

AZT DH API was obtained as a gift from a local pharmaceutical laboratory and used in the experiment without further purification. The Coformers (Gly and ACT) and solvents used in this research were purchased from Sigma-Aldrich, while Distilled water was obtained from the local market.

2.1.1. Preparation method

Equimolar of AZT DH and coformers were added to solvent (water: methanol, v/v 1:1). The solutions were sonicated for 30 min at 50 °C, and solvent evaporated slowly. A few days later, the obtained single crystals were collected from the solvent, dried and stored for further studies.

2.1.2. Preparation of physical mixtures

Physical mixtures (AZT DH-Gly and AZT DH-ACT) of different mole ratios were grinded for 15 min using pestle and mortar. Scheme 1 diagram shows the preparation of AZT-Gly salt, AZT-ACT cocrystal and their physical mixture.

2.2. Characterization of Salt/cocrystals

To assess the thermal behaviour of the pure compounds, physical mixtures, and the prepared salts/cocrystals, melting points were measured using a thermal analysis system (Bibby Scientific Ltd., UK). To better understand ionization state and interactions, the spectroscopic techniques, including FTIR, FT-NIR, Raman, and SSNMR spectroscopy, were employed. FTIR spectra were obtained using a PerkinElmer spectrophotometer (Spectrum version 10.5.1), while Raman spectroscopy was conducted with a LabRAM HR system (Horiba Jobin Yvon, France). Carbon-

13 solid-state NMR spectroscopy was conducted on a Bruker Avance III HD spectrometer using a 4 mm rotor under magic-angle spinning (MAS) conditions. The samples were spun at 10 kHz and analysed at 20 °C. The Cross-polarisation (CP) spectra were acquired with a contact time of 2 milli seconds and a recycle delay of 2 seconds. For confirmation of new solid phase formation and crystalline nature, PXRD patterns were recorded using an EQUINOX 3000 diffractometer (Thermo Scientific), equipped with a CPS-120 detector. The analysis employed Cu-K α radiation ($\lambda = 1.54056 \text{ \AA}$), scanning the samples across a 2θ range of 5–50°, with a step interval of 0.05 seconds. For thermal characterization, DSC was performed using a DSC-60 instrument (Shimadzu, Japan), and the data were analysed with STARe software. Additionally, TGA was conducted using a Diamond Series TG/DTA analyser (PerkinElmer, USA), with aluminium oxide (Al₂O₃) employed as the reference material.

2.3. Theoretical studies

Spin-unrestricted density functional theory, executed via Dmol³ module [45] of Material Studio, was employed to predict molecular structures, confirm feasibility of the co-crystals, and to evaluate the prevailing non-covalent interactions (NCI) between the coformers and AZT. Geometries of the molecules were optimized using the generalized gradient approximation (GGA) of the Perdew-Burke-Ernzerhof (PBE) [46] with double numerical plus polarization (DNP) basis set and the Grimme's D3 dispersion correction [47], along with the 4.6 Å basis set cutoff and smearing of 0.005 Ha. The convergence criteria during geometry optimization for energy, displacement, and force were set as 10⁻⁵ Ha, 0.005 Å, and 0.002 Ha Å⁻¹, respectively. Similarly, the conductor-like screening model (COSMO) [48] was used to incorporate the effect of water as a solvent, with a dielectric constant value of 78.5. While formation energies of the cocrystals were calculated through the following equation.

$$E_{\text{form}} = E_{\text{cocrystal}} - (E_{\text{AZT}} + E_{\text{coformer}}) \quad (1)$$

Where the $E_{\text{cocrystal}}$, E_{AZT} , and E_{coformer} are the ground state energies of the optimized cocrystal, AZT, and coformers (Gly and ACT), respectively.

The quantum theory of atoms in molecules (QTAIM) [49] and the NCI reduced density gradient (NCI-RDG) [50] analyses were performed using the Multiwfn 3.7 and visual molecular dynamics (VMD) programs, whereas the input files were generated by the Gaussian 16 package. The QTAIM topological parameters, such as all electron density “ $\rho(r)$ ”, Laplacian of the electron density “ $\nabla^2\rho(r)$ ”, and the total electron energy density “ $H(r)$ ” at the bond critical points (BCPs) between nuclei, were computed to characterize the nature and strength of molecular interactions.

2.4. In vitro Powder Dissolution Study

To investigate the dissolution rate of AZT DH and its salt/cocrystal forms, a USP type-II paddle apparatus was used. To minimize the influence of particle size, all samples were ground to a fine powder and passed through a 100-mesh sieve. In each test, a total of 50 mg of sample was dissolve in 900 mL of phosphate buffer (pH 6.8) and stirred on 75 rpm at 37 °C. The aliquots of 5 mL were picked at specific intervals and immediately added the same amount of fresh buffer to ensure constant volume. The collected samples were filter and analyzed by High-performance liquid chromatography (HPLC) to measure drug release. The same was procedure was performed with 0.1 mM HCl to test dissolution rate in acidic conditions.

2.5. In vitro antibacterial study

Regarding the antibacterial assay, approximately, 20 mL of sterilized agar solution was added to Petri dishes and allowed to incubate for 24 hours. Further testing was performed using only plates that were not contaminated. The strains of bacteria that were tested were *Escherichia coli* (MTCC

1687), *Salmonella typhi* (MTCC 734), and *Klebsiella pneumoniae* (MTCC 1030). Pure AZT was made as stock solutions (1000 µg/mL) and its salt/cocrystal derivatives and then serially diluted to concentrations of 500, 250, 125, and 62.5 µg/mL. Out of every dilution, 5 µL was pipetted onto sterile 6 mm filter paper discs and then put on the agar plates that were inoculated. After 24 hours of incubation at 37 °C, the zone of bacterial inhibition was measured manually to determine the activity of antibacterial.

3. Results and discussion

3.1. Construction of binary phase diagram and determination of melting point

Determination of most suitable mole ratio for multicomponent system is very important. Therefore, binary phase diagrams were constructed for AZT-Gly and AZT-ACT systems [51, 52]. The melting points varied across mixtures with different molar ratios were measured and plotted these data, as shown in **Figure S1**. The W-shaped curves in the diagrams (for AZT-Gly, and AZT-ACT) suggest that a new solid phase is likely to form at a 1:1 molar ratio[51-54]. Supporting this observation, the melting points of the pure compounds and the synthesized products (**listed in Table S1**) indicate that the new materials melt at temperatures between those of their respective starting components, which is strong evidence that a cocrystal or salt has successfully formed.

3.2. Characterization

3.2.1. Solid State UV-Visible spectroscopy

When an intermolecular interaction is established between API and its counterpart, the UV-Vis absorption spectrum alters in terms of its peak intensity and peak position with respect to the starting material [55, 56]. The solid state UV-Vis absorption spectra of starting material, physical mixture, AZT-Gly and AZT-ACT presented in **Figure 1& Figure 2**. The spectra of the prepared material revealed significance difference (peak intensity decrease and shifted toward lower

wavelength) from their respective starting material spectra. Moreover, AZT-Gly and AZT-ACT spectra do not match with their corresponding physical mixture spectra. These changes indicate the formation of ionic bond or hydrogen bond in AZT-Gly and AZT-ACT.

3.2.2. Vibrational characterization

Vibrational spectroscopic analysis (IR and Raman spectroscopy) is alternative techniques to probe intermolecular interactions. These techniques are used for confirmation of salt/co-crystal especially when a perfect single crystal is hard to obtain.

3.2.2.1. FT-IR analysis

When studying salt or cocrystal formation, one of the most useful tools is FT-IR spectroscopy. Changes in the spectra such as peak shifts, broadening, and the appearance or disappearance of peaks particularly in the carbonyl and amine regions, can reveal how molecules are interacting. To explore the interactions between azithromycin (AZT) and two coformers (glycine (Gly) and acetaminophen (ACT)) The FTIR spectra of as received AZT, Gly and ACT are shown in **Figures S2, S3 and S4** respectively. FT-IR spectra of the obtained complexes like AZT-Gly salt, and AZT-ACT cocrystals are depicted in **Figure 3 & Figure 4**. For the AZT-Gly salt (**Figure 3**), we observed a shift in AZT carbonyl peak from 1719 to 1697 cm^{-1} . At the same time, new peaks appeared at 1619, 2460, and 802 cm^{-1} , which are associated with NH_2 scissoring, NH^+ stretching, and NH_2 wagging vibrations, respectively. Interestingly, the NH_3 combination band at 2177 cm^{-1} disappeared in the salt, all of which points to a proton transfer from glycine to the amine group of AZT. In its neutral form, glycine shows a strong $\text{C}=\text{O}$ stretch near 1780 cm^{-1} . However, this peak is absent in the AZT-Gly salt, suggesting that the carboxylic acid group has been deprotonated to form a carboxylate ion. Supporting this, the glycine carboxylate peaks (originally at 682 and 502 cm^{-1}) shifted to 665 and 477 cm^{-1} in the salt form, indicating their involvement in salt formation.

Moreover, significant changes in the broad band region between $3100\text{--}2605\text{ cm}^{-1}$ typical for NH_3^+ stretching along with the disappearance of glycine's 2177 cm^{-1} peak and a shift in AZT's carbonyl band, suggest that the original glycine homodimers dissociate, enabling the formation of a new AZT–glycine heterodimer. Overall, the presence of NH^+ , COO^- , and NH_2 peaks in the AZT–Gly salt confirms the formation of a salt, in which glycine exists in its anionic form and AZT in its cationic form. This proton transfer has also been documented in previous studies [13]. For the AZT–ACT system (**Figure 4**), the FT-IR spectrum shows bands at 3441 , 3341 , and 3232 cm^{-1} , which differ markedly from those observed in pure ACT and its amide-amide (homosynthon) interactions. These spectral shifts suggest the disruption of ACT's amide dimer and the establishment of new interactions with AZT. In addition, two of ACT's key carbonyl-related peaks at 1670 and 1592 cm^{-1} shifted to 1660 and 1622 cm^{-1} in the cocrystal. Since AZT is IR-inactive in this region, the presence of these shifted peaks clearly indicates that AZT and ACT form a cocrystal.

3.2.2.2. FT-NIR analysis

FTNIR spectroscopy is used as a screening tool for salt/cocrystals. It is used to detect hydrogen bond in multicomponent system based on the spectral changes observed in the spectrum of cocrystal with respect to parent molecules [57, 58]. The FTNIR spectra of the parent molecules, physical mixture, AZT-Gly and AZT-ACT depicted in **Figure 5 & Figure 6**, the spectra of the prepared material shown significant spectral changes (attenuation and shifting of peaks) in comparison to FTNIR spectra of respective parent molecules. The results of FTNIR spectroscopy further indicate the existence of intermolecular interaction between AZT and their corresponding counterpart.

3.2.2.3. Raman analysis

The Raman spectra of the AZT-Gly salt displayed notable differences compared to those of the individual starting materials, both in the high wavenumber and fingerprint regions. These spectra, along with those of the corresponding components, are shown in **Figure S5 & Figure S6**. The AZT-Gly salt spectrum (**Figure S5**) revealed several notable changes with respect to pure AZT DH and Gly in term of their peak position and intensity, these considerable changes strongly suggested that both material in interactions in the complex. In particular, the Gly band at 2986 cm^{-1} was slightly shifted and it became a shoulder peak, and the AZT doublet at 2955 and 2970 cm^{-1} was combined into a singlet. Also, the singlet at 2878 cm^{-1} was subdivided into a doublet at 2865 and 2887 cm^{-1} . A low-intensity peak at 3038 cm^{-1} appeared which can probably be attributed to the transfer of protons to the amine moiety of AZT. Even more can be understood about the 1800-200 cm^{-1} range, which is very sensitive to intermolecular interactions. The interaction between components is high in this region hence changes like attenuation, broadening of the band and position shift are observed. The AZT-Gly salt spectrum is rather complex and, therefore, it is difficult to assign the peaks accurately. However, the spectral changes which are observed relative to the pure materials are a strong indication for intermolecular interactions. Likewise, when comparing the Raman spectrum of the AZT-ACT cocrystal to those of pure AZT DH and ACT (**Figure S6**), the spectra of AZT-ACT system changed significantly. These differences also evidence the occurrence of the physical contacts between the components, which means that a successful cocrystal has been formed.

3.2.3. SSNMR spectroscopy

The SSNMR spectroscopy is important technique to characterize salts/cocrystals, especially when obtaining single crystal is difficult [59]. Solid state ^{13}C NMR spectroscopy revealed the formation of salts or cocrystals, especially nitrogen or carboxylic functional group exist either in the active

pharmaceutical ingredients or in the conformers. This technique is commonly used for detection of proton transfer, as the spectrum of the salt depicted change than corresponding raw material. When carboxylic group is in interaction with their counterpart, the blue chemical shift is observed (around 3-7 ppm) due to hydrogen bond formation. On the other hand, in case of proton transfer from carboxylic group to nitrogen with the formation of COO^{-1} and $\text{NH}^{+}\cdots\text{O}^{-}$ contact the blue chemical shift (around 6-7 ppm) is observed [60]. Furthermore, when very strong hydrogen bond accomplished, the chemical shift pattern usually is similar to that of carboxylate group [61, 62]. The solid-state ^{13}C NMR spectra of the as received AZT-DH, ACT are shown in **Figure S7 and Figure S8**, while the complexes, AZT-Gly and AZT-ACT were obtained and presented in **Figure 8**. The chemical shift value of carboxylic group of Gly (**Figure S8**) shifted from 173.30 to 179.40 ppm (around 6 ppm increase) in AZT-Gly complex spectrum (**Figure 7**) while ACT carboxylic group (**Figure S8**) exhibited shift from 176.19 to 179.16 (around 3 ppm increase) in their respective AZT-ACT complex spectrum (**Figure 8**). Moreover, in AZT-Gly complex and AZT-ACT complex the carbonyl peak of AZT overlap with the carbonyl peak of Gly and ACT. These changes suggested that interactions (transfer of proton and establishment of hydrogen) exist in AZT-Gly and AZT-ACT complexes.

3.2.4. XRD analysis

Preparing detailed crystal structure data with precise dimensions can be a tedious task, with a high possibility of failure. One limitation of single-crystal X-ray diffraction is that the selected crystal may not be representative of the actual bulk product, it could simply be a side product. Moreover, single-crystal analysis is time-consuming and not always readily available in many research settings. Conversely, PXRD is an easily available and strong method, especially when primarily

characterizing the change in phases in the solid state [63-66]. Preferably, reference XRD patterns can be produced using single-crystal data, but it can be difficult to grow appropriate crystals. Therefore, *Wu et al.*[64], and Sathisaran *et al.* [67] used PXRD patterns to confirm the formation of new solid forms. To reduce the effect of preferred orientation and to maximize the visibility of weaker peaks, single crystals have been properly ground into fine powder before PXRD analysis. The new solid phases were confirmed by comparing the PXRD patterns of the final products with those of the starting materials. As shown in **Figure 9 and Figure 10**, The patterns of the AZT-Gly salt and the AZT-ACT cocrystals are markedly different from their respective starting materials. These observations are a strong indication of the successful formation of new solid forms a salt in the case of AZT-Gly and a cocrystal in the case of AZT-ACT.

3.2.5. Differential scanning calorimetry

Differential Scanning Calorimetry is a widely used method for probing intermolecular interactions in newly developed solid forms. In this work, DSC was employed to study the thermal behavior of both the starting materials and the prepared solids. The DSC thermogram of AZT–Gly (**Figure 11**) displayed a broad endothermic signal between 40 and 100 °C, which can be attributed to the removal of crystalline solvate or residual solvent. This was followed by three closely spaced endothermic peaks at 144, 148, and 151 °C. Since these transitions do not match those of the individual components, they suggest the formation of a new solid phase. Similarly, the DSC thermogram of AZT-ACT (**Figure 12**) also shown marked difference than respective raw material.

3.2.6. Thermo gravimetric (TG) analysis

The thermal stability and hydration properties of AZT DH, AZT-Gly salt, and AZT-ACT cocrystal were further examined using TGA, and the TG thermograms were presented in **Figure S9**. The steady mass loss seen up to melting temperature demonstrate the transition of hydrated/solvated to

anhydrous form [72] as the removal of bound and unbound solvate molecules take place up to melting temperature [64], huge weight loss observed onward 240°C occur due to degradation of the material[73]. The ACT-ACT TG thermogram exhibited no considerable weight loss up to the onset temperature. It is suggested that AZT-ACT formed an anhydrous solid. Moreover, the complexes' degradation started at a high temperature compared to pure AZT, exhibited better thermal stability when AZT complexes were formed with Gly and ACT.

3.3. Quantum chemical study analysis

The quantum chemical calculations were adopted to rationalize the existence of the salts/cocrystals. The optimized geometries, along with the molecular electrostatic potential (MEP) maps are presented in **Figure S10**. The MEP maps are associated with red, yellow-green and blue areas, representing electron-rich, moderate, and electron-deficient regions of the AZT, Gly and ACT molecules, respectively. For instance, red (electrophilic centers) and blue (nucleophilic centers) regions are observed over the electronegative atoms (oxygen/nitrogen) and electropositive atoms (hydrogen), respectively. MEP maps of the molecules were used to predict the binding modes between AZT and the coformers [45]. Thus, optimized geometries of the AZT-Gly salt and AZT-ACT cocrystals (**Figure 13**) were obtained by bridging the coformers with the AZT, followed by geometry relaxation calculations. Similarly, the E_{form} of the cocrystals were calculated to be -1.58 and -1.45 eV for AZT-Gly and AZT-ACT complexes, respectively, which are extremely negative than the reported felodipine-glutaric acid cocrystal [46]. The high negative E_{form} values reflect thermodynamic feasibility, confirming successful synthesis of the salt/cocrystal. **Figure 13a** clearly demonstrates that the AZT-Gly salt could be achieved through initial protonation of the nitrogen atoms at the two ends of AZT by Gly coformer and their successive association. It shows that ionic interaction is the major cause of the formation of AZT-

Gly cocrystal, being initiated by close approach of the Gly molecules to AZT up to bond distances of 1.52 and 1.58 Å. In contrast, the AZT-ACT cocrystal (**Figure 13b**) is originated by the intermolecular interactions of ACT molecules with AZT, facilitated by the amine groups of ACT molecules with the two nitrogen atoms of AZT, after preliminary bonds rearrangements. However, discrepancy in bond distances (1.86 and 2.58 Å) between the two ACT molecules and AZT can be observed, which is assigned to the difference in steric hinderance at one end of AZT molecule relative to the other. In addition, the MEP maps of the two AZT-Gly and AZT-ACT are displayed in **Figure 13(c, d)**. The multicolored MEP highlights highest polarity (dipole moments) of the cocrystals, involving polar interactions, which ensure crystal stability (improving shelf life) in their solid state and compatibility with polar solvents in solution-phase[47]. It is noteworthy that cocrystals–solvent interaction is imperative for better solvation that eventually leads to enhanced solvent-related properties, including dissolution rate, solubility, and bioavailability.

The QTAIM and NCI analyses were performed to get insight into the nature and strength of bonding between the coformers and AZT in their respective cocrystals. The QTAIM topological parameters are summarized in **Table 1**. The bond critical points (BCPs) provide substantial information regarding chemical bonds in terms of the topological parameters. Rozas *et al.*, [48] and Koch *et al.*, [49] classified interactions based on the QTAIM parameters [50]; thus, if the $\rho(r) > 0.10$ a.u., $\nabla^2\rho(r) < 0$ (negative), and $H(r) < 0$ (negative) at a given BCP, it would be a shared-shell (covalent) interaction. Alternatively, if the $\rho(r)$ is in the range of 0.02–0.06 a.u., $\nabla^2\rho(r) > 0$ (positive), and $H(r) < 0$ (negative) at a BCP, the interaction would be a closed-shell (electrostatic) interaction, also referred to as medium H-bond. According to these criteria, the BCP307 and 368 along the respective interactions N(29)···H(150) and H(10)···N(28), in AZT-Gly molecular graph (**Figure 14a**), represent (coordinate) covalent interactions, given rise by the protonation of the

AZT nitrogen atoms. Similarly, the BCP313 along O(144)···H(150) and BCP371 along O(4)···H(10) bond paths, elucidate ionic interactions between the two glycinate ions (generated by the proton transfer) and the protonated AZT cation. Moreover, the other BCPs (306, 322, and 332) in AZT-Gly salt molecular graph indicate weak H-bond/van der Waals interactions since their $\nabla^2\rho(r)$ and $H(r)$ both are positive. Conclusively, the AZT-Gly is formed via ionic bonds, as the principal interaction, in addition to other weak van der Waals forces, bridging the two Gly with AZT.

However, in case of AZT-ACT cocrystal, (from **Table 1**) the positive values of $\nabla^2\rho(r)$ and negative values of $H(r)$ at BCPs 157 and 294 along the H(107)···N(142) and N(18)···H(138) interactions imply that H-bonding is the primary force, active in the formation of trimolecular AZT-ACT cocrystal. Likewise, the other BCPs (158, 258, 285, 314) in the molecular graph of AZT-ACT (**Figure 14b**) signify the existence of either weak H-bonding or van der Waals interactions, leading to overall stability of the AZT-ACT cocrystal.

Furthermore, the NCI-RDG analysis [50, 74] visualizes graphically the non-covalent interactions in the form of isosurfaces in real-space, which are characterized to have red, green, and blue colors that demonstrate repulsive-steric, van der Waals, and H-bonding interactions, respectively. Such as isosurfaces in **Figure S11(a, b)** clearly displays the interactive forces in both the cocrystals. However, the NCI 2D scatter plots (**Figure S12**) also differentiate bonding interactions through colored regions, based on the values of $(\text{sign } \lambda_2)\rho$, such as attractive interactions on the left with $(\text{sign } \lambda_2)\rho < 0$, while repulsive interactions are plotted on the right side in red with $(\text{sign } \lambda_2)\rho > 0$. Hence, in **Figure S12-a**, the prominent spike on left in blue at -0.04 a.u. substantiates the existence of ionic interactions in AZT-Gly salt, in addition to the weak H-bonding and van der Waals interactions, denoted by minor light-blue and green spikes in between -0.03 to -0.01 a.u.,

respectively. Analogously, the noticeable spike at around -0.03 a.u. (in **Figure S12-b**) is attributed to the dominant H-bonding in AZT-ACT cocrystal, along with van der Waals interactions (-0.02 to -0.01 a.u.). In conclusion, the NIC-RDG analysis agrees well with the above assessments, suggesting that ionic interaction is the major attractive force in AZT-Gly salt, whereas H-bonding in AZT-ACT co-crystal, further validating their effective synthesis.

3.4. Solubility studies

To evaluate the solubility of AZT DH for pure API and salt/cocrystal, its solubility was determined in both distilled water and a phosphate buffer solution (pH 6.8). Measurements were conducted for the pure drug, as well as its cocrystal and salt forms. As presented in **Table 2**, both cocrystallization and salt formation resulted in a marked enhancement of AZT solubility.

3.5. Powder dissolution study

To evaluate the dissolution characteristics of the new materials, we tested the *in vitro* release of AZT-DH and the synthesized AZT-Gly and AZT-ACT in both phosphate buffer (pH 6.8) and 0.1 mM HCl. As shown in **Figure 15**, the new formulations dissolved noticeably faster than pure AZT dihydrate. The original form of AZT dehydrate exhibited poor dissolution within the first hour, likely due to agglomeration and poor wettability[12]. In contrast, the improved performance of the cocrystal and salt forms can be attributed to alterations in crystal structure, particle size, and shape[64], as well as the hydrophilic nature of the coformers (Gly and ACT) [75].

3.6. Antibacterial studies

The antibacterial activity of the original drug and the new materials was compared through zone of inhibition assay. These tests were performed against *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S. typhi*), and *Klebsiella pneumoniae* (*K. pneumoniae*). The results, presented in **Table 3**, demonstrated that the newly synthesized materials exhibited superior antibacterial performance

relative to the parent drug, despite containing a lower amount of AZT. This improvement may be attributed to enhanced dissolution[76], potential chemical interactions [77], or improved diffusion into bacterial cells [78]. Collectively, these findings underscore the potential of cocrystal and salt strategies to enhance antibiotic efficacy, particularly in addressing multidrug-resistant strains (MDRS) in future applications.

Conclusion

The AZT-Gly salt and AZT-ACT cocrystal have been prepared successfully in a 1:1 molar ratio through the solvent evaporation method. The characterization techniques like Solid state UV-Visible, FTIR, FTNIR, Raman, SSNMR, PXRD, DSC, and TGA were used to evaluate various aspects such as interaction, crystallinity, and hydrated/solvated nature of AZT-Gly and AZT-ACT complexes. The Density functional theory results also proved that the interactions among AZT-Gly and AZT-ACT are energetically favorable. The solubility (pure water and phosphate buffer solution having pH 6.8) and dissolution rate (phosphate buffer solution of pH 6.8 and 0.1 mM HCl) of AZT improved through salt/cocrystal formulation in comparison with the pure antibiotic. Moreover, the antibacterial activity improved with the salt and cocrystal synthesis. Hence, the described salt and cocrystal were found to be potential entities for the next step in formulation development. Though the techniques used for the characterization provided ample evidence to confirm salt/cocrystal synthesis, single XRD analysis should be required to ascribe exact interactions and lattice structure. Further, experiments are needed to evaluate the observed potentials in animal models along with toxicological considerations.

Declaration

Funding Declaration

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Declaration of competing interest:

The authors declare that they have no competing interests.

Data availability:

All data generated or analyzed during this study are included in this published article and its supplementary information file (SI).

Consent to Publish Declaration

Not applicable.

Consent to Participate Declaration

Not applicable.

Ethics Declaration

Not applicable.

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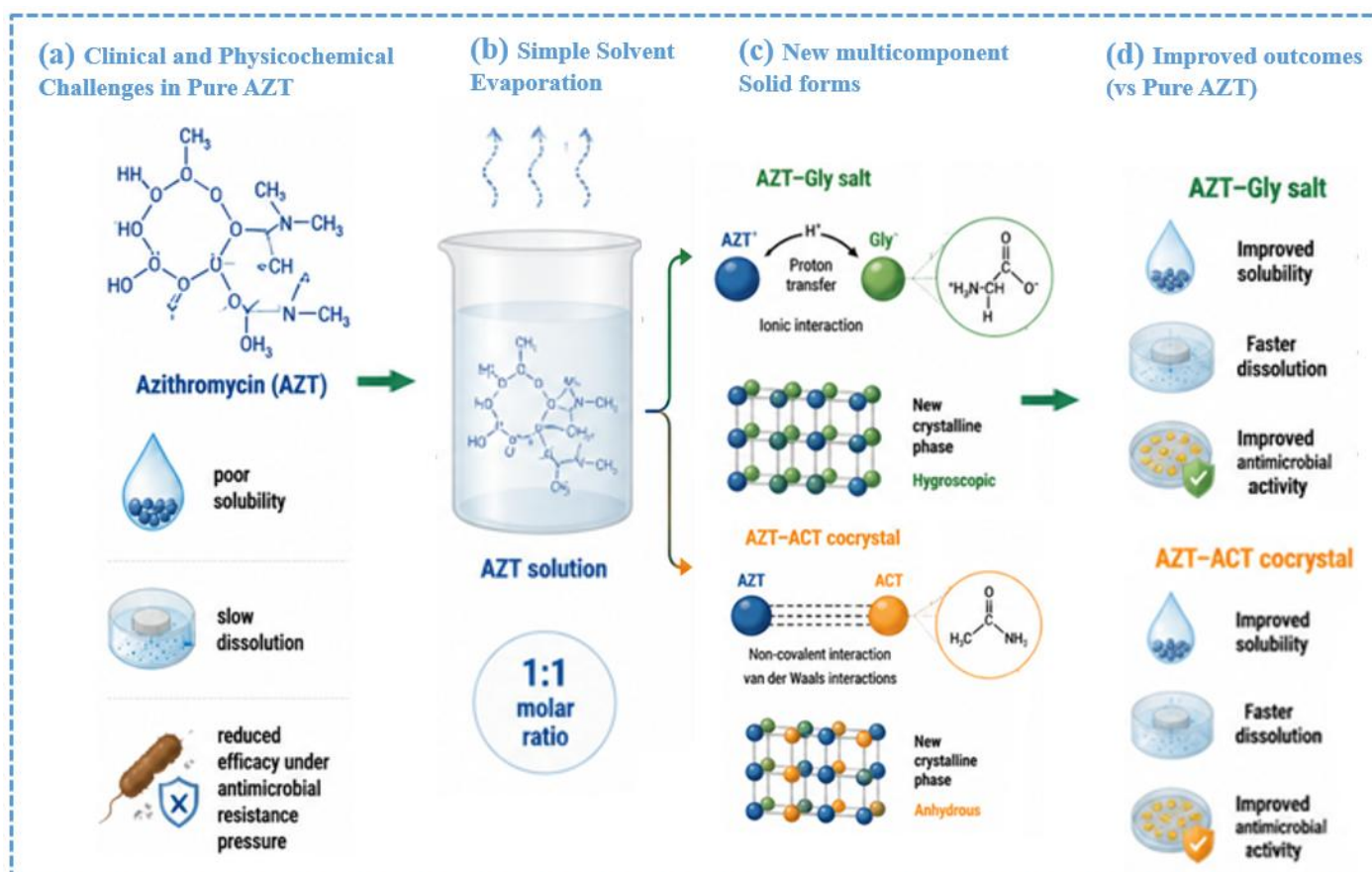
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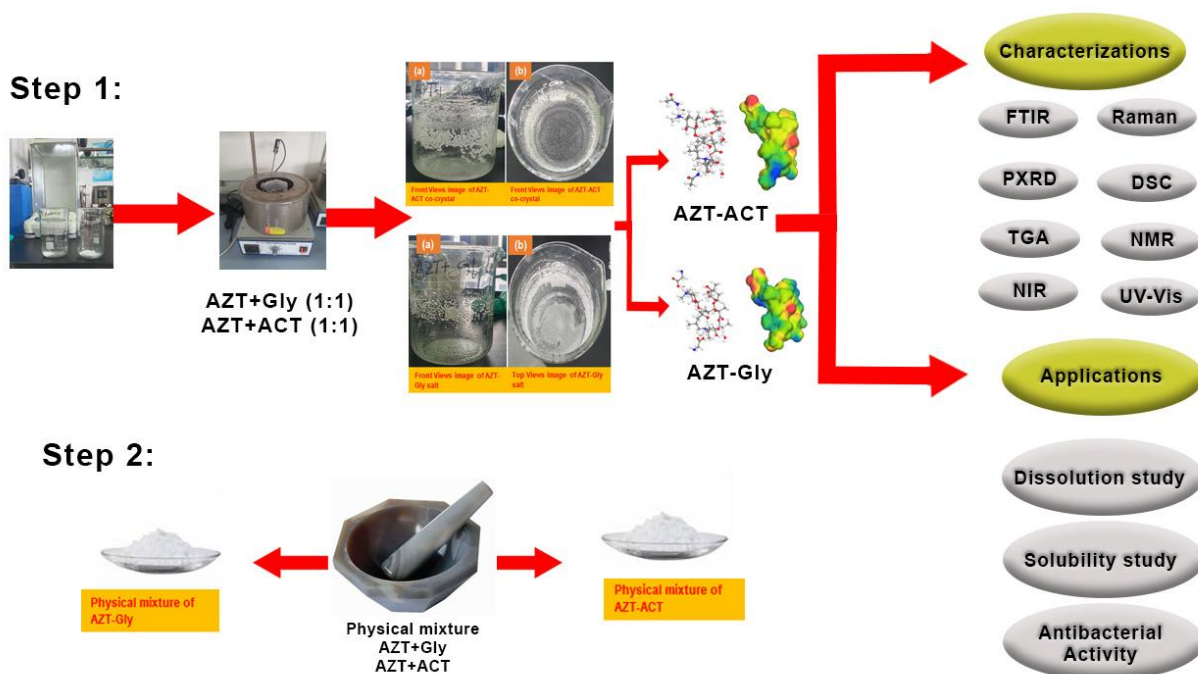
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Graphical abstract: AZT salt/co-crystal engineering is an effective strategy to enhance AZT

physicochemical properties and antimicrobial performance.



Scheme 1. Schematic diagram showing the preparation of AZT-Gly salt, AZT-ACT co-crystal and their physical mixtures.

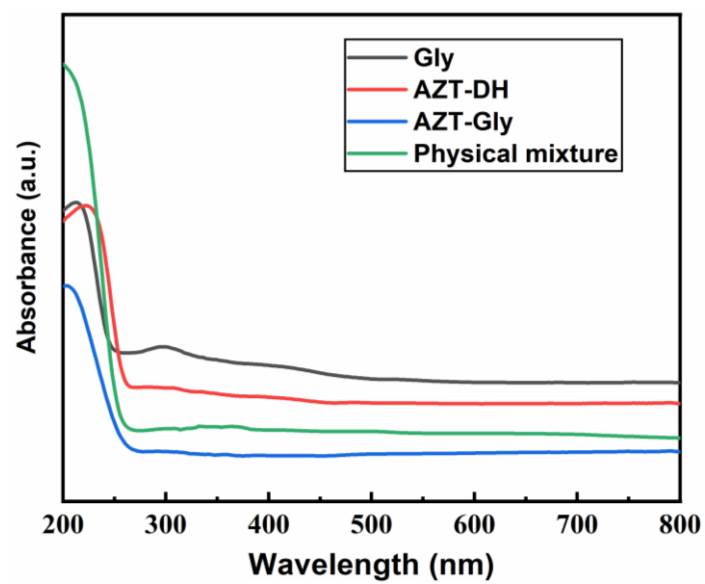


Figure 1: Solid state UV-Visible spectra of Gly, AZT DH, and AZT-Gly salt and their physical mixture.

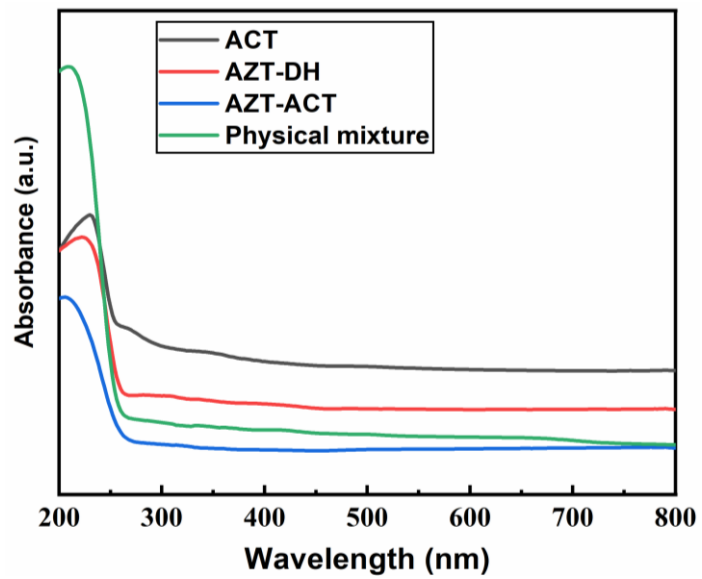


Figure 2: Solid-state UV-Visible spectra of ACT, AZT DH, AZT-ACT co-crystals, and their physical mixture.

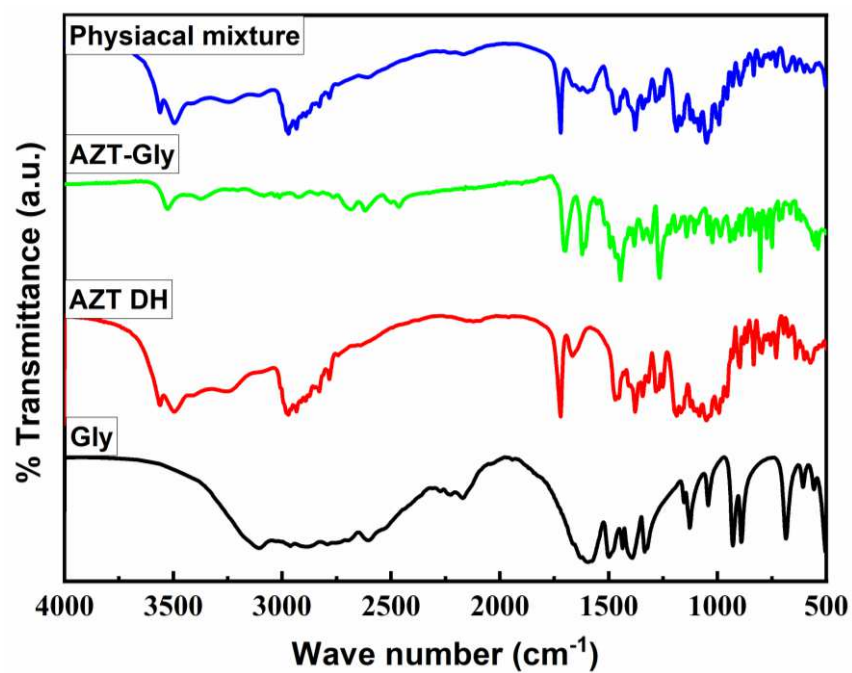


Figure 3: FTIR spectra of Gly, AZT DH, AZT-Gly salt and their physical mixture.

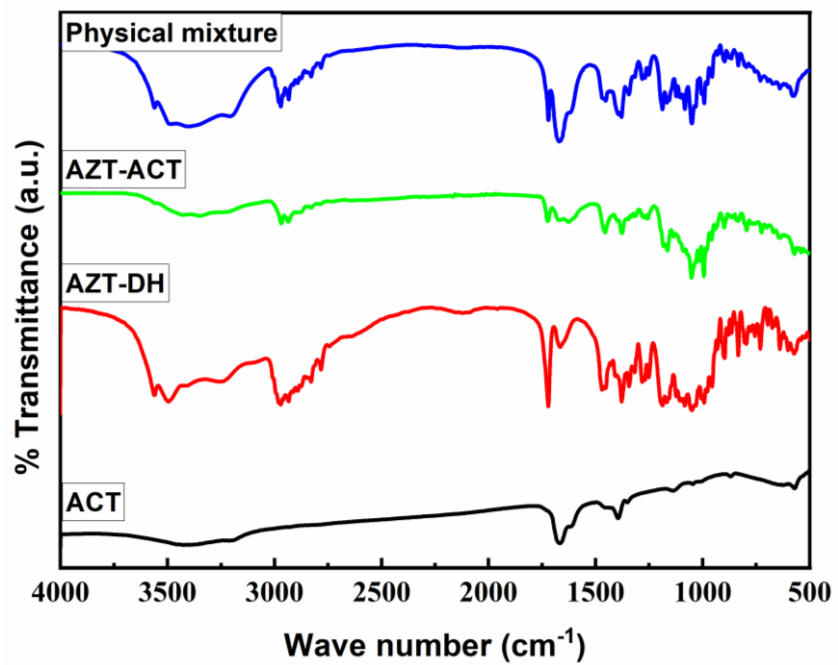


Figure 4: FTIR spectra of ACT, AZT DH, AZT-ACT cocrystal and their physical mixture.

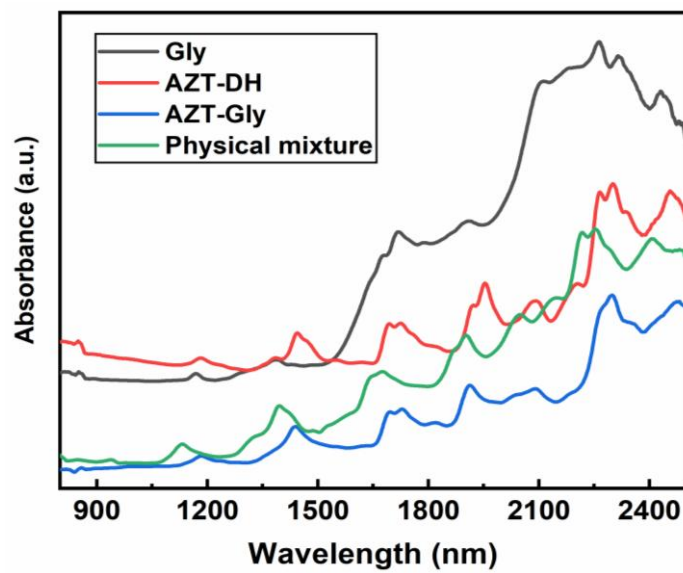


Figure 5: FT-NIR spectra of Gly, AZT DH, AZT-Gly salt, and their physical mixture.

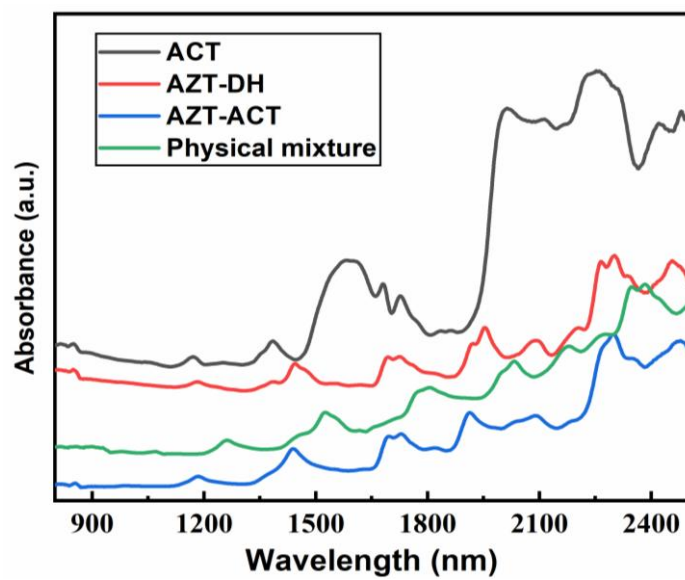


Figure 6: FT-NIR spectra of ACT, AZT DH, AZT-ACT co-crystal and their physical mixture.

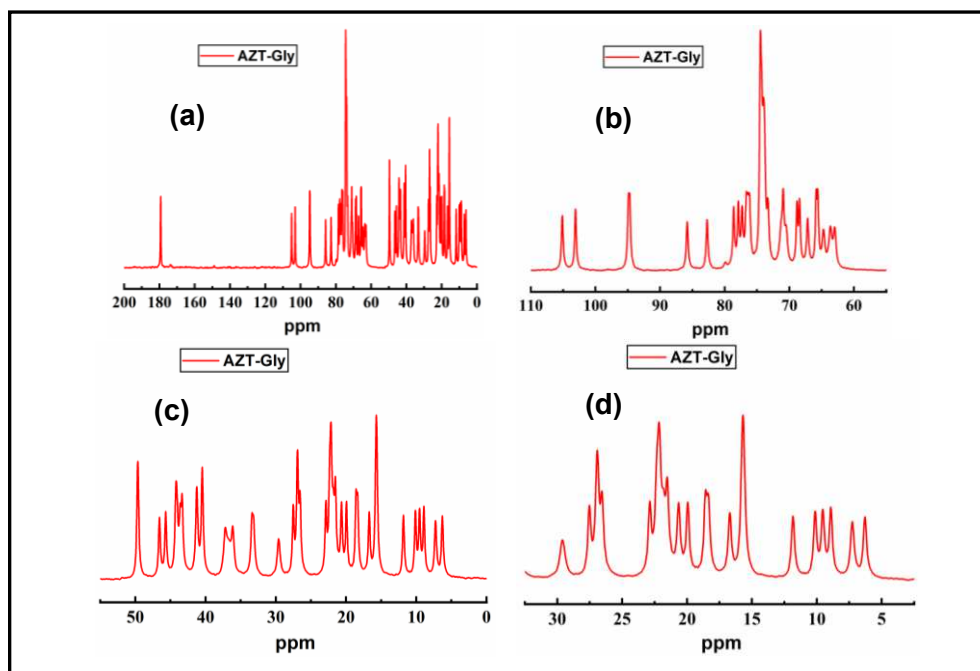


Figure 7: ^{13}C CP-MAS SSNMR spectra of AZT-Gly salt.

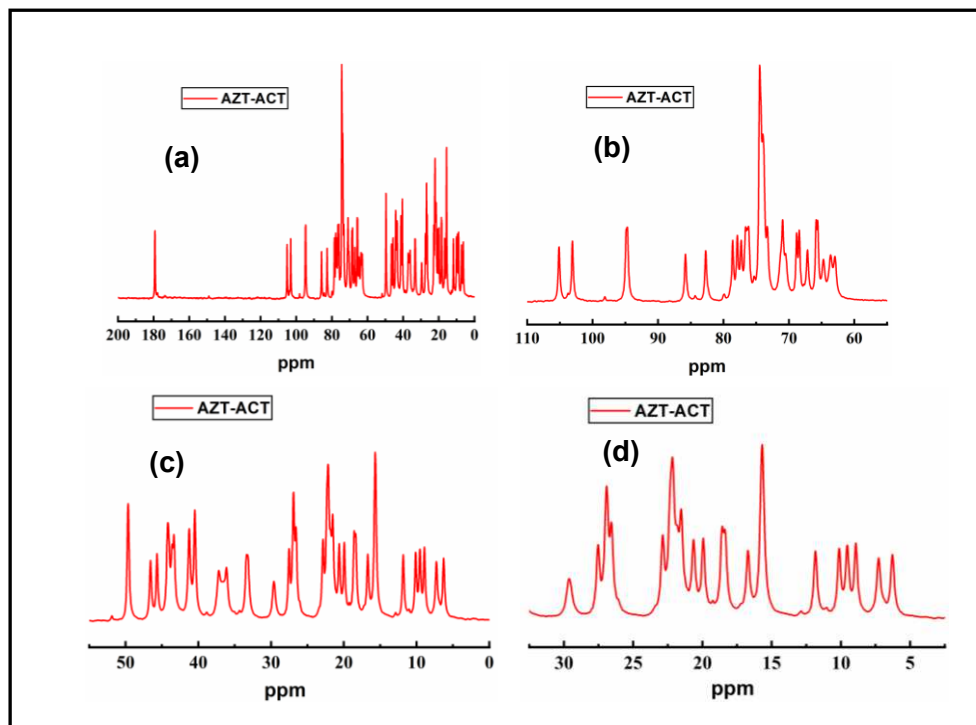


Figure 8: ^{13}C CP-MAS SSNMR spectra of AZT-ACT cocrystal.

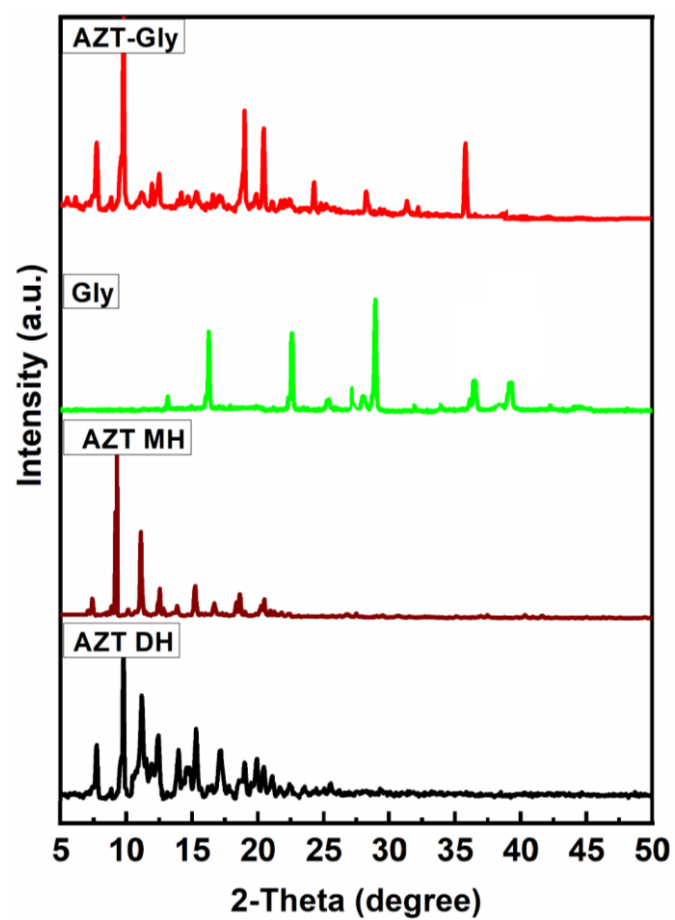


Figure 9: PXRD patterns of AZT DH, AZT MH, Gly, and AZT-Gly salt.

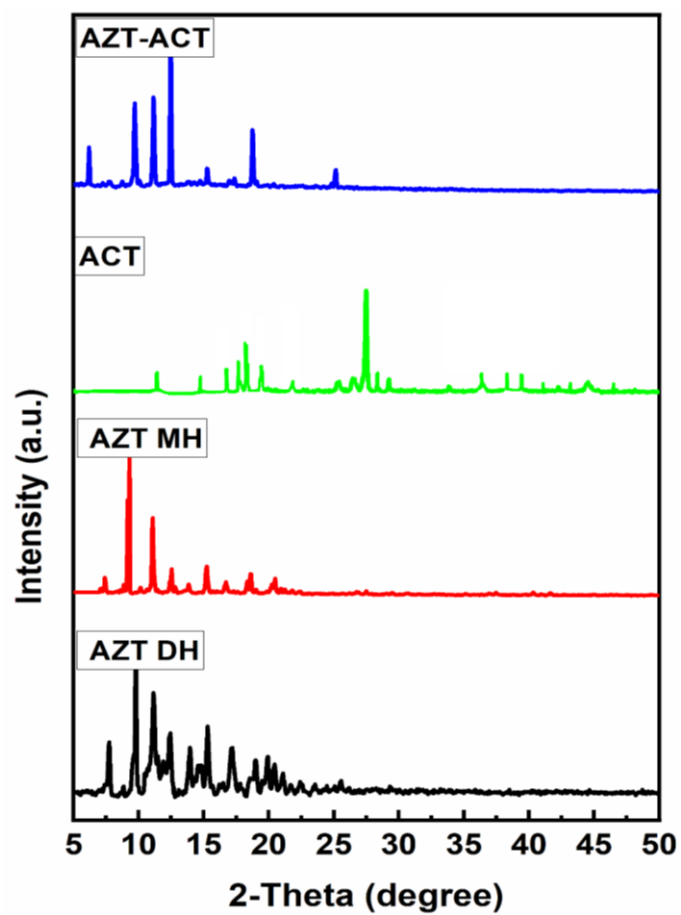


Figure 10: PXRD patterns of AZT DH, AZT MH, ACT, and AZT-ACT cocrystal.

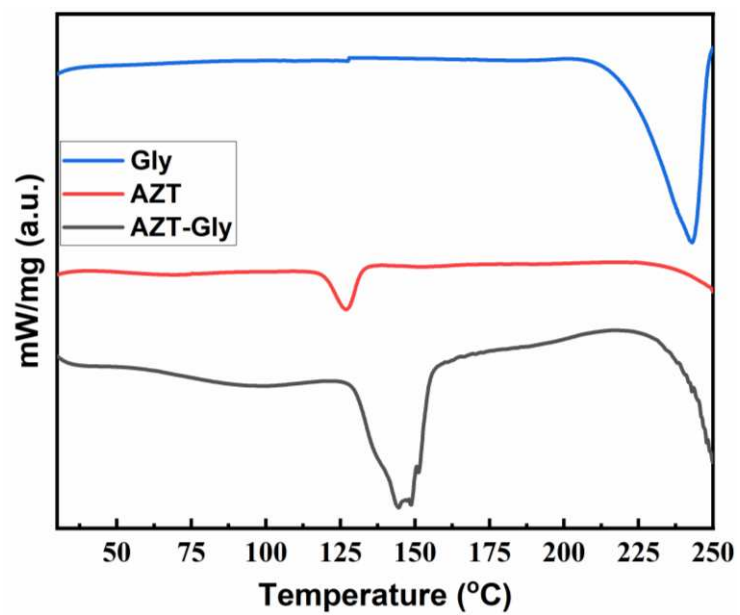


Figure 11: The DSC thermograms AZT DH, Gly and AZT-Gly salt.

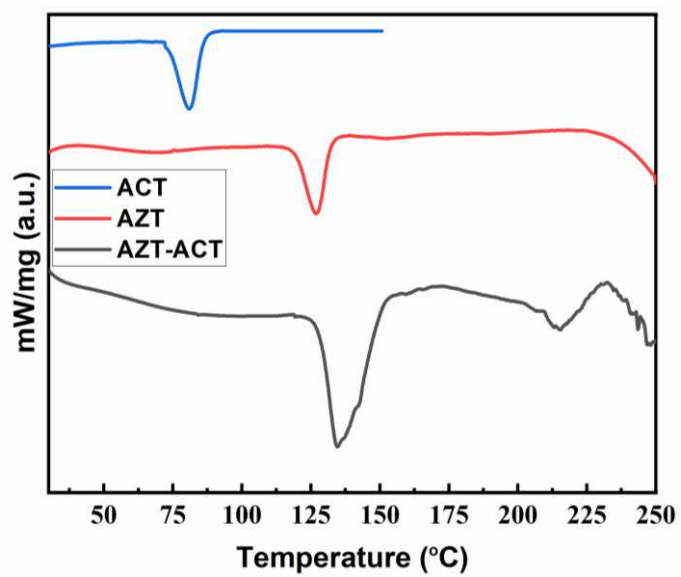


Figure 12: DSC thermograms of AZT DH, ACT and AZT-ACT cocrystal.

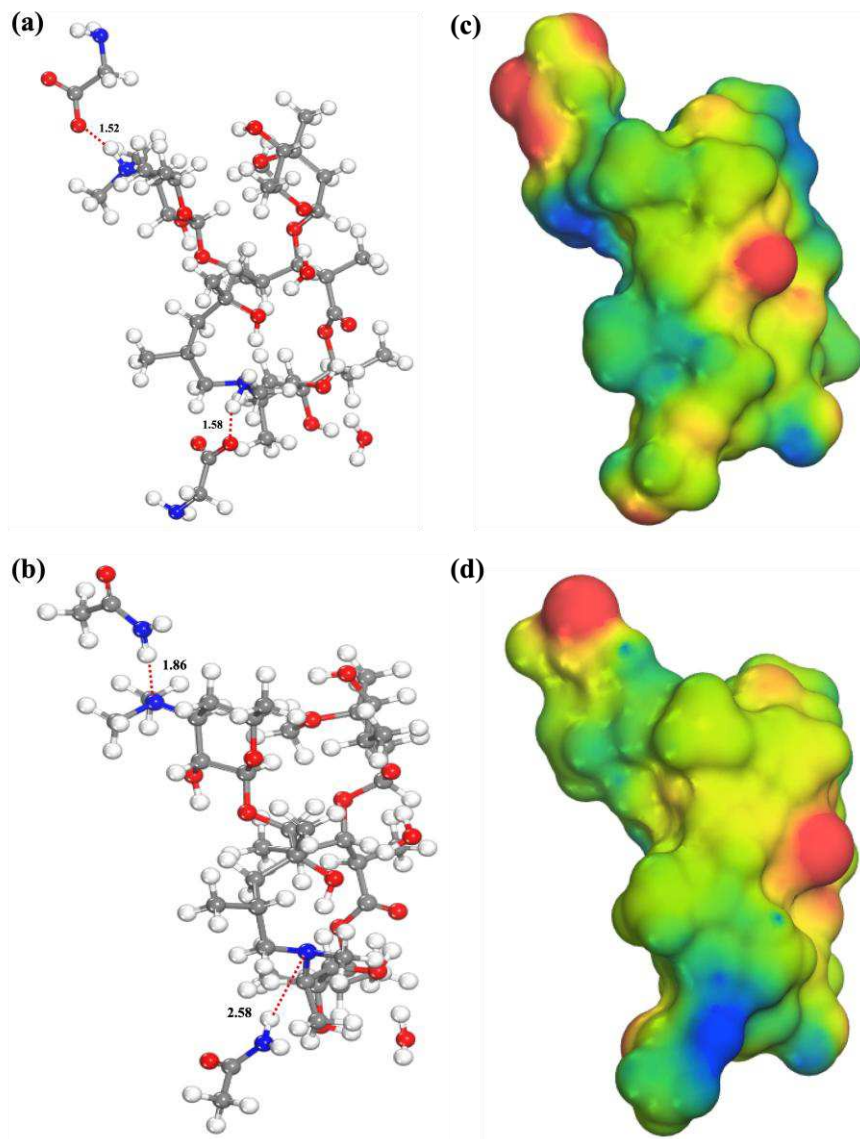


Figure 13: The optimized geometries (**a**, **b**), and MEP maps (**c**, **d**) of AZT-Gly salt and AZT-ACT cocrystal.

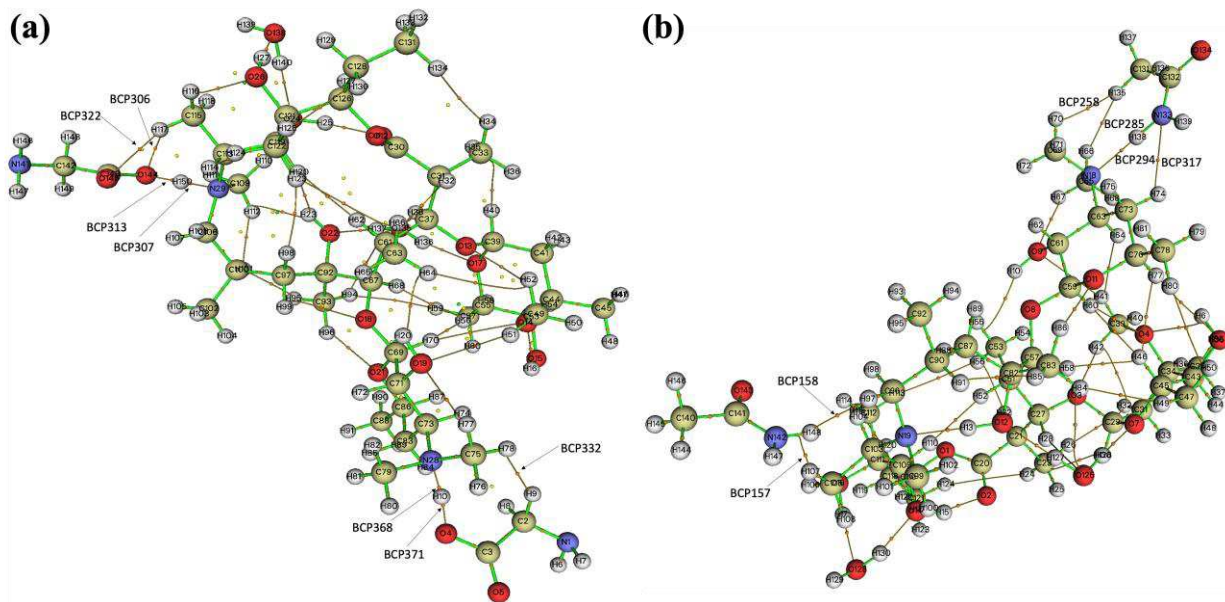


Figure 14. Molecular graphs of AZT-Gly salt and AZT-ACT cocrystal along with their BCPs, represented by orange dots.

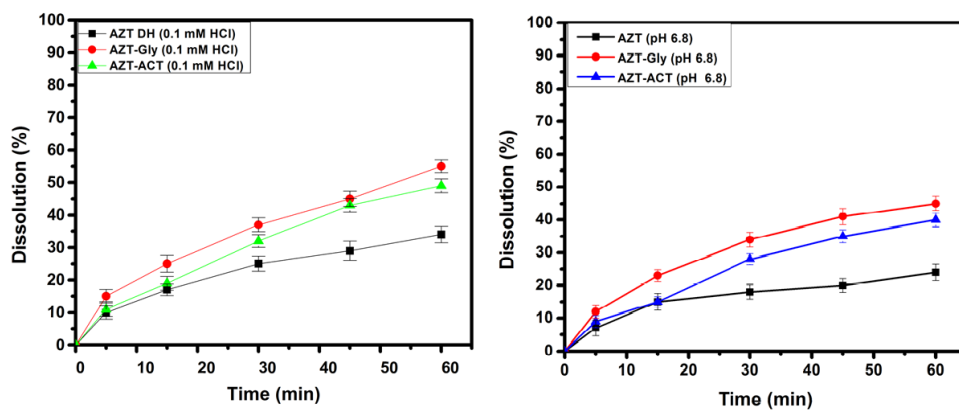


Figure 15: The in vitro drug studies of AZT DH and the prepared materials in phosphate buffer solution (pH 6.8) and 0.1 mM HCl.

Table 1: Calculated quantum chemical parameters of AZT-Gly salt and AZT-ACT cocrystals.

Cocrystal	E_{form} (eV)	E_{gap} (eV)	BCP	$\rho(\mathbf{r})$ (a.u.)	$\nabla^2\rho(\mathbf{r})$ (a.u.)	$\mathbf{H}(\mathbf{r})$ (a.u.)
Gly-AZT	-1.58	3.91	BCP306, H(117)···O(144)	0.020	0.077	0.00148
			BCP307, N(29)···H(150)	0.181	-0.467	-0.19596
			BCP313, O(144)···H(150)	0.038	0.396	-0.19263
			BCP322, H(117)···O(145)	0.019	0.067	0.00094
			BCP332, H(9)···H(78)	0.007	0.022	0.00123
			BCP368, H(10)···N(28)	0.167	-0.371	-0.16784
			BCP371, O(4)···H(10)	0.028	0.472	-0.21365
ACT-AZT	-1.45	3.79	BCP157, H(107)···N(142)	0.041	0.078	-0.00688
			BCP158, H(97)···H(148)	0.005	0.017	0.00103
			BCP258, H(70)···H(135)	0.011	0.041	0.00110
			BCP285, H(66)···H(135)	0.002	0.051	0.00177
			BCP294, N(18)···H(138)	0.040	0.074	-0.69243
			BCP317, H(74)···O(133)	0.035	0.002	0.00019

Table 2: Solubility of AZT-DH, AZT-PCM, AZT-Gly and AZT-ACT.

Solubility medium	Pure AZT DH ($\mu\text{g/ml}$)	AZT-PCM ($\mu\text{g/ml}$)	AZT-Gly ($\mu\text{g/ml}$)	AZT-ACT ($\mu\text{g/ml}$)
Distilled water	55 $\pm$ 1.31	73 $\pm$ 1.42	120 $\pm$ 2.1	105 $\pm$ 1.4
Phosphate Buffer (pH 6.8)	60 $\pm$ 1.28	80 $\pm$ 1.40	115 $\pm$ 1.6	97 $\pm$ 1.36

Table 3: Zone of inhibitions of AZT DH, AZT-Gly and AZT-ACT against different bacterial strains.

Bacterial strain	Sample amount (µg)	AZT DH (100%) Zone inhibition (mm)	of AZT-Gly (91%) zone of inhibition (mm)	AZT-ACT (93%) zone inhibition (mm)	of
<i>K. pneumoniae</i>	5	21±0.45	24±0.45	23±0.40	
	2.5	17±0.40	20±0.37	19±0.38	
	1.25	14±0.35	16±0.40	16±0.30	
	0.62	10±0.25	13±0.30	12±0.40	
<i>E. coli</i>	5	15±0.32	18±0.36	17±0.33	
	2.5	10±0.30	13±0.40	13±0.34	
	1.25	8±0.25	9±0.28	9±0.23	
	0.62	--	--	--	
<i>S. typhi</i>	5	13±0.35	16±0.39	14±0.37	
	2.5	9±0.30	12±0.27	10±0.37	
	1.25	7±0.24	8±0.22	8±0.31	
	0.62	--	--	--	

The percent concentration of active antibiotic in the formulations present in the bracket in first row of column two, three, four and five of the table 3.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supportinginformationfile.docx](#)