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COMMUNICATION

Drug-drug salt forms of ciprofloxacin with diflunisal and indoprofen†

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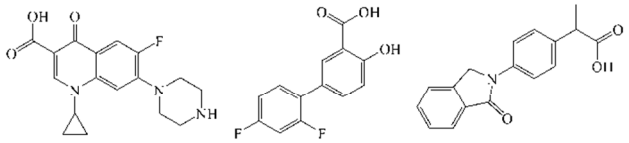
Two salt forms of fluoroquinolone antibacterial drug, ciprofloxacin (CIP) with non-steroidal anti-inflammatory drugs, diflunisal (CIP/DIF) and indoprofen (CIP/INDP/H_2O) are synthesized and characterized by PXRD, FTIR, DSC and TGA. Crystal structure determination allowed us to study the drug-drug interactions and the piperazine based synthon (protonated piperazine-carboxylate) in the two forms, which is potentially useful for the crystal engineering of new salt forms of many piperazine based drugs.

The pharmaceutical multicomponent forms consisting of an active pharmaceutical ingredient (API) and an inactive co-former, which is ideally a generally recognized as safe (GRAS) substance, have been well explored in recent times.[1-3] Formation of co-crystals and salt forms can improve the API's physicochemical properties, such as solubility, bioavailability and mechanical properties of individual drugs, without changing any covalent bonds in either of the species.[4-7] Due to the simple and convenient preparation methods, there is an increased interest in the discovery of multi-API forms as evident from recent rise in number of publications and patent applications.[8,9] These examples include the crystalline forms of theophylline with phenobarbital,[10] ethenzamide with gentisic acid,[11] meloxicam with aspirin,[12] acetylsalicylic acid with (L)-theanine,[13] acetaminophen with theophylline,[14] lamivudine with zidovudine,[15] sulfamethazine with theophylline,[16] isoniazid with 4-aminosalicylic acid[17] and pyrazinamide with isoniazid.[18] Salt formation is the most widely practiced method to greatly improve the solubility and stability of drugs.[19]

Crystal engineering approach has been effectively utilized in recent times for the synthesis of new forms particularly by exploiting the supramolecular synthons. Hence the identification of synthons that can be transferred across different systems is important. For example, synthon transferability in cytosine and lamivudine salts was recently demonstrated by Desiraju and co-workers by IR spectroscopy studies.[20a] Aakeröy and co-workers successfully established the role of synthon transferability (intermolecular amide···amide synthons) for the assembly and organization of bidentate acetylacetonate (acac) and acetate "paddlewheel" complexes of a variety of metal(II) ions.[20b] Recently Das *et al*. have reported the gelation behaviour in various diprimary ammonium monocarboxylate salts by exploiting the synthon transferability.[20c]



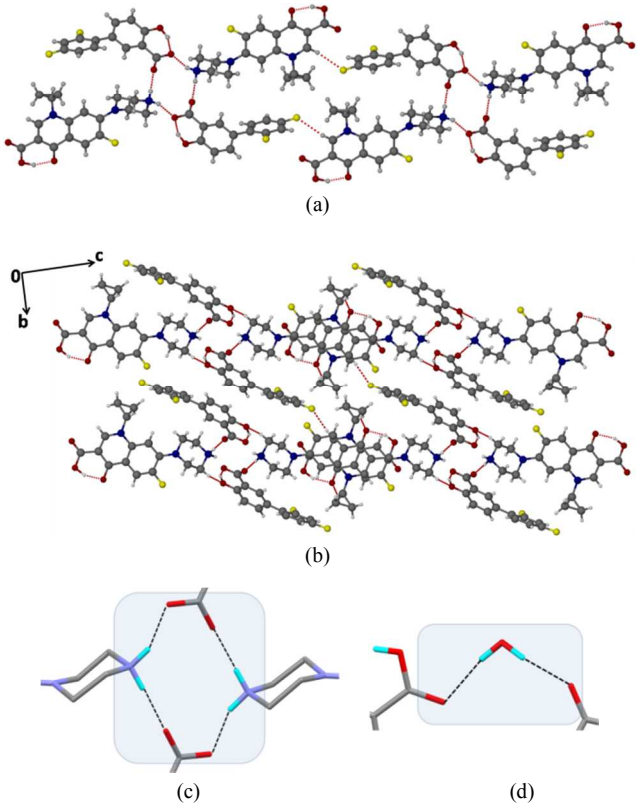
Scheme 1. The chemical structures of APIs used in this study.

Here we report two drug-drug salt forms of ciprofloxacin (CIP) with diflunisal and indoprofen. Ciprofloxacin is a widely used drug, belonging to fluoroquinolone antibacterial family (Scheme 1).[21] It is one among the broad-spectrum antibiotic drugs and is active against both Gram-positive and Gram-negative bacteria, but is known to have poor solubility and absorption particularly in basic media. CIP is available in several brand names, such as Cifran Od, Ciprolet, etc in India, and Cipro and Cipro XR in the USA. Diflunisal and indoprofen are non-steroidal anti-inflammatory drugs (NSAID). A search in Cambridge Structural Database (CSD; version 5.35) revealed 82 structure reports containing ciprofloxacin. Among them, 17 structures belong to the multi-component forms of CIP, which include hydrates,[22] solvates,[23] co-crystals and salts,[24] while the remaining structures belong to metal complexes. In our study we attempted to prepare co-crystals/salts of CIP with a few APIs as co-formers (Table S2). We succeeded to obtain only two salt forms of CIP with diflunisal (DIF) and indoprofen (INDP). The salt formation of these drugs is consistent with the Δ*p*Ka rule (see supporting information, Table S2). These two new solid forms, CIP/DIF (salt) and CIP/INDP/H_2O (salt hydrate) are investigated by various characterization techniques.

The solid form screening was conducted by neat grinding (NG),[25a] liquid assisted grinding (LAG)[25b] in presence of methanol, and fast evaporation method (FE)[26] using rotary evaporation technique (Table S2). The resulted solid powders were characterized by PXRD and FT-IR (Figs. S1, S2). We observed that the NG method did not produce the new salt forms, but the LAG and FE methods were successful. This manifests the efficiency of the latter two methods for screening purposes. In some recent systematic studies, we demonstrated the effectiveness of the FE method (which is widely used, but poorly documented) for screening of polymorphs, solvates and co-crystals.[26]

For single crystal preparation, 50 mg (0.1509 mmol) of CIP and the same equivalents of co-former DIF or INDP were dissolved in 10 mL of acetonitrile/methanol mixture by heating

the flask until a clear solution was obtained. The good quality single crystals suitable for X-ray diffraction studies were obtained in 3 to 4 days from the slow evaporation method at ambient conditions. The crystallographic data for the two salt forms, CIP/DIF and CIP/INDP/H_2O, are listed in Table S3, Supporting Information (SI). Hydrogen bond table (Table S1) and the ORTEP diagrams (Fig. S3–S4) are also given in SI.

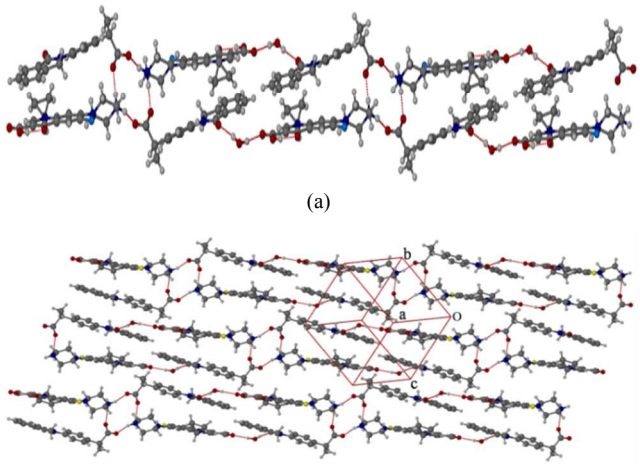


(a) (b) (c) (d)

Figure 1. (a) Crystal packing in CIP/DIF salt shows the centrosymmetric tetrameric motifs formed by hydrogen bonding among two CIP and two DIF molecules, and (b) the close packing of adjacent tetramers, viewed along *a*-axis. Notice the supportive C–H···F interactions formed between tetramers in (a). (c) Synthon 1, observed in both CIP/DIF and CIP/INDP/H_2O, and (d) synthon 2, observed in CIP/INDP/H_2O.

The salt CIP/DIF crystallizes in triclinic *P*-1 space group with each of an ionised drug molecule in the asymmetric unit (Fig. 1). Transfer of proton from acid group of DIF to the secondary N-atom on piperazine ring of CIP results in the ionization of molecules. In the structure, piperazine ring of CIP adopts a chair conformation. The two phenyl rings of DIF are tilted with respect to each other by ~39.63º while the hydroxyl group of DIF forms intramolecular hydrogen bond with carboxylate group *via* O–H···O interactions (O(6)–H(6)···O(5)=C(18); 1.62 (4) Å, 151(4)º). The carboxylic acid group of CIP also forms an intramolecular hydrogen bond with the adjacent carbonyl group (O(2)–H(2)···O(3); *d*/Å, *θ*/º: 1.62(4) Å, 156(4)º). The carboxylate group of DIF interacts with one axial and one equatorial H–$N_{(piperazine)}$ groups of two adjacent CIP molecules, respectively, *via* synthon 1 (N(3)–H(3B)···O(4); 1.83 Å, 180º and O(5) ··· H(3A)–N(3); 1.81 Å, 159º). The hydrogen bonding between CIP and DIF generates a ($R^4_4(12)$) ring motif (Fig. 1). These two successive centrosymmetric tetrameric motifs are connected by weak C–H···F interactions (C(10)–H(10)···F(3); 2.44 Å, 160º) (Fig. 1a).[27]

The salt hydrate CIP/INDP/H_2O crystallizes in triclinic *P*-1 space group with one molecule of each component in the asymmetric unit (Fig. 2). The piperazine ring of CIP exists in chair conformation and N(3) is protonated. The carboxylic acid group of CIP forms an intramolecular hydrogen bond with carbonyl group (O(2)–H(2)···O(3); 1.74 Å, 155º) by accepting proton from the carboxyl group of INDP. The carboxylate group of INDP interacts with both axial and equatorial H–$N_{(piperazine)}$ groups of two adjacent CIP molecules respectively, *via* synthon 1 (N(3)–H(3B)···O(4); 1.91 Å, 148º and N(3)–H(3A)···O(5); 1.79 Å, 170º) to form a centrosymmetric tetrameric ring motif ($R^4_4(12)$). Notably the synthon 1 which involves the protonated piperazine rings is very similar to that seen in CIP/DIF, demonstrating its transferability (Fig. 2a).[27] In this structure water molecule acts as a bridge between CIP and INDP *via* O–H···O interactions by synthon 2 (O(7)–H(35)···O(6); 1.96(4) Å, 172(5)º and O(7)–H(35A)···O(1); 1.98(5) Å, 171(4)º). Interestingly, the water molecule here does the job of C–H···F interactions in CIP/DIF to link the tetramers. This leads to the formation of ribbons running parallel to each other along [111] axis (Fig. 2b).

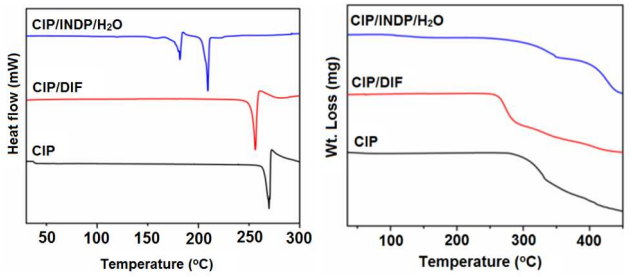


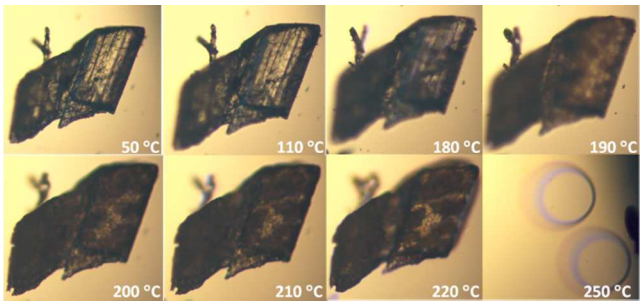
(a) (b)

Figure 2. Crystal packing in CIF/INDP/H_2O. (a) Centrosymmetric tetrameric synthon 1 formed by head-to-head interactions among two CIP and two INDP molecules. Notice the chair conformation adopted at the synthon 1, and the linkage of tetramers by water molecule. (b) Crystal packing to show the arrangement of adjacent ribbons.

The new forms were also characterized by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) (Fig. 3a and 3b). In the DSC experiment, the endothermic peak for melting of CIP/DIF was found at 255.9 ºC. In case of CIP/INDP/H_2O the first endothermic peak in DSC was found at 182 ºC, followed by a second large endotherm at 209 ºC. TGA experiment for CIP/INDP/H_2O showed the first weight loss (2.92%) from about 100 ºC, which matches well with the expected weight loss (2.85 %) for one water molecule in the lattice, and the second weight loss from 230 ºC most likely corresponding to the sublimation of INDP (mp = 208-210 ºC) from the melt. Further evaluation of this salt hydrate by hot stage microscopy (HSM) revealed that the integrity of the crystal is not lost upon solvent evaporation from about 100 ºC, but a phase

transition at 182 ºC (see the morphology changes at 180-190 ºC, in Fig. 3c) is apparent, following which the melting occurs upon further heating. This suggests that the loss of water from the crystal probably forms an anhydrate form (salt/co-crystal), which upon further heating, undergoes a phase transition before melting, which we plan to investigate further in future by variable temperature PXRD.





(c)

Figure 3. Thermal properties of ciprofloxacin salts. (a) DSC and (b) TGA plots of the two salts, CIP/DIF (red lines) and CIP/INDP/H_2O (blue lines). (c) Host stage microscopy images of the salt hydrate, CIP/INDP/H_2O.

A search in CSD (version 5.35) for structures containing both piperazine ring and carboxyl group found 238 hits, out which majority (233) were salts with the protonated piperazine ring (by acid group), while only 5 structures were neutral. This is not surprising as the piperazine group is highly basic (pKa of unsubstituted piperazine is 9.8) and the protonation is expected by acid groups (due to the large ΔpKa). The cyclic synthon 1 was found in 19 of the salts. This finding demonstrates that synthon 1 is not rare and shows some degree of transferability, especially when at least one secondary $N_{(piperazine)}$ group is available.[28] Hence the salt formation using the general ΔpKa rule can be exploited for the crystal engineering of large library of piperazine based drugs (such as trimetazidine, amoxapine, 6-nitroquipazine etc.). For example, by carefully choosing acid co-formers with the ΔpKa > 3 the formation of salts maybe promoted in piperazine drugs, where synthon 1 may play a role.

In conclusion, the two new drug-drug salt forms, CIP/DIF and CIP/INDP/H_2O are characterized by single-crystal X-ray diffraction, DSC and TGA. The DSC and host stage microscopy experiments on the salt hydrate indicate to a phase transition before melting. Notably, in the screening process, the liquid assisted grinding and fast evaporation methods have successfully identified the two new forms, whereas the neat grinding failed. This study demonstrates the effectiveness of the FE method for screening salt forms. The ability of piperazine based drugs to form salts as well as the transferability of synthon 1 is established by analyzing the present and CSD structures, which is important in the context of crystal engineering to generate new salt forms of the large group of piperazine based drugs. As the drug-drug forms of CIP are important in the context of drug development, we further plan to study their properties, such as solubility and stability under different relative humidity conditions, in future.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental method, geometrical parameters of molecules from crystal structures, powder X-ray diffraction patterns, infrared spectra, ORTEP diagrams. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b000000x/

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