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Norfloxacin salts with benzenedicarboxylic acids: charge-assisted hydrogen-bonding recognition and solubility regulation†

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The crystallization of norfloxacin, an antibacterial fluoroquinolone compound, with different benzenedicarboxylic acids yields five novel pharmaceutical salts *via* molecular recognition. X-ray single-crystal diffraction analyses reveal that these pharmaceutical agents present uniform charge-assistant hydrogen-bonding networks, which are in 1 : 1 stoichiometry of norfloxacin and dicarboxylate therein (with the exception of that with terephthalate). Unsubstituted benzenedicarboxylates (phthalate in **1**, isophthalate in **2**, and terephthalate in **3**, respectively) are likely to crystallize with norfloxacin without lattice solvents, while the substituted isophthalate moieties (2-aminoisophthalate in **4** and 5-aminoisophthalate in **5**) tend to form supramolecular adducts with the inclusion of water. Aromatic stacking interactions are present in all these structures, occurring between the fluoroquinolone and carboxylate phenyl rings (in **1–4**) as well as between the fluoroquinolone planes (in **5**). Thermal stability and solubility of all crystalline binary adducts have been determined. The effect of carboxylate counterions, substituents, and hydration states on the solubility and dissolution profile of drug salts **1–5** are investigated in pure water and 0.1 M HCl. After the formation of salts, the solubility increases at near neutral pH in almost all cases (except **3**), but the order is changed in acidic medium. Phthalic acid shows evidence of a good candidate to enhance the solubility of fluoroquinolone drugs for the solubility of **1** is approximately 39 times as large as that of norfloxacin in pure water and also larger than that of norfloxacin in acidic system. For the isophthalates series, **2** has very poor solubility in 0.1 M HCl (only 0.06 times as that of Nf), while amino substituted isophthalates result in the enhancement of the solubility. Remarkably, crystal packing analyses of these pharmaceutical salts allow a possible correlation between the H-bonding prototypes and the solubility to be established.

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Introduction

In view of the importance of hydrogen bonds in crystal engineering, tremendous efforts have been devoted to the understanding of hydrogen-bonded architectures and the exploitation of them in material science, molecular recognition and biological entities as well as the pharmaceutical industry.[1–4] During the last two decades, an increasing interest has focused on the crystalline phases of active pharmaceutical ingredients (APIs),[5] including polymorphs,[6] hydrates[7], solvates,[8] salts,[9] and cocrystals.[10] These solid-state forms of APIs are defined as structurally homogeneous crystalline materials that are constructed from at least one active pharmaceutical ingredient and other solvents or solid components (coformers) with a well-defined stoichiometry. Notably, APIs and coformers are sustained by noncovalent interactions such as hydrogen bond, halogen bond, and π···π stacking[6–10] instead of by covalent forces.

Meantime, the ''supramolecular synthons''[11] strategy has become a promising tool to design new solid forms and generate novel structures of APIs. A supramolecular synthon is ''... a structural unit within supermolecules, which can be formed and/or assembled by known or conceivable synthetic operations involving intermolecular interactions''.[11] Significant numbers of documents have indicated that the

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† Electronic supplementary information (ESI) available: PXRD patterns, IR spectra, DSC curves, H-bonding metrics, molar extinction coefficient and crystallographic data in CIF format for **1–5** are included. CCDC 926036–926040. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3ce40567b

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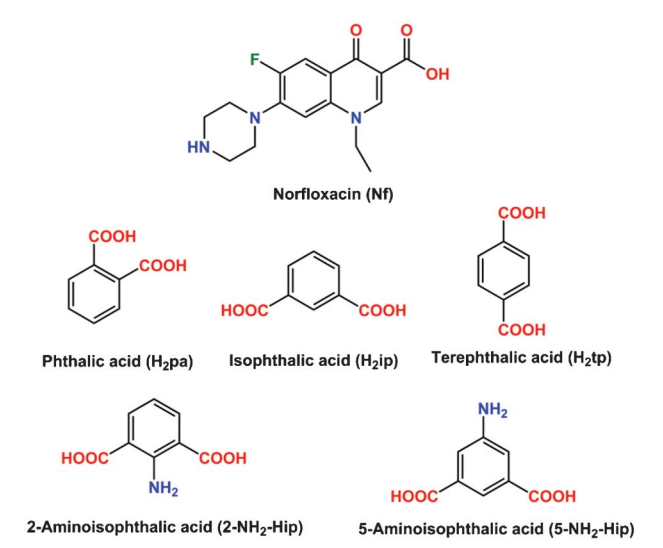
physical and chemical properties of the APIs can be altered by the formation of suitable salts.[12] Enhancing the poor solubility of active drugs is primarily important for optimal delivery when the APIs serve in bioavailability. One effective approach to improve the dissolution and solubility of an API candidate is to prepare its cocrystal or salt forms driven by neutral or charge-assisted hydrogen-bonding interactions.[12–14]

As a synthetic broad antibacterial compound, norfloxacin (Nf, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, Scheme 1), is a poorly soluble zwitterionic fluoroquinolone marketed as Noroxin® by Merck, which has been widely used in clinical practice for the treatment of various infections. The reduced dissolution behavior in water (0.28–0.40 mg mL^{-1})[15] of norfloxacin counters significant challenges in the formulation of conventional dosage forms, *e.g.* tablets, and obstructs the design of liquid dosage forms, such as parenteral and ophthalmic solutions. Therefore, a number of efforts have been devoted to the improvement of the aqueous solubility of norfloxacin, mainly through the preparation of different solid pharmaceutical forms of norfloxacin such as polymorphs, salts, cocrystals and solvates for the dosage forms.[16–18] For example, norfloxacin is known to exhibit polymorphism in many hydrates of changeable stoichiometry water molecules[19] and a varied degree of bioavailability including its anhydrate and hydrates.[19b] Notably, norfloxacin has a fluorine atom and piperazine ring, which is likely to generate complex adducts in salt-form with acidic moieties. In recent cases, proton transferring from carboxylic acid to piperazine base was confirmed by the crystal structure of such fluoroquinolone drugs.[17] Among all the binary charge-transferred crystalline products of norfloxacin, improvement of the solubility based on benzoic acid is the highest with about 40-fold improvement in pure water and phosphate buffer media. It could be anticipated that norfloxacin drugs with aromatic acids may have a considerable improvement in solubility and it is also reasonable to speculate that the higher solubility of the salts could translate into higher bioavailability.[20]



Scheme 1 Structures of norfloxacin and benzenedicarboxylic acids.

Taking all these points into account, we would like to investigate the supramolecular assemblies of molecular adducts with norfloxacin and various benzenedicarboxylic acids. In this contribution, we demonstrate the syntheses, characteristics, and solubility of pharmaceutical salts assembled by zwitterionic hydrogen-bonding interactions between norfloxacin and diverse aromatic dicarboxylic acids (see Scheme 1), namely phthalic acid (H_2pa), isophthalic acid (H_2ip), terephthalic acid (H_2tp),[21] 5-aminoisophthalic acid (5-NH_2-H_2ip), and 2-aminoisophthalic acid (2-NH_2-H_2ip). In all the resulting compounds, ion-pair N^+–H···O^- hydrogen bonds are observed with N^+···O^- type H-bond donor and acceptor groups, which can be considered to be salt bridges[22] and are stronger than those involving neutral acceptors and donors. These binary adducts exhibit different types of H-bonding patterns with diversiform structural features and stabilities, influenced by the geometric effect or the H-bonding competition of involved water solvents (in **4** and **5**).

Experimental section

General materials and methods

All the chemicals and solvents were commercially available and used as received. Crystalline form salts based on both norfloxacin and various aromatic dicarboxylic acids were generally obtained by slow evaporation of water–CH_3OH or water–DMF (in the case of **3**) solution of the reactants in a 1 : 1 ratio. Fourier transform (FT) infrared data were collected on an AVATAR-370 (Nicolet) spectrometer by transmission through the sample deposited on a KBr pellet. Elemental analyses were performed on a CE-440 (Leemanlabs) analyzer. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) experiments were performed on a TG/DTA 6300 thermoanalyzer from room temperature to 800 °C under nitrogen atmosphere at a heating rate of 10 °C min^{-1}. Powder X-ray diffraction (PXRD) of bulk samples were recorded on a SMART Bruker D8 Advanced powder X-ray diffractometer using Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 30 mA. Each sample was scanned in the 2θ range 5–50° with a step size of 0.02°.

The following procedure for the preparation of [HNf]·[Hpa] (**1**) is typical.

Synthesis of [HNf]·[Hpa] (1)

Phthalic acid (16.6 mg, 0.1 mmol) was dissolved in a methanol solution (6 mL), to which an aqua solution (3 mL) of norfloxacin (Nf) (31.9 mg, 0.1 mmol) was added with stirring. The mixture was then filtered and left to slow evaporation. Colorless block single crystals suitable for X-ray analysis were afforded after several weeks. Yield: 44% (21.3 mg, based on Nf). Anal. calcd for $C_{25}H_{24}FN_2O_7$: C, 62.11; H, 5.00; N, 5.79%. Found: C, 62.44; H, 5.10; N, 5.61%. FT-IR (cm^{-1}): 3438b, 2728w, 2642w, 2490b, 1728vs, 1627s, 1476s, 1377s, 1268vs, 1032s, 934m, 787m, 737s, 621m, 572s, 444m, 410s.

[HNf]·[Hip] (2)

Yield: 90% (43.5 mg, based on Nf). Anal. calcd for $C_{25}H_{24}FN_2O_7$: C, 62.11; H, 5.00; N, 5.79%. Found: C, 62.40; H, 5.11; N, 5.71%. FT-IR (cm^{-1}): 3425b, 2997s, 2840s, 2665w, 2553b, 1693vs, 1631w, 1479s, 1273vs, 1220m, 1145w, 937s, 837w, 746s, 730m, 690s, 574s, 528w, 410s.

[HNf]·[Htp]·[H_2tp]$_{0.5}$ (3)

The synthetic media are water–DMF (3 mL : 3 mL) system. Yield: 88% (33.4 mg, based on H_2tp). Anal. calcd for $C_{28}H_{27}FN_3O_9$: C, 59.15; H, 4.79; N, 7.39%. Found: C, 59.12; H, 4.67; N, 7.43%. FT-IR (cm^{-1}): 3404b, 3065w, 2855w, 2750w, 2593w, 2453b, 1710vs, 1631s, 1584w, 1482s, 1362m, 1325w, 1273vs, 1223s, 932m, 766s, 739s, 508m, 416w.

[HNf]·[2-NH_2-Hip]·H_2O (4)

Yield: 85% (43.9 mg, based on Nf). Anal. calcd for $C_{25}H_{27}FN_3O_8$: C, 58.14; H, 5.27; N, 8.14%. Found: C, 58.12; H, 5.33; N, 8.17%. FT-IR (cm^{-1}): 3435b, 2995w, 2857w, 2770w, 2505b, 1918w, 1699s, 1631vs, 1548s, 1479vs, 1380s, 1270vs, 1205m, 1109w, 1037w, 928w, 893w, 809m, 773m, 755m, 681m, 569w.

[HNf]$_2$·[5-NH_2-Hip]$_2$·7H_2O (5)

Yield: 70% (39.3 mg, based on Nf). Anal. calcd for $C_{50}H_{64}F_2N_6O_{21}$: C, 53.47; H, 5.74; N, 7.48%. Found: C, 53.44; H, 5.70; N, 7.43%. FT-IR (cm^{-1}): 3434vs, 3308s, 3144w, 3046w, 2968w, 2834w, 2458b, 1912w, 1687vs, 1626s, 1433s, 1366m, 1300w, 1268s, 1192s, 1137s, 1039m, 930w, 873m, 753s, 685s, 599m, 492s, 404m.

X-Ray crystallography

X-Ray single-crystal diffraction data for **1–5** were collected on a Bruker Apex II CCD diffractometer at room temperature by using a fine-focus molybdenum Kα tube (λ = 0.71073 Å). There was no evidence of crystal decay during data collection. A semiempirical absorption correction was applied (SADABS), and the program SAINT was used for integration of the diffraction profiles.[23] All structures were solved by direct methods with SHELXS and refined by full-matrix least-squares on F^2 with SHELXL program of the SHELXTL package.[24] Notably, in the structure of **5**, the disorder lattice water O atoms (O10, O11, and O12) are assigned to partial site-occupancy of 0.50, and the amount of water molecules (3.5 H_2O in the asymmetric unit) is also consistent with the results of TG and elemental analyses. All H atoms were first found in difference electron density maps, and then placed in the calculated sites and included in the final refinement with fixed thermal factors. Crystallographic data and structural refinement parameters are summarized in Table 1. Hydrogen-bonding geometries are listed in Tables S1 and S2, ESI.†

Solubility studies

The solubility studies were performed by a UV-1201 spectrophotometer. The bulk phase of each salt was washed by water and methanol 3 times before the solubility and dissolution experiment and each sample matches well with its single crystal form as revealed by the X-ray powder diffraction (PXRD) pattern (Fig. S1, ESI†). The concentrations of norfloxacin for **1–5** were calculated by means of a standard graph, which was made by measuring the absorbance of varied concentrations of norfloxacin at their respective λ_{max}. These absorbance values were plotted against the concentration to determine the calibration curve. From the slope of the calibration curves, the molar extinction coefficient (Table S3, ESI†) for each salt/drug was calculated in different media. An excess amount of all the samples was added to the solvent of interest (pH 1.2, 0.1 mol L^{-1} HCl and pH 6.4, distilled water). The solutions were stirred at 300 rpm using a magnetic stirrer at 25 °C (±1). After 72h, the suspensions were filtered through filter paper. The

Table 1 Crystal data and structure refinement parameters for compounds **1–5**

	1	2	3	4	5
Formula	$C_{24}H_{24}FN_3O_7$	$C_{24}H_{24}FN_3O_7$	$C_{28}H_{27}FN_3O_9$	$C_{24}H_{27}FN_4O_8$	$C_{48}H_{64}F_2N_8O_{21}$
Formula weight	485.46	485.46	568.53	518.50	1127.07
Crystal system	Triclinic	Orthorhombic	Triclinic	Monoclinic	Monoclinic
Space group	$P\bar{1}$	*Pbca*	$P\bar{1}$	$P2_1/n$	$P2_1/c$
a/Å	8.998(12)	13.9940(17)	9.8768(19)	9.5670(8)	9.964(3)
b/Å	9.624(13)	13.6288(18)	10.2489(19)	8.1152(6)	14.760(4)
c/Å	13.044(18)	22.537(3)	13.674(3)	30.906(3)	18.275(5)
α/°	94.09(3)	90	89.319(4)	90	90
β/°	106.38(3)	90	74.752(4)	92.476(2)	90.283(5)
γ/°	91.93(3)	90	71.679(4)	90	90
V/Å^3	1079(3)	4298.3(9)	1263.9(4)	2397.3(3)	2687.6(13)
Z	2	8	2	4	2
ρ_{calcd}/g cm^{-3}	1.494	1.500	1.494	1.437	1.393
F(000)	508	2032	594	1088	1188
μ/cm^{-1}	0.117	0.117	0.117	0.114	0.114
Measured reflections	6088	26 842	7289	13 418	14 525
Independent reflections	3768	4914	4654	4437	4888
R_{int}	0.0264	0.1042	0.0214	0.0250	0.0528
R^a	0.0549	0.0592	0.0585	0.0472	0.0797
$R_w{}^b$	0.1769	0.1332	0.1782	0.1507	0.2345
GOF	1.087	0.995	1.079	1.102	1.039

^{a} $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. ^{b} $R_w = [\Sigma[w(F_o{}^2 - F_c{}^2)^2]/\Sigma w(F_o{}^2)^2]^{1/2}$.

filtered solutions were diluted sufficiently and the absorbance was measured at their respective λ_{max} (276 nm for **1–3** and 274 nm for **4–5**). Finally, the concentrations of the norfloxacin salts after 72h were calculated using their respective calibration curves.

For the powder dissolution experiments, the solids of norfloxacin and **1–5** were milled to powder and sieved using standard mesh sieves to provide samples with approximate particle size ranges of 75–150 μm. Excess amounts (500 mg) of the samples were suspended in 10 mL of water or 0.1 mol L^{-1} HCl in flasks. These flasks were maintained at 25 °C (±1) and were stirred at 300 rpm using a magnetic stirrer. At each time, the solution was withdrawn from the flask and filtered through a 0.2 μm nylon filter. A 0.1 mL portion of the filtered aliquot was diluted to 10 mL with different media and was measured with UV-vis spectrophotometry.

Results and discussion

The crystallization of all the binary organic salts can be achieved in the water and methanol solvents, with the exception of compound **3** where water–DMF solvent is propitious to the formation of suitable single crystals for X-ray diffraction. All the crystalline materials were prepared by using 1 : 1 norfloxacin : acid molar ratio for the reaction, while the final charge-assisted hydrogen-bonding products were obtained as 1 : 1 or 1 : 1.5 (**3**) norfloxacin : acid binary compounds. The purities of these were confirmed by PXRD analyses, in which the experimental data are consistent with the single-crystal-simulated spectra (Fig. S1 in the ESI†). Generally, the final molecular molar ratio is consistent with the amido : carboxylate ratio and independent of the original agent ratio. Unexpectedly, the 1 : 1 ratio of hydrogen-bonding donor and acceptor was destroyed in **3** for the $[HNf]\cdot[Htp]\cdot[Htp]_{0.5}$ system, despite no metal salt being been added in the synthetic procedure comparing with the formerly published experiment.[21] The intense infrared (IR) peaks at about 1700 and 1270 cm^{-1} indicate that the COOH group of norfloxacin is unionized in all these cases (Fig. S4, ESI†). The two characteristic peaks at 1584 and 1339 cm^{-1} of carboxylic acid are absent in all salts with the exception of **3**, which has a carboxylic acid moiety. Moreover, the broad peaks at 2450–2553 cm^{-1} in **1–5** should be ascribed to the protonated piperazine nitrogen (NH_2^+) *via* proton transfer from carboxylic acid, which is absent in norfloxacin anhydrate.[16]

Molecular and supramolecular structural description of [HNf]·[Hpa] (1)

In the molecular structure of **1** (Fig. 1a), the asymmetric unit contains one protonated HNf^+ cation and one phthalate anion, resulting from the proton transformation of phthalic acid to the piperazine ring of norfloxacin. Within each HNf^+ cation, the carboxylic acid group is coplanar with the quinolone moiety (torsion angle O2–C15–C13–C14 0.17(4)°) and participates in intramolecular hydrogen bonding (O2–H2···O3, known as S(6) graphic set,[25] synthon I, Scheme 2) with the carbonyl oxygen atom of the quinolone moiety. Multiple weak

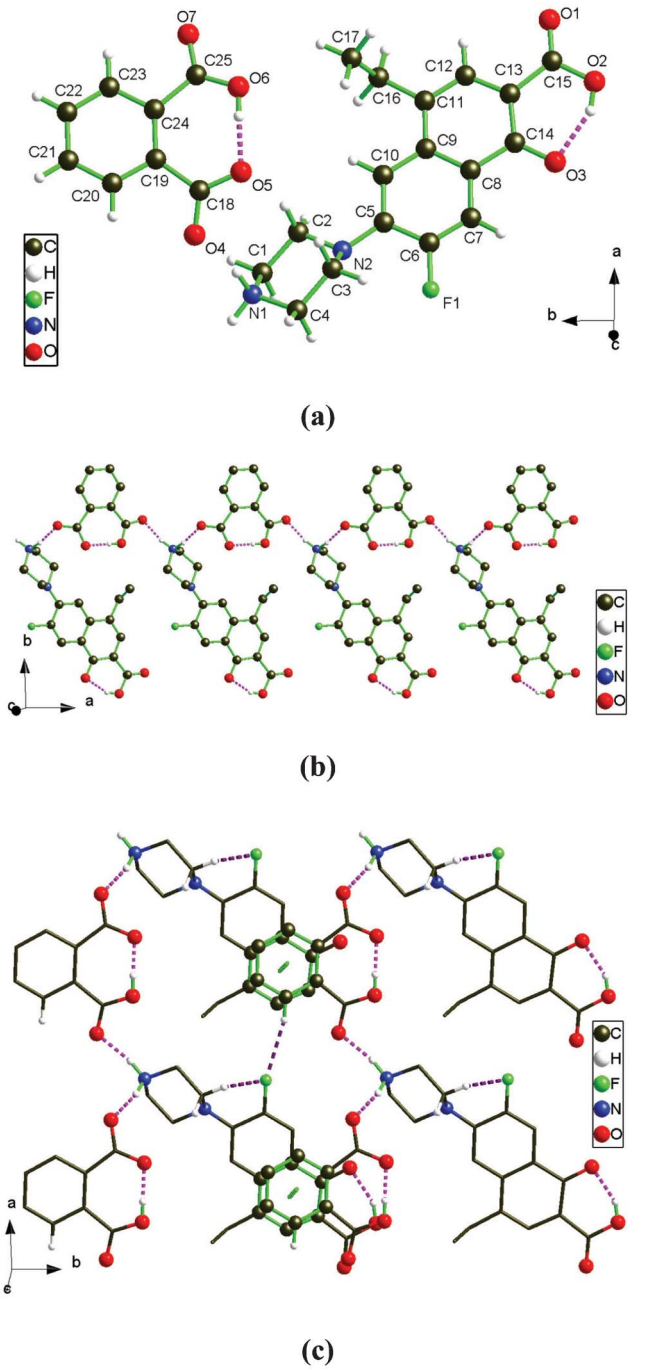
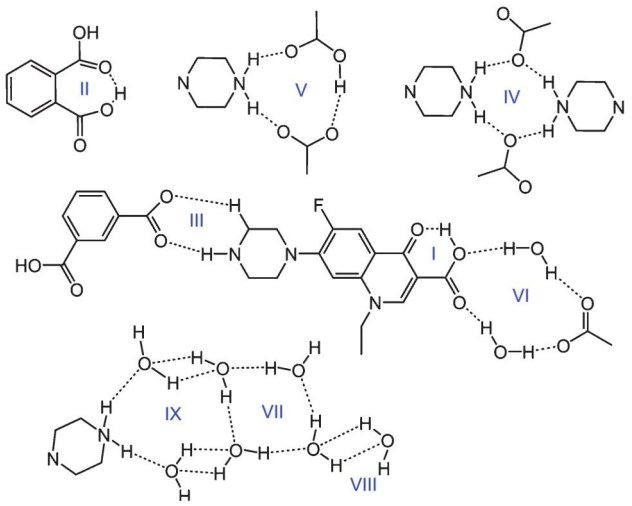


Fig. 1 (a) Molecular structure of **1** with atom labeling of the asymmetric unit. (b) A chain structure of **1** in which norfloxacin molecules are connected by N–H···O hydrogen bonds. The intramolecular O–H···O hydrogen bonds are also illustrated. (c) A 3D supramolecular network constructed by π–π stacking interactions (highlighted in green dashes) between the acid and the piperazine components.

intramolecular interactions based on fluorine atoms are found within the cationic norfloxacin. Meanwhile, the intramolecular S(7) hydrogen bond motif (O6–H6···O5, synthon II, Scheme 2), which is a common occurrence in the 1,2-disubstituted dicarboxylic acids form,[26] is found in the phthalate moiety.



Scheme 2 Possible hydrogen-bonding synthons of norfloxacin cation with coformers.

Each phthalate forms intermolecular interactions with two adjacent norfloxacin cations through the connection between carboxylate oxygen atoms and protonated piperazine nitrogen atoms. A herring skeleton-like supramolecular motif is afforded to run along the crystallographic [100] direction, as shown in Fig. 1b. Meanwhile, the piperazine ring takes a chair conformation with the exposed protonated nitrogen, facilitating the formation of H-bonding with the carboxylate group. These 1D motifs arrange in a parallel fashion along the *b* axis with π–π stacking interactions (the center to center distance of 3.64 Å) between the adjacent fluoroquinolone and phthalate benzene rings, which fulfill the 2D supramolecular network. Examination of this structure with PLATON[27] shows that there is no solvent-accessible void in the crystal lattice.

Molecular and supramolecular structural description of [HNf]·[Hip] (2)

The molecular structure of **2** is composed by one proton transferred HNf cation and one anionic isophthalate (Hip) component (Fig. 2a). Similar intramolecular O2–H2···O1 hydrogen bonds are found to connect the carboxylic acid group with the carbonyl oxygen atom of the quinolone moiety within the HNf^+ cation. Unlike in compound **1**, the weak C1–H1B···O6 interactions provide support for the directional N1–H1D···O7 hydrogen bonding between the two components [synthon III, Scheme 2, $R_2^2(7)$].[25] A pair of inversion-related isophthalate and piperazinyl arrange alternately along the [001] direction to generate a double-chain motif [N1–H1C···$O7^i$/N1–H1D···O7, symmetry code: i = $-x + 1$, $-y + 2$, $-z + 1$, synthon IV $R_4^2(8)$, see Scheme 2], as indicated in Fig. 2b. Interestingly, π–π interactions (the center to center distance of 3.82 Å) between fluoroquinolone and isophthalate rings extend the 1D arrays to form a 2D network parallel to the *bc* plane (Fig. 2c).

Molecular and supramolecular structural description of [HNf]·[Htp]·[H_2tp]$_{0.5}$ (3)

The structure of **3** has been reported previously,[21] but for the consistency of the systematic investigation, we have re-

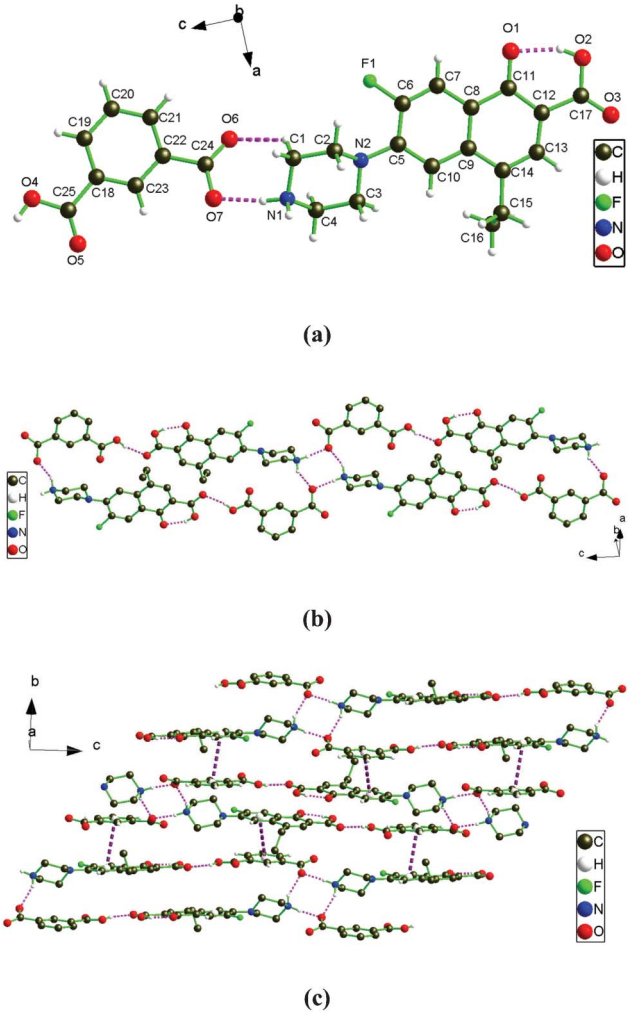


Fig. 2 (a) Molecular structure of **2** with atom labeling of the asymmetric unit, showing the hydrogen-bonding synthon $R_2^2(7)$. (b) A double-chain motif of **2** running along the [001] direction, in which a synthon $R_4^4(8)$ of piperazine amino and carboxylate groups joins two adjacent acid–base alternating chains. (c) A 2D supramolecular network built by π–π stacking interactions between the acid and the piperazine components.

determined this structure. The asymmetric unit contains a cationic HNf molecule, an anionic Htp component and a centrosymmetric H_2tp molecule, as indicated in Fig. 3a. Each piperazine moiety adopts the familiar chair-conformation and builds a charge-assistant N–H···O interaction with the carboxylic moiety from another cationic HNf molecule. In this way, the HNf molecules are head-to-tail joined into a 1D hydrogen-bonding array running along the crystallographic [001] direction. The hydrogen-bonding chains are further penetrating the supramolecular ladder constructed by the linkage of multiple O–H···O interactions between Htp and H_2tp units (Fig. 3b). An additional N–H···O hydrogen bond (between one of the Htp carboxylate O atoms and a piperazine nitrogen atom of HNf) completes the final 3D H-bonding network, as depicted in Fig. 3c. Notably, there are significant π–π interactions (center to center distance: 3.41 Å) between the

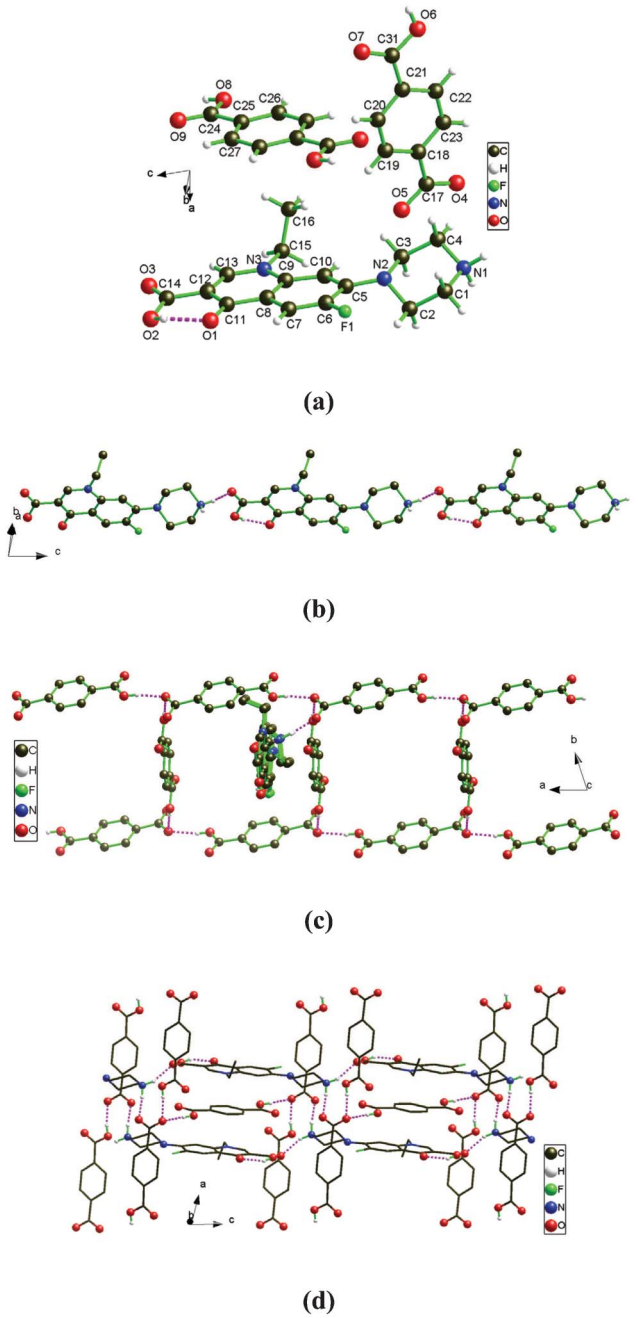


Fig. 3 (a) Molecular structure of **3** with atom labeling of the asymmetric unit. (b) A norfloxacin chain of **3** through the N–H···O interactions running along the [001] direction. (c) A supramolecular ladder of the terephthalate moieties with the penetration of the norfloxacin chain. (d) A portion view of the 3D hydrogen-bonding network in **3**.

fluoroquinolone and terephthalate benzene rings, which further stabilize the resultant 3D network.

Molecular and supramolecular structural description of [HNf]·[2-NH_2-Hip]·H_2O (4)

The structure of **4** has one norfloxacin cation, one mono-deprotonated 2-aminoisophthalate anion, and one H_2O molecule in the asymmetric unit, as indicated in Fig. 4a. One of the two carboxylic acid groups of 2-aminoisophthalic acid transfer one proton to the piperazinyl ring N atom of the norfloxacin molecule, thereby forming a 1D hydrogen-bonding motif that is an acidic chain decorated by norfloxacin cations, in which two hydrogen-bonding patterns are formed as the graphic set S(6) within an acid anion and $R_3^3(10)$ (synthon V, Scheme 2) among two acid and one norfloxacin molecules. The two norfloxacin cations and two lattice water molecules form a cyclic tetramer synthon $R_4^4(12)$ (VI, Scheme 2) and connect the adjacent two inversion-related 1D hydrogen-bonding motifs into a 1D supramolecular ladder along the [100] direction (Fig. 4b). An additional N–H···O interaction joins the neighboring ladder motifs into a complicated 3D hydrogen-bonding arrangement, as depicted in Fig. 4c. Within this 3D network, the quinolone rings are stacked in a parallel fashion as that in **4** with the center-to-center separations of 3.66(2) and 3.75(2) Å, respectively, which will further strengthen the extended 3D host–guest network.

Molecular and supramolecular structural description of [HNf]$_2$·[5-NH_2-Hip]$_2$·7H_2O (5)

The structure of **5** (Fig. 5a) contains a cationic pharmaceutical agent norfloxacin, a mono-deprotonated 5-aminoisophthalate and 3.5 lattice water molecules, including two crystallographically independent O8 and O9 molecules and three water sites (O10, O11 and O12) with half-occupancy. The dihedral angle between the substituent phenyl ring of 5-NH_2-Hip and that of HNf is 76.2(2)°. A hydrogen-bonding motif of herring skeleton is shaped along the [010] direction, linked by the interactions of N1–H1C···O5^{i} and O7–H7A···O5^{iv} (symmetry code: i = x + 1, y, z; iv = $-x$, y − 1/2, $-z$ + 1/2), where a head-to-tail chain of acid moieties and the decorated norfloxacin molecules are involved (Fig. 5b).

Calculation with the PLATON[27] program shows that the solvent-accessible-void in **5** (438.9 Å, 16.3% of the unit cell) is occupied by lattice water moieties. Analysis of the crystal packing indicates a distinct channel residing along the [100] direction. Two fully occupied water sites (O8 and O9) are anchored to the host chains through N–H···O and O–H···O interactions (Table S2, ESI†) accommodated outside the channels (Fig. 5c). While the partially-occupied O10, O11 and O12 are hydrogen bonded to each other to form a water chain running along the straight channel centered at (x, 1/2, 1/2) (see Fig. 5c and 5d). With respect to the channel, a water tetramer [O10–H10A···O11 and O11–H11A···O10] with the graphic set $R_4^4(8)$ (synthon VII, Scheme 2) and two sets of double hydrogen-bonded bridges (synthon VIII, Scheme 2) [$R_2^2(4)$ between the O11 and O12 water molecules, and between each two O12 water molecules] are found to have a planar configuration because of their crystallographic symmetry. The hydrogen-bonding behavior within the channel spaces must have a considerable effect on the dehydration behavior of **5**, although the H atoms of the water molecules could not be reliably placed. It is reasonable to suggest that the loss of the channel water molecules should be favored in such a channel hydrate form.[28]

An extended 3D network is eventually constructed *via* multiple O–H···O and N–H···O hydrogen bonds involving the lattice water and the amido of 5-NH_2-Hip, which is also

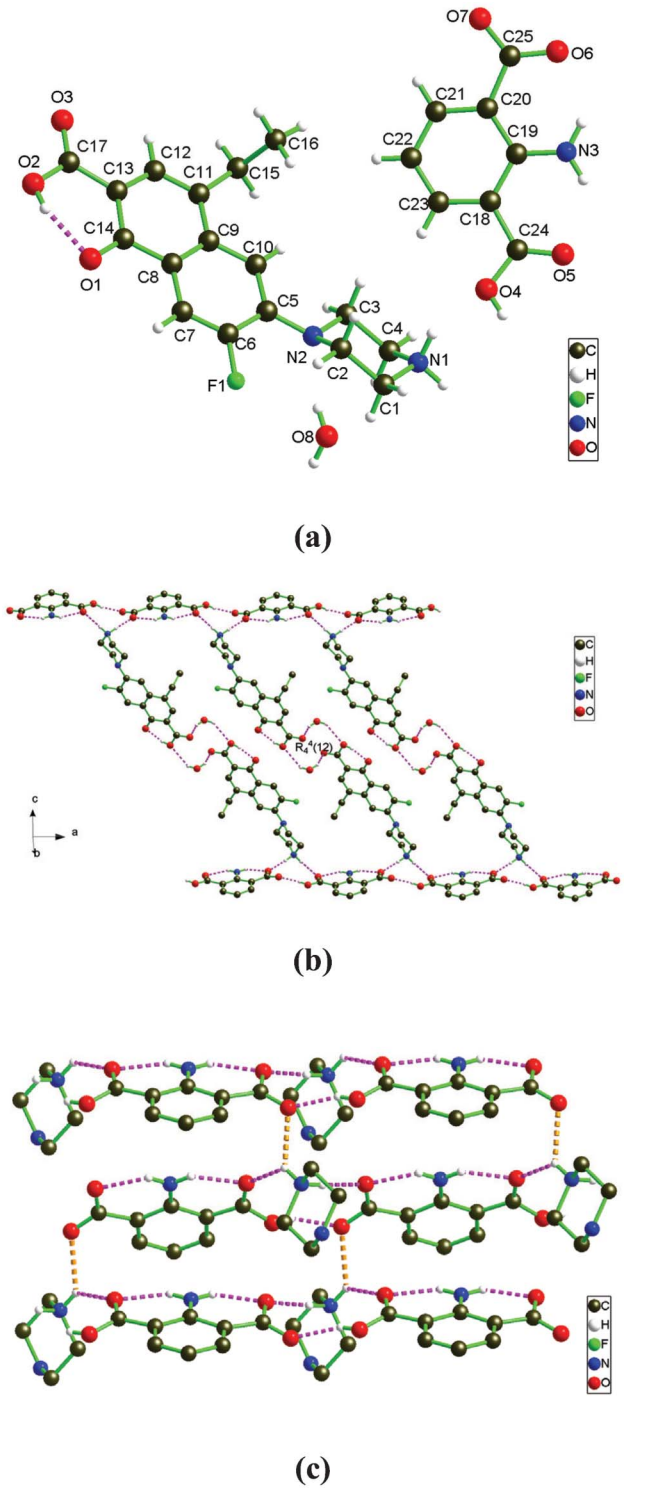


Fig. 4 (a) Molecular structure of **4** with atom labeling of the asymmetric unit. (b) A supramolecular ladder involving guest water molecules running along the [100] direction. (c) A portion view of the H-bonding interactions between acid chains and norfloxacin with only the piperazine rings shown for clarity.

consolidated by the aromatic stacking forces with the center-to-center separations of 3.58(2) and 3.70(2) Å, between both of the fluoroquinolone benzene rings or between one of the

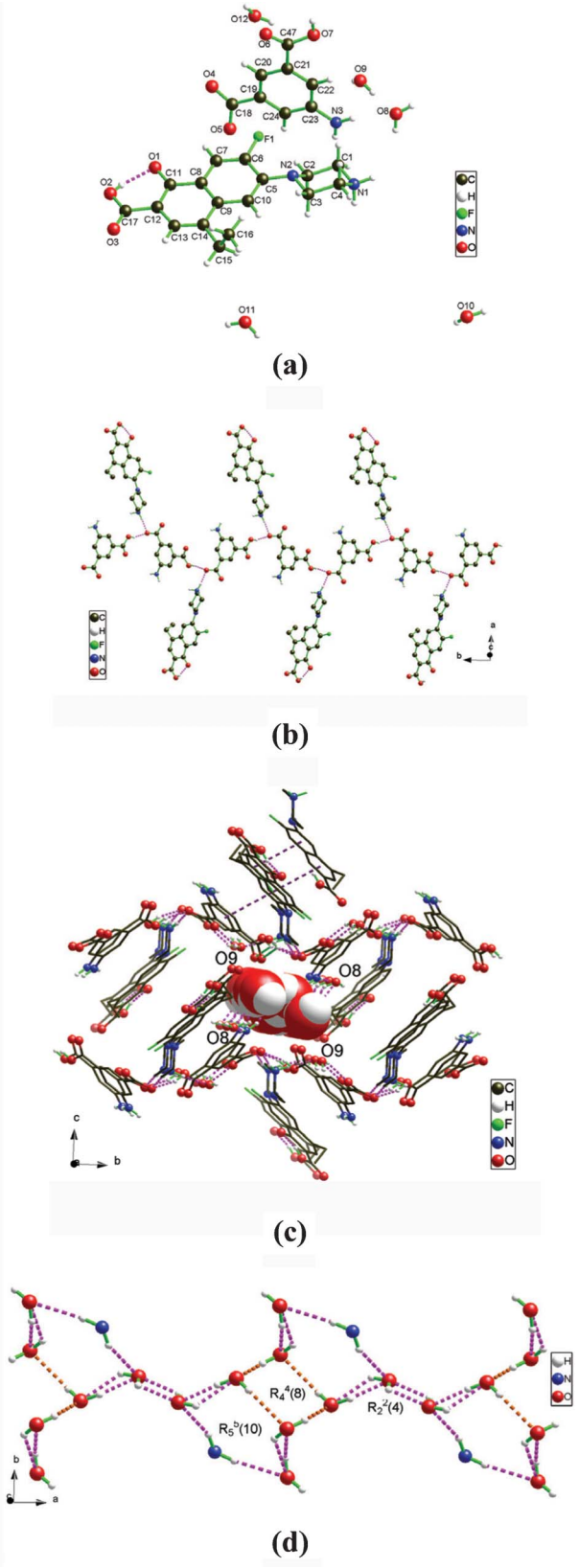


Fig. 5 (a) Molecular structure of **5** with atom labeling of the asymmetric unit. (b) An aminoisophthalate chain running along the [010] direction with decoration of norfloxacin molecules through the N–H···O interactions. (c) A supramolecular host–guest system of **5** with a hydrogen-bonding water chain highlighted in the space-filling mode. (d) A portion view of the hydrogen-bonding array including water moieties in **5**.

fluoroquinolone benzene rings and the phenyl rings of 5-NH_2-Hip molecules (Fig. 5c). Meanwhile, the aqua molecule O10 also forms a similar double hydrogen-bonded bridge (O10–H10B···O8 and O8–H8A···O10) with the O8 water molecule (Fig. 5d). Moreover, this hydrogen-bonded bridge combines with another bridge of the 1D water chain motif to connect the same amino group of the 5-NH_2-Hip moiety (Fig. 5d). In this way, a novel H-bonded pattern notated as the graphic set $R_5^5(10)$ (synthon IX, Scheme 2)[25] is afforded to further consolidate such 1D water motifs within the extended 3-D host network.

Structural comparison of norfloxacin salts

As described above, a similar components ratio has been used for the crystallization of norfloxacin and the five aromatic acids. With the exception of **3** (norfloxacin : acid molar ratio = 1 : 1.5), all these crystalline products were yielded in a 1 : 1 ratio with the salt forms. Several attempts have been carried out to obtain a 1 : 1 ratio salt of norfloxacin terephthalate in the water–DMF or water–methanol system with the norfloxacin : acid molar ratio of 2 : 1, 1 : 1 and 1 : 2, respectively. X-ray single-crystal diffraction of the resultant crystalline products indicates that all the attempts failed, which may partly arise from the structural nature of terephthalic acid for the tendency to crystallize with more than one molecule in the asymmetric unit, with slight conformational dissimilarity.[29] It is generally known as the Rule of Three that the reaction of an acid with a base will be expected to form a salt if ΔpK_a (ΔpK_a = pK_a(base) − pK_a(acid)) is greater than 3, while either a cocrystal or a salt will form when ΔpK_a is between 0 and 3.[30,31] Estimated ΔpK_a values between the piperazine base[32] and carboxylic acids[33] (pK_{a1}) suggested salt formation (Table 2). Also, X-ray single-crystal diffraction gives evidence that all the crystalline compounds are ionized, with proton transfer occurring from the carboxylic acid to the chair-conformational piperazine NH group.

In all cases, the carboxylic acid group of norfloxacin is in the neutral state for C–O and C=O distances differ by *ca.* 0.1 Å and the intramolecular hydrogen bond with the quinolone C=O presents as S(6) graphic set. The C–O distances of the carboxylate moiety in mono-deprotonated dicarboxylate are near-equal (difference < 0.03 Å), which can distinguish the carboxylate salts from the carboxylic acids of cocrystals or solvates. The inclusion of water was found in two substituted isophthalate cases, leading to a cyclic tetramer synthon $R_4^4(12)$ (VI in Scheme 2) in **4** and a supramolecular water chain (Fig. 5d) in **5**. For **1** and **2**, similar hydrogen-bonding chains are observed with the acid and piperazine base arranging alternately. For **3–5**, the lack of acid–base alternating chains may be due to the unexpected components ratio in **3** and the inclusion of water guests in **4** and **5**, respectively. Additionally, π-stacking interactions are found to be the most prominent in all the five novel pharmaceutical salts.

Table 2 pK_a values of the norfloxacin and benzenecarboxylic acids used

Norfloxacin[a]	Carboxylic acid[b]	ΔpK_a[c]
6.30 (COOH)	2.98 (phthalic acid)	5.40
8.38 (piperazine)	3.46 (isophthalic acid)	4.92
	3.51 (terephthalic acid)	4.87
	3.69 (5-aminoisophthalic acid)	4.69
	4.27 (2-aminoisophthalic acid)	4.11

[a] Ref. 32. [b] First ionization constant for diacids. [c] ΔpK_a is calculated as pK_a (piperazine NH^+) − pK_{a1} (carboxylic acid).

Thermogravimetric (TG) and differential scanning calorimetry (DSC) analyses

These binary pharmaceutical salt materials are air stable at ambient conditions and thermogravimetric experiments were implemented to investigate their thermal stabilities. Thermal analysis (Fig. 6 and S5, ESI†) shows that the TGA and DSC curves are consistent with the salt composition. The thermogravimetric (TG) curves of **1–3** reveal a first weight loss of 33.6%, 34.7% and 28.9% in the temperature range from 201–367 °C, 207–367 °C, and 188–300 °C, respectively, which can be ascribed to decomposition of the crystalline samples and elimination of a benzene dicarboxylate molecule. While for **4** and **5**, the first weight losses in the region from 85 to 160 °C and 40 to 120 °C, respectively, correspond to the removal of the guest water molecules (for **4**, calculated: 3.5%; observed: 4.3%; for **5**, calculated: 11.2%; observed: 12.1%). The decompositions of the retained hydrogen-bonding networks start at 245 and 230 °C with different speeds of the weight losses for **4** and **5**, respectively. The data of DSC analyses show the endothermic melting peaks at 230.8, 282.6, 312.9 and 269.1 °C for **1–4**, respectively, which have lower melting points and demonstrate the same trend as those of acidic coformers. The endothermic peak at 64.3 °C for **5** is consistent with the release of lattice water molecules followed by a blurred endothermic melting transition at around 195 °C, while no significant endothermic transition has been found to indicate the loss of guest water for **4**.

Solubility of norfloxacin salts 1–5

The solubility data for norfloxacin salts **1–5** were measured by estimating the drug concentration in pure water (pH 6.4) and 0.1 M HCl (pH 1.2). All salt samples used for the powder dissolution profiles are shown in Fig. 7 and the apparent solubility data are listed in Table 3. The undissolved salt materials analyzed by PXRD after the dissolution experiment show that all the carboxylate salts are stable to the dissolution conditions in pure water (Fig. S2, ESI†). Partial transformation to the norfloxacin dihydrate form may occur in salts **1** and **5** after the dissolution experiment in 0.1 M HCl solution, but salt **2** is stable. However, the PXRD patterns of salts **3** and **4** are different from the original patterns after the dissolution experiment in acidic medium.

The solubility profile in pure water medium shows a clear increase in the solubility of the drug when in the form of most of the carboxylate salts. From Fig. 7a, it can be found that both the dissolution rate and solubility values of the salts (with the exception of **3**) are larger than those of norfloxacin in pure water medium, indicating a clear increase in the solubility of norfloxacin as carboxylate salts. Especially for **1**, the solubility value is approximately 39.04 times as large as that of norfloxacin. The very poor solubility of **3** may be due to the

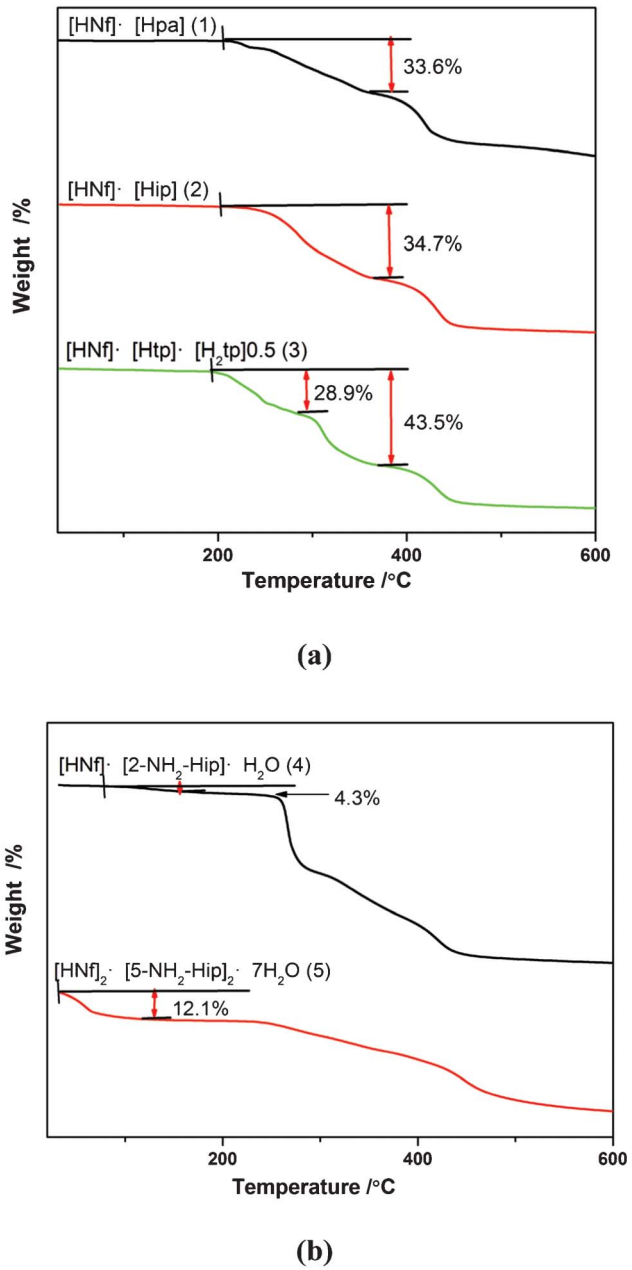


Fig. 6 TG curves for norfloxacin salts **1–3** (a) and **4–5** (b).

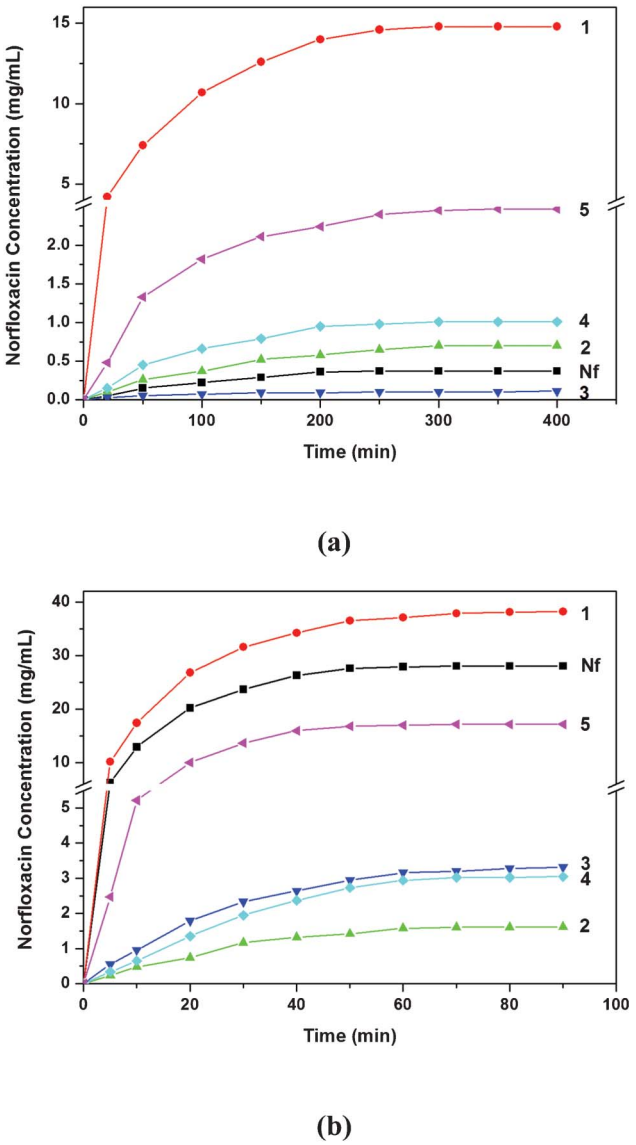


Fig. 7 Powder dissolution profiles of norfloxacin salts in (a) pure water and (b) 0.1 M HCl.

undeprotonated H_2tp moiety in the molecule and the resultant H-bonding assembly. The solubility of **1–5** increases as the pH decreases (from 6.4 to 1.2) but the values for the salts are higher than that for the reference drug at near neutral pH, which is expected because the counterions in the salts are conjugate bases. A further understanding of the crystal packing for all Nf salts indicates a reliable correlation between the hydrogen-bonding networks and the solubility. In comparison with the anhydrous analogues (**2** and **3**), the highest solubility of **1** may be caused by the absence in intermolecular hydrogen-bonding for the carboxylic group of norfloxacin. Correspondingly, the highest solubility of **5** comparing with **2** and **4** could also be an effect of the norfloxacin carboxylic group not being involved in intermolecular hydrogen-bonding. It seems that more structural data are needed in order to find a plausible explanation for these differences. Revisiting the previously published structures and solubility of Nf salts reveals that the carboxylic group of norfloxacin is absent in intermolecular H-bonding interaction in the solid states of norfloxacin maleate hydrate[16] and norfloxacin benzoate monohydrate,[17] both of which show the highest solubility (Table 3) in the respective work.[16,17]

In the acidic medium (0.1 M HCl, pH 1.2) the dissolution rate order changed, following the order **1** > Nf > **5** > **3** > **4** > **2** (Fig. 7b), with the salts showing faster dissolution at this pH value because of the protonated feature. For the normally less soluble norfloxacin, the higher dissolution is due to protonation of the piperazine ring in acidic medium. Meaningfully, most of the salts have generally lower solubility in relation to

Table 3 Solubility (mg mL^{-1}) of norfloxacin and norfloxacin salts measured at 72 h in different pH medium (25 ± 1 °C)

Compound	Pure H_2O	Number that Nf is multiplied by to give the compound solubility value in pure H_2O	0.1 M HCl	Number that Nf is multiplied by to give the compound solubility value in 0.1 M HCl
Nf	0.38	—	28.19	—
1	14.84	39.04	38.28	1.36
2	0.71	1.87	1.65	0.06
3	0.11	0.28	3.32	0.12
4	1.02	2.68	3.06	0.11
5	2.48	6.53	17.17	0.61
Norfloxacin oxalate dihydrate[a]	2.93	—	21.84	—
Norfloxacin tartarate dihydrate[a]	5.50	—	14.53	—
Norfloxacin benzoate monohydrate[a]	12.80	—	29.97	—
Norfloxacin 0.5 (succinate) hydrate[b]	6.60	—	—	—
Norfloxacin malonate dihydrate[b]	3.90	—	—	—
Norfloxacin maleate hydrate[b]	9.80	—	—	—

[a] Ref. 17. [b] Ref. 16.

norfloxacin with the exception of **1**, which has a solubility approximately 1.36 times that of the parent drug in the 0.1 M HCl system. Comparing with the norfloxacin salt of benzoate (lower solubility in the acidic system),[17] **1** demonstrates a higher solubility than that of norfloxacin either in pure water or acidic systems, indicating phthalic acid is a good candidate to enhance the solubility of fluoroquinolone drugs in acidic environment. For the isophthalates series, norfloxacin salts with amino substituted isophthalates have the enhanced solubility, while norfloxacin isophthalate shows very poor solubility in 0.1 M HCl (only 0.06 times that of Nf).

Conclusions and perspectives

A series of norfloxacin salts of benzenedicarboxylates were synthesized to confirm the proton transfer of hydrogen-bonding and control their solubilities. Pharmaceutical agents **1–5** were obtained and their structures were determined by single crystal X-ray diffraction, in which **1** and **2** form similar 1D H-bonding chains with the acid and piperazine base alternating and a further 2D supramolecular network *via* π–π interactions, while for **3–5**, the lack of acid–base alternating chains leads to very complicated 3D H-bonding architectures stabilized by π-stacking. In summary, norfloxacin turns out to be a reliable H-bonded agent capable of forming charge-assisted H-bonded networks by recognizing various benzene-carboxylates. The aqueous solubility of norfloxacin is increased after the formations of all salts except **3**. To establish how the hydrogen-bonding interactions influence the solubility, crystal packing analyses were carried out. These indicate that the absence of intermolecular H-bonding for the carboxylic group of norfloxacin is helpful to improve the solubility of related salts. Consequently, **1** shows considerable solubility either in pure water or acidic medium, indicating the solubility of Nf can be enhanced *via* the formation of salts with suitable carboxylate agents. Although the preliminary investigations show that these norfloxacin salts have a certain security, further study to improve their safety are in progress for the coformers do not belong to the GRAS (generally regarded as safe) compounds. As solubility and bioavailability are often correlated, it is anticipated that the bioavailability of norfloxacin may also be increased after the formation of such salts, and further studies on other fluoroquinolone salts are encouraged.

Acknowledgements

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