Cocrystals and alloys of nitazoxanide: enhanced pharmacokinetics

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Electronic Supplementary Information (ESI) †

Table S1 Crystallographic parameters.

	NTZ-PABA	NTZ-PASA	CA1(0.67:0.33)	CA2 (0.75:0.25)
Emp. form.	$C_{12}H_9N_3O_5S$-$C_7H_7NO_2$	$C_{12}H_9N_3O_5S$-$C_7H_7NO_3$	$C_{12}H_9N_3O_5S$-$C_7H_7NO_{2.33}$	$C_{12}H_9N_3O_5S$-$C_7H_7NO_{2.25}$
Form. wt.	443.42	460.42	449.62	448.89
Cryst. system	Triclinic	Triclinic	triclinic	triclinic
Space group	*P*-1	*P*-1	*P*-1	*P*-1
T (K)	298(2)	298(2)	298(2)	100(2)
a (Å)	7.4607(6)	7.2396(4)	7.3750(8)	7.377(2)
b (Å)	11.3799(12)	11.6008(9)	11.4414(12)	11.371(5)
c (Å)	13.9457(15)	13.8909(12)	13.8757(12)	13.888(5)
α (º)	66.488(10)	109.021(7)	108.729(8)	108.933(13)
β (º)	83.048(8)	97.558(6)	97.485(8)	97.742(13)
γ (º)	71.106(9)	104.385(6)	107.618(10)	108.643(19)
Z	2	2	2	2
V (Å^3)	1027.2(2)	1039.19(15)	1022.72(19)	1006.5(6)
Rflns. collect	3343	6589	7161	11091
Unique rflns.	2551	2950	4006	3622
Obsd. rflns.	1930	3974	1880	1202
Parameters	209	298	289	290
R_1	0.0478	0.0487	0.0593	0.0781
wR_2	0.1310	0.1428	0.1070	0.1857
GOF	1.008	1.025	0.870	0.842
Diffractometer	Oxford Xcalibur Gemini	Oxford Xcalibur Gemini	Oxford Xcalibur Gemini	Bruker D8 Quest

Table S2 Selected geometric parameters characterizing hydrogen bonds in NTZ cocrystals and cocrystal alloys.

D–H···A	D···A (Å)	H···A (Å)	D–H···A (°)	symmetry code
NTZ-PABA				
N1–H1A···O6	2.850(4)	2.00	168	intra
N4–H4A···O3	3.133(4)	2.30	162	x,y,-1+z
N4–H4B···O4	3.075(5)	2.22	169	-1+x,1+y,-1+z
O7–H7A···N2	2.747(4)	1.93	174	intra
C3–H3···O2	3.229(5)	2.59	127	-x,1-y,1-z
C15–H15···O2	3.502(5)	2.60	164	1-x,-y,1-z
NTZ-PASA				
N1–H1A···O6	2.833(3)	2.00	162	1-x,1-y,-z
N4–H4A···O3	3.184(4)	2.33	168	1-x,1-y,1-z
N4–H4B···O5	3.098(4)	2.19	162	1-x,2-y,1-z
O7–H7A···N2	2.722(3)	1.88	169	1-x,1-y,-z
O8–H8A···O6	2.591(6)	1.90	146	intra
C6–H6···O8	3.464(3)	2.57	161	-1+x,-1+y,z
C18–H18···O2	3.421(8)	2.52	164	1+x,y,z
CA1 = NTZ-PABA : NTZ-PASA (0.67:0.33)				
N1–H1A···O6	2.832(3)	1.94	174	1-x,1-y,1-z
N4 –H4B···O3	3.145(4)	2.37	150	1-x,1-y,-z
N4–H5A···O4	3.056(4)	2.44	129	1-x,-y,-z
O7–H7A···N2	2.724(3)	1.91	175	1-x,1-y,1-z
C5–H5···O2	3.258(5)	2.60	129	2-x,2-y,1-z
C18–H18···O2	3.464(5)	2.56	164	-1+x,y,z
CA2 = NTZ-PABA : NTZ-PASA (0.75:0.25)				
N1–H1A···O6	2.805(4)	1.94	169	1-x,1-y,1-z
O7–H017A···N2	2.724(4)	1.89	174	1-x,1-y,1-z
N4–H4A···O4	3.057(7)	2.41	134	1-x,1-y,-z
N4–H4B···O3	3.091(4)	2.33	140	1-x,-y,-z
C5–H5···O2	3.187(7)	2.51	128	1-x,1-y,1-z
C18–H18···O2	3.424(5)	2.49	166	x,1+y,z

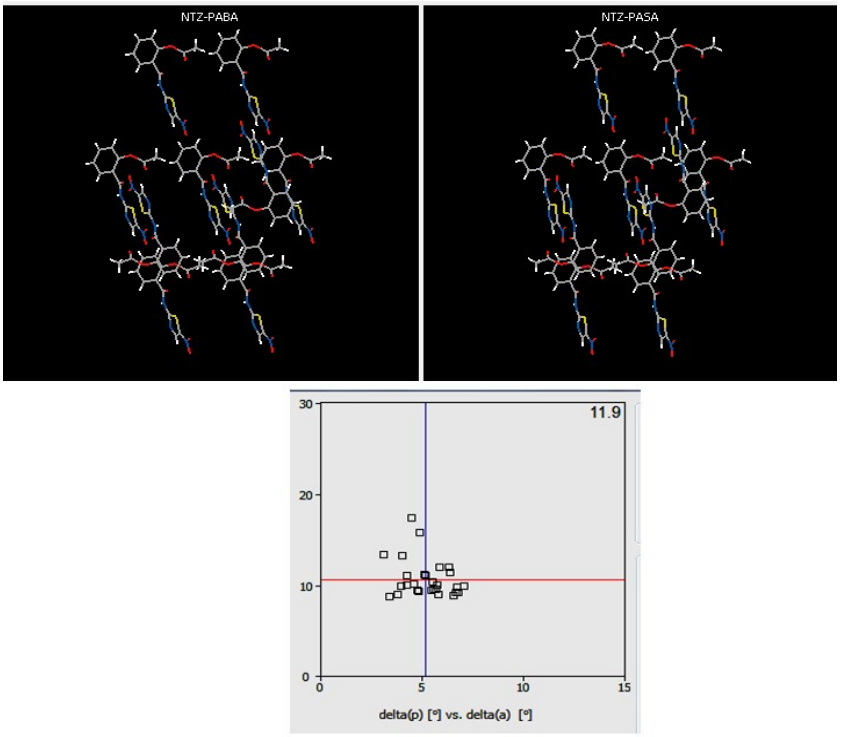


Figure S1 3D supramolecular construct of NTZ-PASA and NTZ-PABA cocrystals and the plot of the interplanar angular deviation vs. angular deviation (u) in XPac with a dissimilarity index of 11. Normally dissimilarity index is lower for single component systems (<5) but higher for multi-component cocrystals, and more so when one of the molecular components is chemically different. Isostructurality wasalso determined by unit cell similarity index (Π) to give a value close to zero (Π = 0.001) indicating the isomorphous nature of cocrystals.

References

(1) T. Gelbrich, T. L. Threlfall and M. B. Hursthouse, *CrystEngComm*, 2012, **14**, 5454-5464

(2) T. Gelbrich and M. B. Hursthouse, *CrystEngComm*, 2006, **8**, 448-46.

(3) T. Gelbrich and M. B. Hursthouse, *CrystEngComm*, 2005, **7**, 324-336.

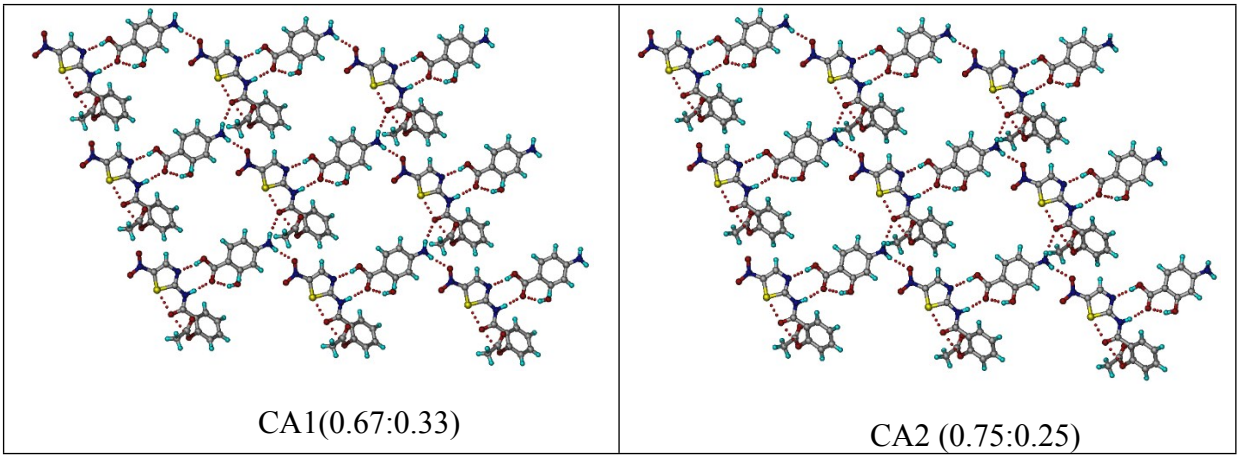


Figure S2 The sheet structure of CA1 and CA2 cocrystal alloys extends through N–H···O and O–H···N hydrogen bonds.

Conformational analysis

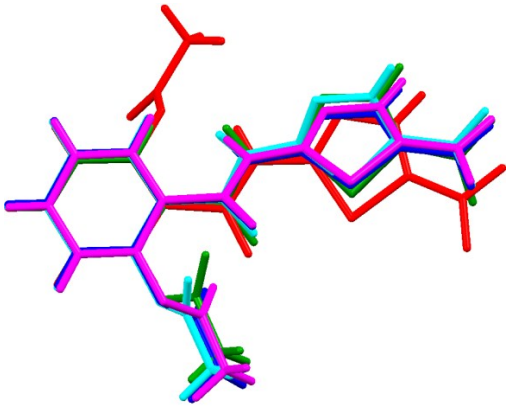


Figure S3 Overlay of NTZ molecules extracted from crystal structures. NTZ: Red, NTZ-PABA: Green, NTZ-PASA: Cyan, CA1: Blue and CA2: Magenta.

The main conformational change that occurs in the cocrystals compared to the pure drug is that the ester group is oriented anti to the amide C=O in NTZ while the phenyl ring rotates about the C–C bond and the ester group is on syn side of the amide C=O in the other four cocrystal/ alloy structures. There are other minor torsion angle changes, but they are more in the way of adjustments to fit the overall crystal packing in each case.

Table S3 Melting point of NTZ cocrystals and alloys.

S. No	Cocrystal/ Cocrystal alloy	Melting Point (°C)	Coformer	Melting Point (°C)
1	NTZ-PABA	158-160	PABA	187-189
2	NTZ-PASA	159-163	PASA	150-151
3	CA1	153-155	--	--
4	CA2	152-156	--	--

Melting point of nitazoxanide 200-202 °C

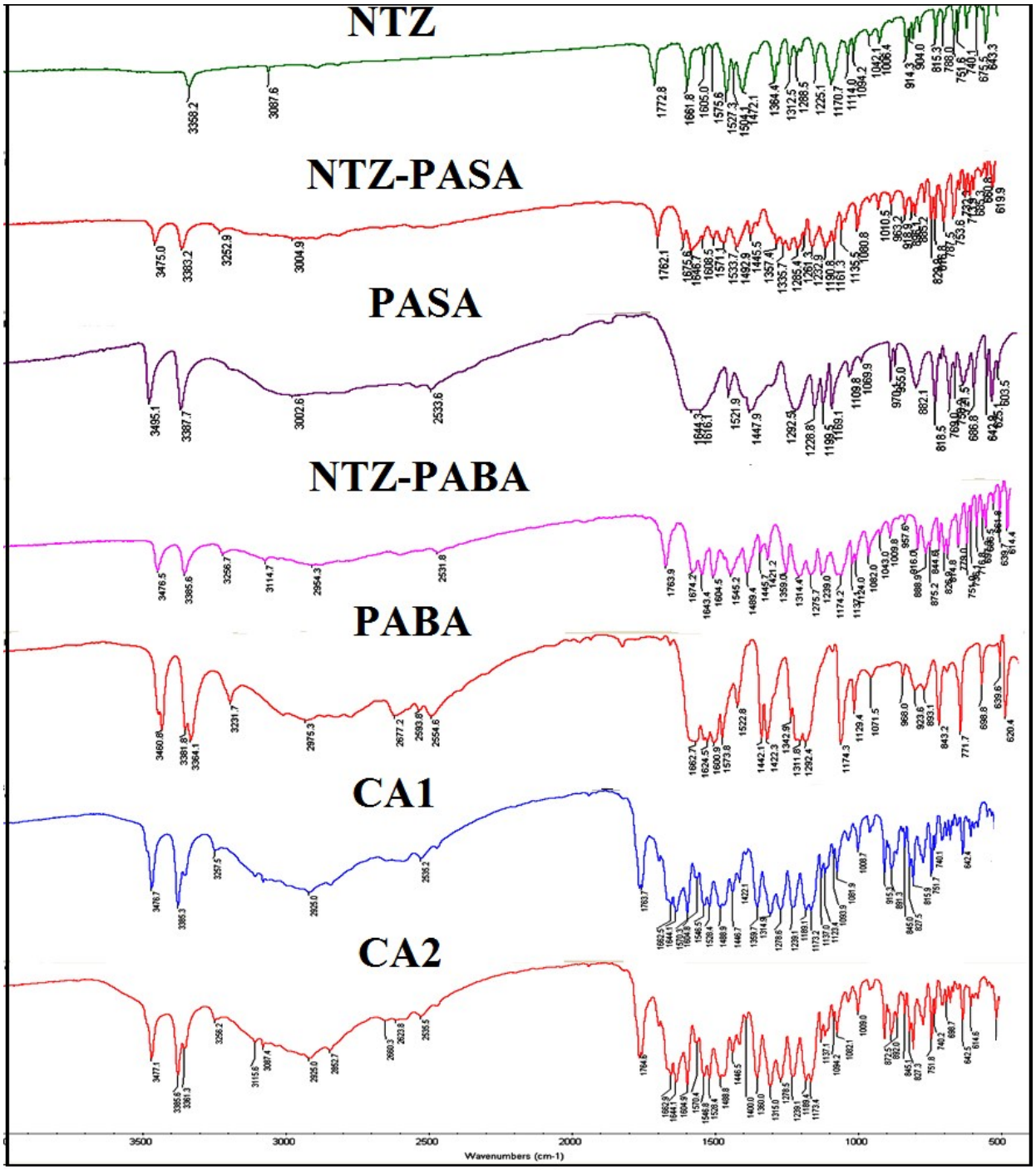


Figure S4 FT-IR spectra of NTZ cocrystal / alloy compared with the starting materials.

Table S4 IR stretching frequencies of CONH / $OCOCH_3$in NTZ and $COOH/NH_2$ functional groups of conformers (see Figure S4 for spectra).

Solid form	N-H/O-H stretch of acid/amide/amine	C=O stretch of amide	C=O stretch of ester	C=O stretch of acid
NTZ	3358	1661	1772	-
NTZ-PASA	3475, 3383,3252	1646	1762	1675
PASA	3495, 3387	-	-	1644
NTZ-PABA	3476, 3385, 3258	1643	1764	1674
PABA	3460, 3381,3363	-	-	1666
CA1	3476, 3385,3257	1644	1763	1662
CA2	3477, 3383,3256	1644	1764	1662

Table S5 Intrinsic dissolution rate of NTZ cocrystals and alloys. The enhancement compared to NTZ is given in parenthesis.

Compound	Solubility of coformer in water (mg/mL)	Molar Extinction coefficient (mM^{-1} cm^{-1})	Intrinsic dissolution rate, IDR (mg/cm^2)/min ($x10^{-3}$)	Amount of NTZ dissolved at the end of dissolution (240 min, %)
NTZ	0.007	27.9	0.073	3.8
NTZ-PASA	1.690	27.2	0.087 (x1.19)	6.2 (x1.63)
NTZ-PABA	6.100	23.9	0.099 (x 1.35)	6.7 (x1.76)
CA1	--	21.7	0.114 (x1.56)	8.7 (x2.28)
CA2	--	21.5	0.125 (x1.71)	9.9 (x2.60)

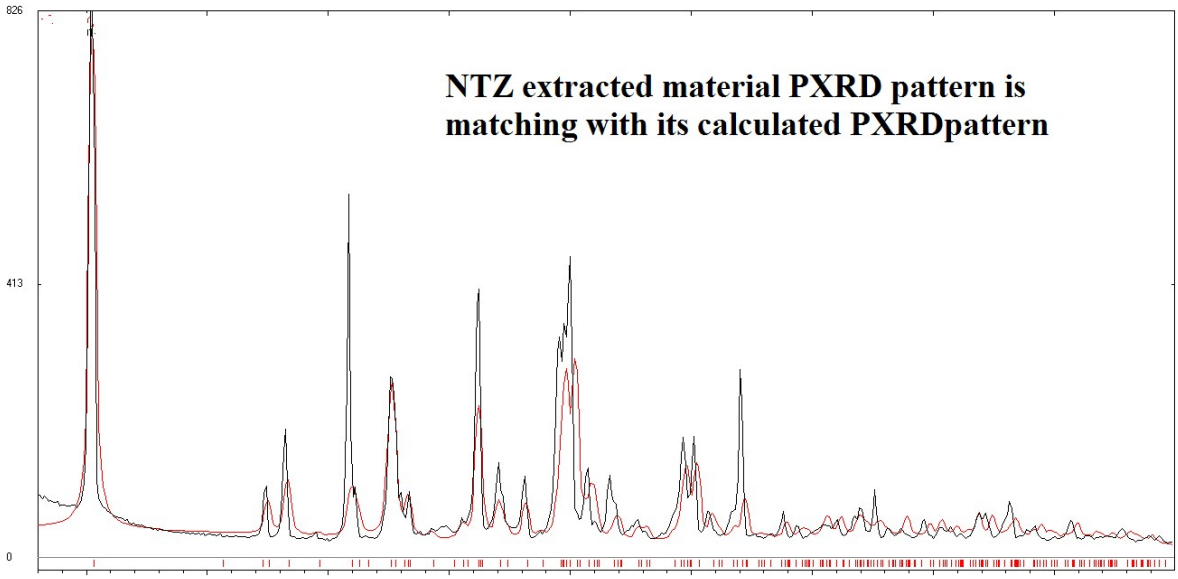
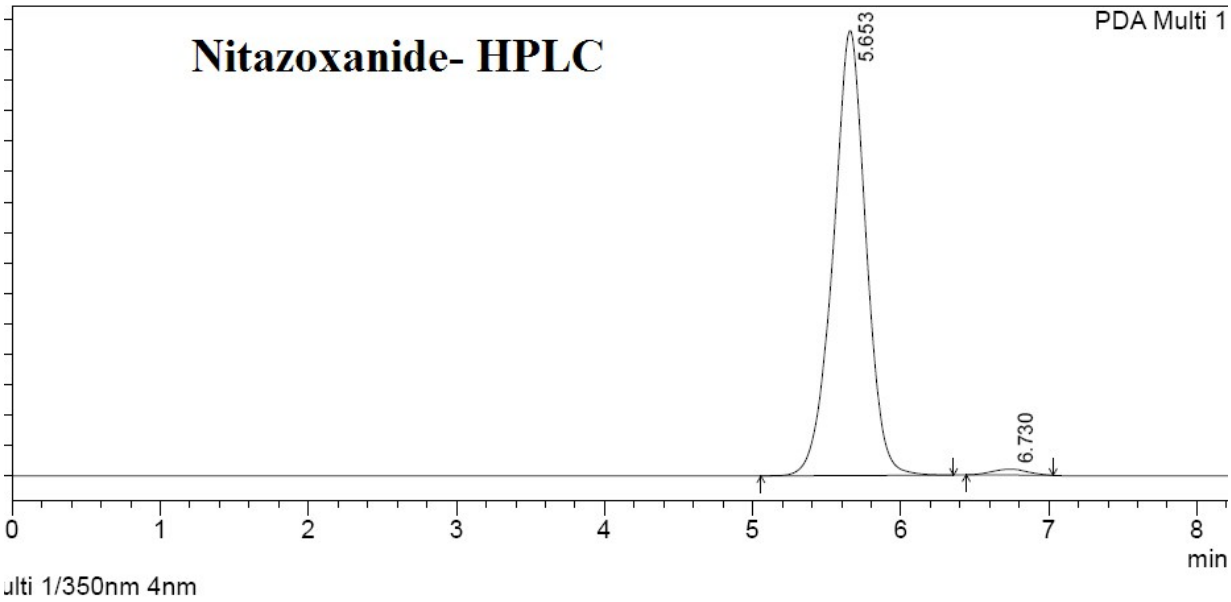


Figure S5 Overlay of the powder XRD line profile of extracted and purified nitazoxanide with that of the reported X-ray crystal structure (Refcode QUZWOY) shows excellent match.



PeakTable

h1 350nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %
1	NTZ	5.653	22579596	1461855	98.576
2	Impurity	6.730	326277	19850	1.424
Total			22905873	1481705	100.000

Figure S6 Nitazoxanide HPLC purity chromatogram (mobile phase is 5% acetic acid–acetonitrile, 40:60).

Experimental Section

Nitazoxanide was extracted from Nizonide tablets (500 mg) using chloroform solvent. The pure drug was crystallized from methanol. This crystalline powder was used for all experiments (PXRD match of extracted and purified nitazoxanide with the reported X-ray crystal structure of Refcode QUZWOY is shown in Figure S5 and purity is 98.6% in Figure S6). PABA (>99%), PASA (99%), CTAB (>99%) compounds were purchased from Sigma-Aldrich (Hyderabad, India). Solvents (purity >99%) were purchased from Hychem Laboratories (Hyderabad, India). Water filtered through a double deionized purification system (Aqua DM, Bhanu, Hyderabad, India) was used in all experiments.

Preparation of cocrystals and cocrystal alloys

NTZ-PABA:NTZ (307.2 mg) and PABA (137.1 mg) in 1:1 molar ratio were ground in slurry of 5 mL chloroform for 8 h (n=6). The formation of cocrystal was confirmed by PXRD and DSC. 30 mg of this material was dissolved in 5 mL chloroform and left for slow evaporation at ambient conditions. Single crystals suitable for X-ray diffraction were obtained after 4-5 days.

NTZ-PASA:NTZ (307.2 mg) and PASA (153.1mg) in 1:1 molar ratio were ground in slurry of 5 mL acetone for 2 h (n=6). The formation of cocrystal was confirmed by PXRD and DSC. 30 mg of this material was dissolved in 5 mL chloroform and left for slow evaporation at ambient conditions. Single crystals suitable for X-ray diffraction were obtained after 4-5 days.

Physical mixtures of NTZ-PABA and NTZ-PASA in an equivalent ratio (1:1) were dissolved in 5 mL chloroform and left for slow evaporation at ambient conditions. Single crystals suitable for X-ray diffraction were obtained after 4-5 days. Two crystals were solved and refined as CA1 (0.67:0.33) and CA2 (0.75:0.25) with the composition NTZ-PABA: NTZ-PASA. The presence of other stoichiometry alloys in the crystals harvest is being explored.

CA1 (0.67:0.33): The cocrystal alloy bulk material was obtained by grinding NTZ-PABA and NTZ-PASA in 0.67:0.33 stoichiometry with a few drops of n-hexane added in a liquid-assisted method for 30 min (n=6).

CA2 (0.75:0.25): The cocrystal alloy bulk material was obtained by grinding NTZ-PABA and NTZ-PASA in 0.75:0.25 stoichiometry with a few drops of n-hexane added in a liquid-assisted method for 30 min (n=6).

Powder X-ray diffraction

Powder X-ray diffraction was recorded on Bruker D8 Advance diffractometer (Bruker-AXS, Karlsruhe, Germany) using Cu-Kα X-radiation (λ = 1.5406 Å) at 40 kV and 30 mA power. X-ray diffraction patterns were collected over the 2θ range 5–50° at a scan rate of 5°/min. Powder Cell 2.4(Federal Institute of Materials Research and Testing, Berlin, Germany) was used for Rietveld refinement of experimental PXRD and calculated lines from the X-ray crystal structure.

Reference

(1) Powder Cell, a program for structure visualization, powder pattern calculation and profile fitting. Accessed at www.ccp14.ac.uk/tutorial/powdercell

Vibrational spectroscopy

Thermo-Nicolet 6700 FT-IR-NIR spectrometer with NXR FT-Raman module (Thermo Scientific, Waltham, MA) was used to record IR spectra. IR spectra were recorded on samples

dispersed in KBr pellets. Data were analyzed using the Omnic software (Thermo Scientific, Waltham, MA).

Thermal analysis

Differential scanning calorimetry was performed on Mettler-Toledo DSC 822e module, (Mettler-Toledo, Columbus, OH). Samples were placed in crimped but vented aluminum pans for DSC experiments. The typical sample size is 3-5 mg for DSC. The temperature range for the heating curves was 30-250 °C, and the sample was heated at a rate of 5 °C/min. Samples were purged in a stream of dry nitrogen flowing at 80 mL/min.

X-ray crystallography

X-ray reflections were collected on Oxford CCD X-ray diffractometer (Yarnton, Oxford, UK) equipped with Mo-Kα radiation (λ = 0.71073 Å) and Cu-Kα X-radiation (λ = 1.5406 Å) source. Data reduction was performed using CrysAlisPro 171.33.55 software.[1] Crystal structures were solved and refined using Olex2-1.0 with anisotropic displacement parameters for non-H atoms.[2] Hydrogen atoms were experimentally located through the Fourier difference electron density maps in all crystal structures. All O–H and C–H atoms were geometrically fixed using HFIX command in SHELX-TL program of Bruker-AXS. X-ray reflections for CA2 cocrystal alloy were collected on Bruker D8 Quest diffractometer equipped with a graphite monochromator and Mo-Kα fine-focus sealed tube (λ = 0.71073 Å). Data reduction was performed using Bruker SAINT Software. Intensities were corrected for absorption using SADABS, and the structure was solved and refined using SHELX-97. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms on hetero atoms were located from difference electron density maps and all C−H hydrogens were fixed geometrically. Hydrogen bond geometries were determined in Platon. X-Seed was used to prepare packing diagrams. X-Seed[3,4] was used to prepare packing diagrams.

References

(1) CrysAlis CCD and CrysAlis RED, Versions 1.171.33.55, Oxford Diffraction, Oxford, **2008.**
(2) O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Crystallogr.*, **2009**, *42*, 339-341.
(3) L. J. Barbour, *Supramol. Chem.*, **2001**, *1*, 189-191.
(4) L. J. Barbour, X-Seed, Graphical Interface to SHELX-97 and POV-Ray, University of Missouri-Columbia, Columbus, MO, **1999**.

Dissolution and solubility measurements

The solubility curves of NTZ and binary solids were measured using the Higuchi and Connor method in 3% CTAB (cetyltrimethyl ammonium bromide) phosphate buffer (pH 7) medium at 30 °C. First, the absorbance of a known concentration of the salt was measured at the given λ_{max} (NTZ 435 nm) on Thermo Scientific Evolution 300 UV-vis spectrometer (Thermo Scientific, Waltham, MA). These absorbance values were plotted against several known concentrations to

prepare the concentration vs. intensity calibration curve. From the slope of the calibration curves, molar extinction coefficients for NTZ, cocrystals, and cocrystal alloys were calculated. Intrinsic dissolution rate experiments were carried out on a USP certified Electrolab TDT-08L Dissolution Tester (Mumbai, MH). Dissolution experiments were performed for 240min in 3% CTAB buffer medium at 37 ºC. Prior to IDR estimation, standard curves for all the compounds were obtained spectrophotometrically at their respective λ_{max}. The respective molar extinction coefficients were used to determine the IDR values. For IDR measurements, 150 mg of the compound was taken in the intrinsic attachment and compressed to a 0.5 cm^2 disc using a hydraulic press at pressure of 4.0 ton/$inch^2$ for 5 min. The intrinsic attachment was placed in a jar of 500 mL medium preheated to 37 °C and rotated at 100 rpm. 5 mL aliquots were collected at specific time intervals and concentration of the aliquots were determined with appropriate dilutions from the predetermined standard curves of the respective compounds. This experiment was done single time.

Reference
(1) X. L. Yu, A. S. Carlin, G. L. Amidon and A. S. Hussain, *Int. J. Pharm.*, **2004**, *270*, 221-227.

Animal protocol

This pharmacokinetic study was carried in strict compliance of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (546/02/A/CPSCEA), Government of India. The animal handling procedures were reviewed and approved by the Institutional Animal Ethical Committee ofVirchow Biotech Private Limited, Department of Preclinical Toxicology, Hyderabad, India. Adult male Sprague Dawley rats (about 200 ± 50 g) were purchased from Sainath Agency Limited, Hyderabad, India. The rats were maintained on a 12 h light/dark cycle in a specific free pathogen facility. The rats had free accesses to food and water throughout the study unless rats were fasted overnight with free access to water before the experiment.

Pharmacokinetic profile

To assess the pharmacokinetic profile, 12 rats are selected randomly and divided equally in to two groups (n=6).Each group received single oral dosage of NTZ, cocrystals, and cocrystal alloys through oral gavage at the dose of 45 mg/kg (equivalent to human dosage of 500 mg/kg) for rat body weight. Serial blood (400µL) samples were collected from retro-orbital plexus into lithium heparin tube of two groups before dosing and at 30, 60, 90, 120, 150, 180, 240, 360, 480 and 720 min. Blood samples were centrifuged at 2,000 g (4 ºC) for 15 min, the harvested plasma was collected and stored at −80 °C until HPLC analysis. The plasma exposure was calculated by linear trapezoidal method (area under the plasma concentration-time plots from 0 to 12h ($AUC_{0\rightarrow12h}$) in all rats receiving oral NTZ and its cocrystal or alloy.

HPLC assay

HPLC was carried out on a Shimadzu LC-20AD liquid chromatography, Diode Array SPD-M20A detector, degasser DGU-20A3 with a RP-HPLC column C18G (250 x 4.6 mm, 5 µm

particle size), which was protected by a guard columnof 33 mm × 4.6 mm. UV absorbance at 350nm was used to quantify the drug. For liquid extraction, 200 μL acetonitrile solution was added to 200μL plasma. After vigorous vortex, the sample was centrifuged at 8000g at 4 °C for 15 min. Finally, the supernatant liquid was placed in a glass insert, and injected into HPLC. The calibration curve was obtained by TIZ (linearity $R^2 > 0.999$). The mobile phase consists of 5% acetic acid–acetonitrile (40:60), which was filtered through 0.45 μm membrane filter, degassed in a sonicator, and delivered at a rate of 1.5 mL/min. The supernatant plasma of 20 μL was injected into HPLC with a run time of 12 min.