Electronic Supplementary Information

Capturing a novel metastable polymorph of an anticancer drug gefitinib

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Data for compound 1 :

M.P. Form I = 193-195 ^{o}C, Form II = 191-193 ^{o}C; **IR** (solid) = $\bar{\nu}$ 3350 cm^{-1}; **^{1}H NMR (DMSO-d_6 500 MHz)**: δ 1.97-2.0 (m, 2H, $CH_2CH_2CH_2$), 2.39-2.50 (m, 6H, $N(CH_2)3$), 3.58 (m, 4H,$O(CH_2)2$), 3.93 (s,3H,OCH_3),4.15-4.17 (m, 2H, $ArOCH_2$),7.18 (s,1H, HAr),7.41-7.44 (m,1H, HAr),7.77-7.79 (m, 2H, HAr),8.11-8.12 (m,1H,HAr), 8.49 (s, 1H, HAr), 9.53 (s, 1H, HAr) ppm. **^{13}C NMR (DMSO-d_6 125 MHz)**: δ 26.3, 53.61, 55.15, 56.03, 66.36, 67.30, 102.65, 107.45, 108.96, 116.58, 116.74, 118.87, 119.02, 122.45, 123.64, 137.02, 147.15, 148.52, 152.35, 152.77, 154.67, 156.17 ppm.

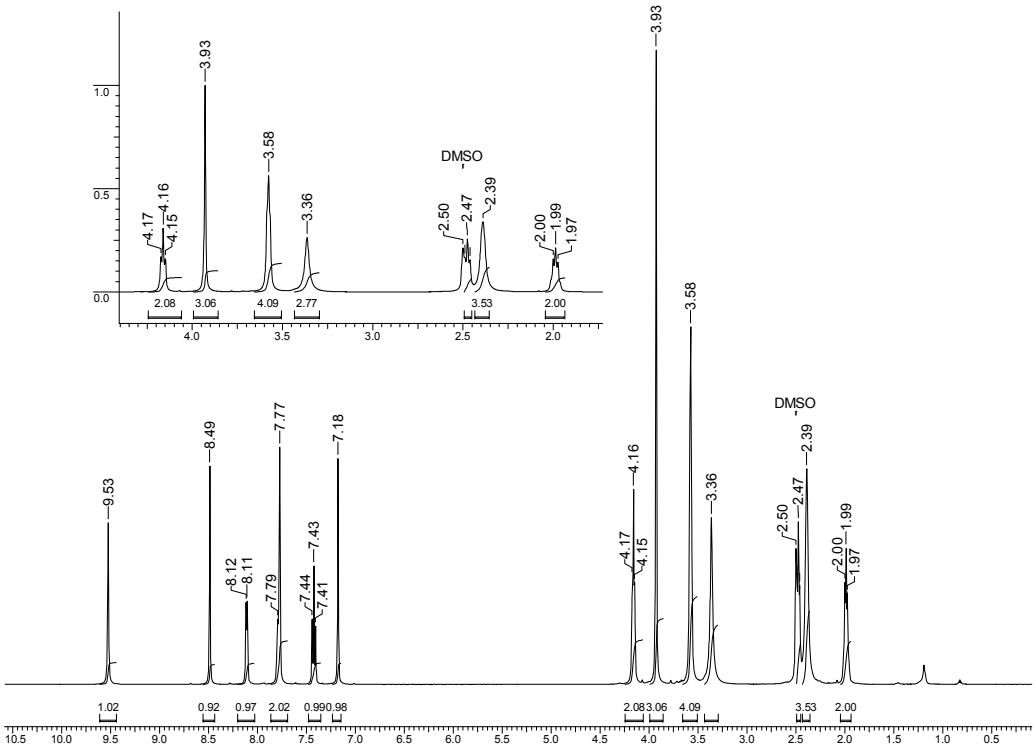


Figure S1. ^{1}H NMR spectra of **1** in DMSO-d_6 solvent.

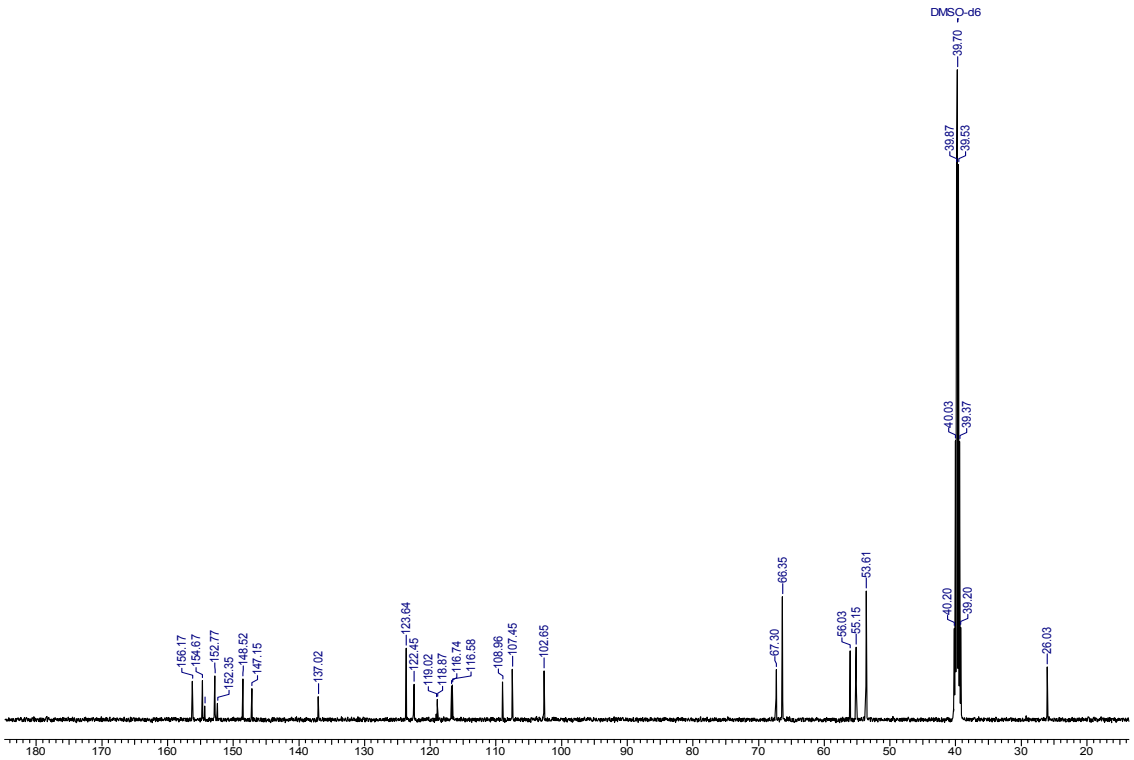


Figure S2. ^{13}C NMR spectra of **1** in DMSO-d_6 solvent.

Crystallization Experiment

Crystallization of gefitinib **1** was attempted from almost all common organic solvents such as methanol, ethanol, acetone, ethyl acetate, acetonitrile, chloroform, dichloromethane, DMF, DMSO, dioxan, nitromethane, isopropanol, *n*-propanol, n-butanol, nitrobenzene, *o*, *m* and *p*-xylene, benzene and toluene. A clear solution was obtained by vigorous shaking/stirring, warming or filtration in the desired solvent depending on the solubility of the **1** and the filtrate was allowed to evaporate at room temperature over 1-3 days. Solvents such as ethanol, acetone, ethyl acetate, acetonitrile, chloroform, dichloromethane, DMF, dioxan, nitromethane, *n*-propanol, nitrobenzene, *o*, *m* and *p*-xylene yielded known Form I crystals (Fig. S3a) exclusively. Crystallization from methanol, isopropanol, DMSO, n-butanol produced solvated crystals of **1**. However, crystallization from benzene and toluene gave two types of crystals concomitantly, thick plates (Form I, Fig. S3a) and thin plates (Form II, Fig. S3b). Thin plates obtained at the wall of the sample vial within few hours of crystallization whereas thick plates (blocky) were seen at the bottom of the sample vial after 1-3 days of crystallization. The proportion of the Form I and Form II crystals was found to be 70:30 and 80:20 in toluene and benzene respectively.

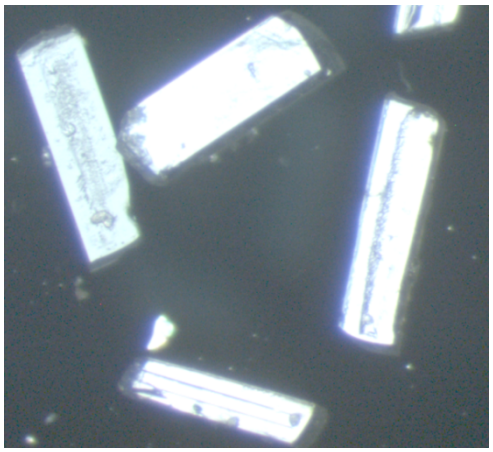
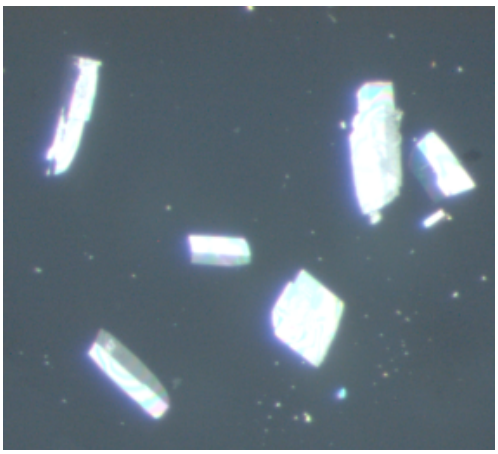
(a)  (b) 

Figure S3. Photomicrographs of Form I (a) and Form II (b) crystals of **1.**

DSC Analysis

The thermal behavior of gefitinib (Form I) and its novel crystalline polymorph (Form II) was investigated by measuring the enthalpy change on a Mettler Toledo Differential Scanning Calorimeter instrument. Crystals (~ 3-5 mg) were placed in a sealed aluminum pan (40 μL) with crimped pan closure and were analyzed from room temperature 25–225 °C using an empty pan as the reference. The heating rate was 10 °C min^{-1} and nitrogen gas was used for purging.

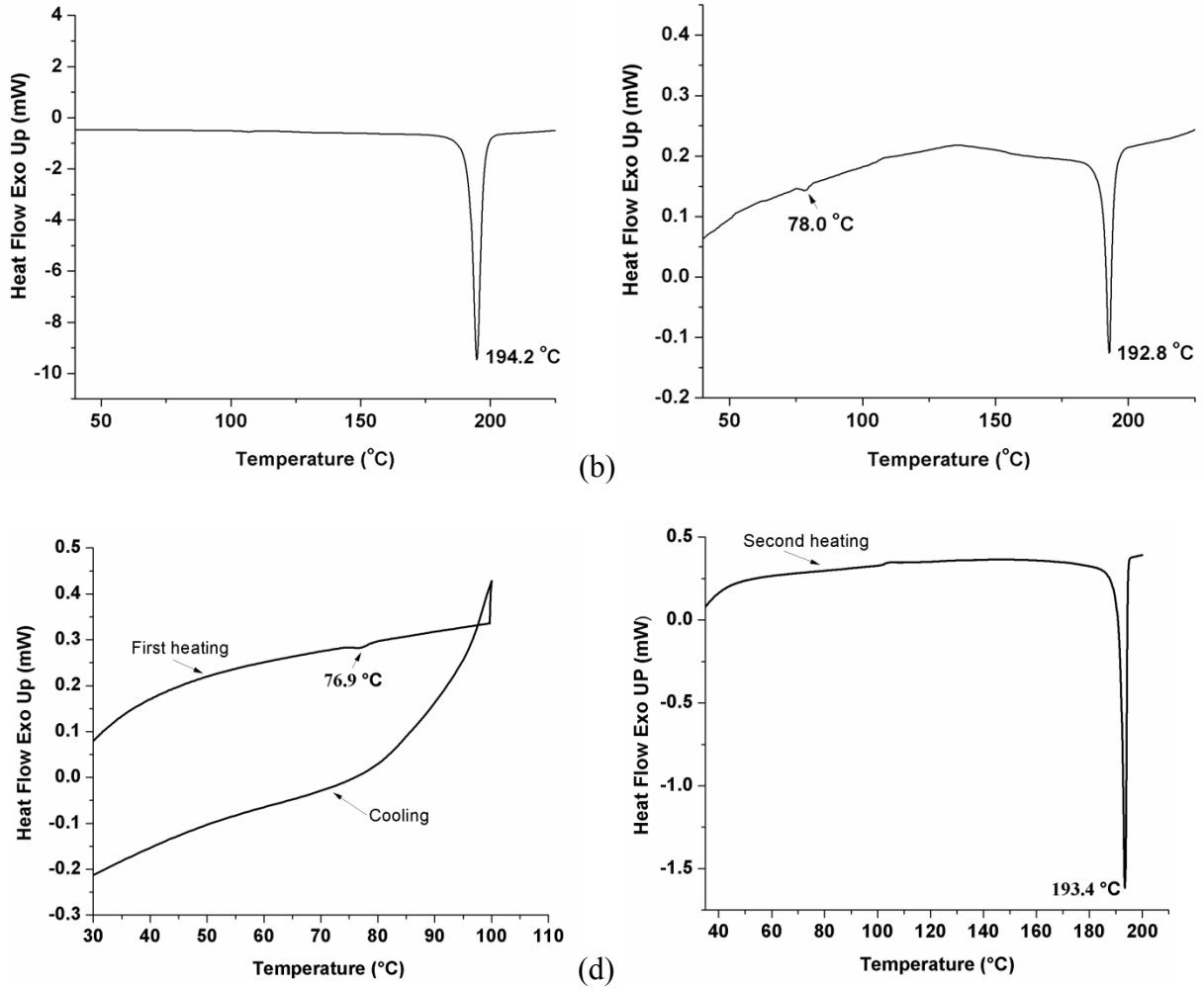


Figure S4. DSC profiles of (a) Form I crystals, (b) Form II crystals, (c) first heating (beyond transition temperature) and cooling cycle of Form II crystals and (d) second heating cycle of Form II crystals.

PXRD Analysis

The experimental Powder X-ray diffraction patterns were recorded on Rigaku Micromax-007HF instrument (High intensity microfocous rotating anode X-ray Generator) with R-axis detector IV++ at a continuous scanning rate of 2° 2θ/min using Cu Kα radiation (40 kV, 30 mA) with the intensity of the diffracted X-ray being collected at intervals of 0.1° 2θ. A nickel filter was used to remove Cu K$_\beta$ radiation. The powder X-ray diffraction patterns Form I and Form II crystals is displayed in the Figure S5a and b.

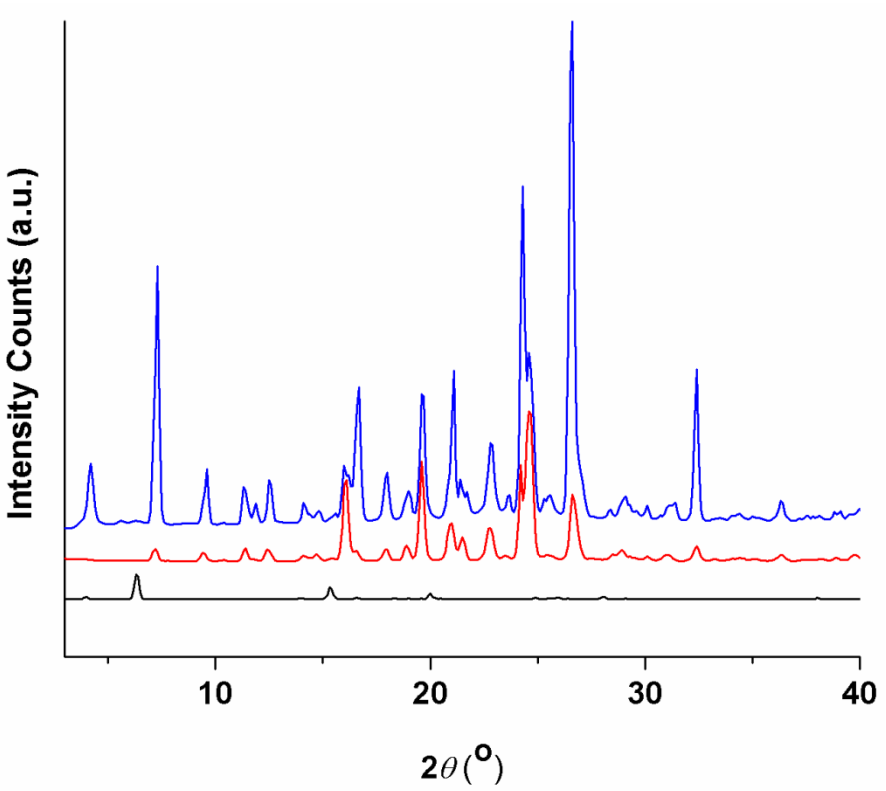


Figure S5. PXRD plots of Form I (blue), cooled crystals of Form II after heating upto 150 °C (red) and Form II (black) crystals of **1**.

Hot Stage Microscopy

The hot stage microscopy a study is performed on Leica polarizing microscope MZ75 equipped with heating P350. Form II crystals of **1** were heated beyond the transition temperature i.e. up to 130 °C with the heating rate 5 °C min^{-1}. Figure S6 displays the behaviors of Form II crystals at different temperatures. The fragmentation of the crystals initiated around 77 °C but continued over wide range of temperature. Firstly the hairline crack was developed along the crystal main axis incepted at one end roughly from the crystal center, thus split the crystal into two nearly equal halves (plates) followed by segmentation of these plates transverse to their length. This fragmentation feature was consistent in all the crystals. Interestingly, the unit cell parameters determination of these fragments revealed it to be Form I crystals. Reproducibility of this irreversible crystal-to-crystal transition was confirmed by repeating this experiment on several fragments.

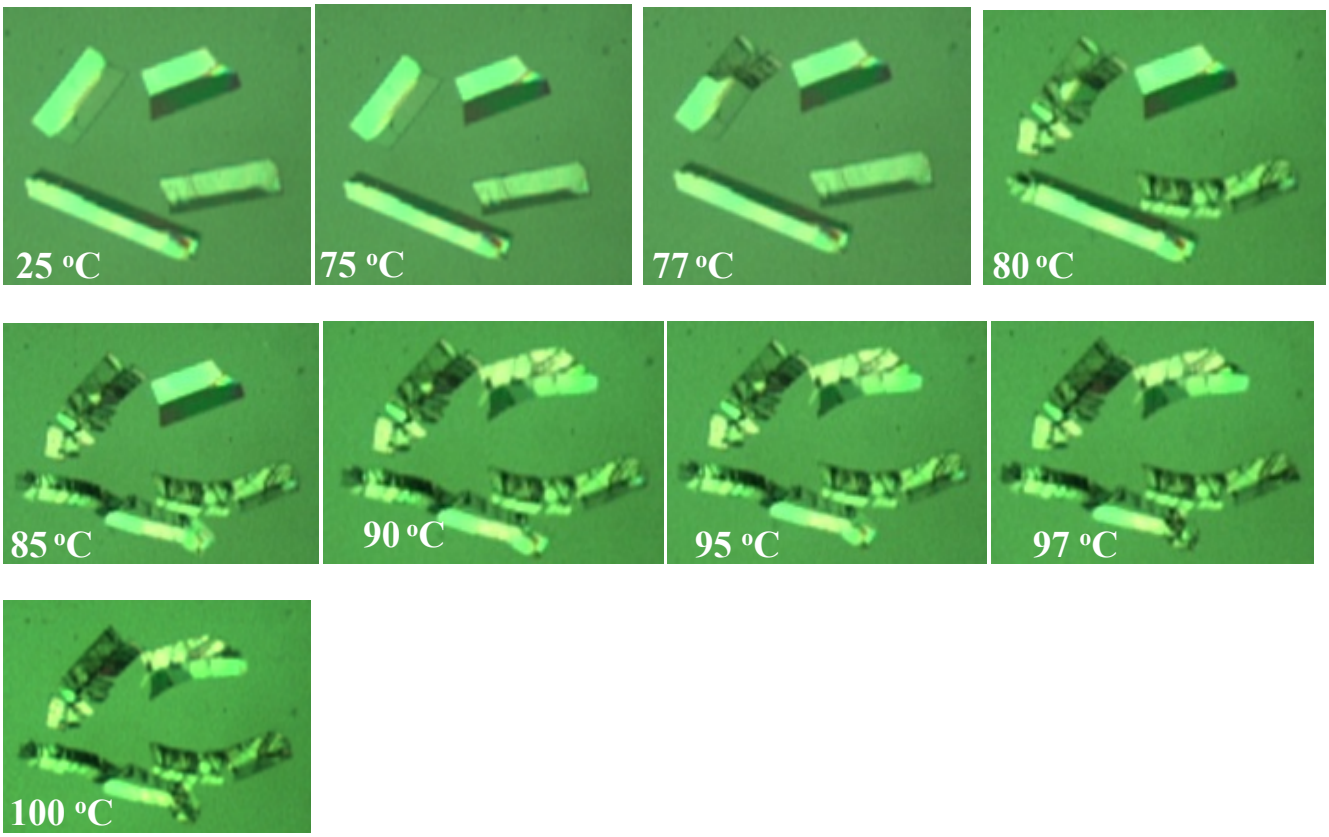
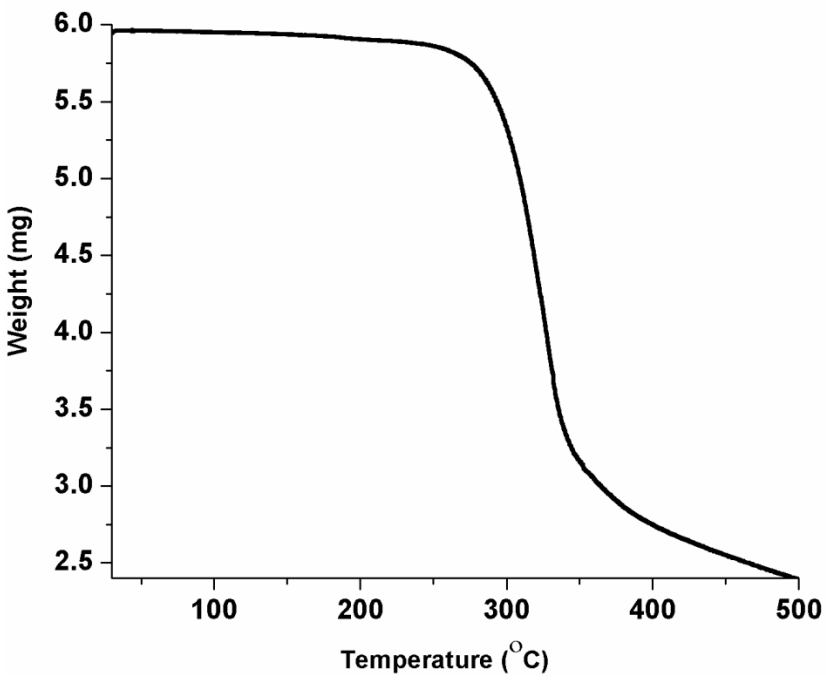


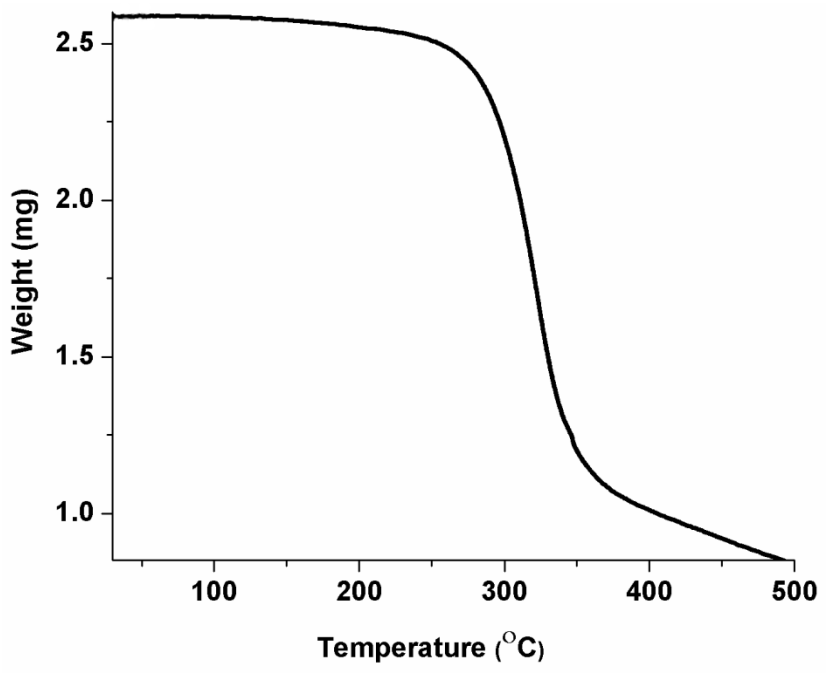
Figure S6. Photomicrographs of Form II crystals of **1** during hot stage microscopy at different temperatures.

TGA Analysis

Samples were prepared by placing 3-6 mg of material in a standard 180 μL aluminum pan. Thermogravimetric analysis was performed using a Perkin Elmer **STA 6000 TGA** instrument with a nitrogen air purge flow rate of 30 cm^3 min^{-1}. The samples were heated from ambient temperature (30 °C) to 500 °C with a heating rate 10 ^oC/min.



(a)



(b)

Figure S7. TGA graphs of Form I (A) and Form II (B) crystals of **1.**

X-ray Crystallography

Single crystal X-ray structure of Form I of **1** was determined by measuring X-ray intensity data on a Bruker SMART APEX II single crystal X-ray CCD diffractometer having graphite-monochromatised (Mo-Kα = 0.71073 Å) radiation at 100(2) K. The X-ray generator was operated at 50 kV and 30 mA. A preliminary set of cell constants and an orientation matrix were calculated from total 36 frames. The optimized strategy used for data collection consisted different sets of φ and ω scans with 0.5° steps in φ/ω. Data were collected with a time frame of 20 sec keeping the sample-to-detector distance fixed at 5.00 cm. The X-ray data acquisition was monitored by APEX2 program suit.[1] All the data were corrected for Lorentz-polarization and absorption effects using SAINT and SADABS programs integrated in APEX2 package.[1] The structures were solved by direct methods and refined by full matrix least squares, based on F^2, using SHELX-97.[2] All the hydrogen atoms were located in difference Fourier and refined isotropically. Molecular diagrams were generated using ORTEP-32[3] and Mercury programs.[4] Geometrical calculations were performed using SHELX[2] and PLATON.[5]

Single crystal X-ray structure of Form II of **1** was determined by measuring X-ray intensity data on a Bruker SMART APEX four-circle diffractometer with a micro-focus Cu K-α radiation (λ= 1.5418 Å) and equipped with a PHOTON-100 CMOS detector at 100(2) K. The intensity data collection was performed in the scanning mode with the goniometer and detector angular settings optimized using the program COSMOS. Data were collected with a time frame of 30 sec keeping the sample-to-detector distance fixed at 4.00 cm. The diffraction spots were measured in full with a high accuracy, scaled, corrected for Lorentz-polarization correction, and integrated using Bruker SAINT (2013).[6] Absorption effects were empirically corrected by using multi-scan, SADABS (Sheldrick, G. M., *SADABS*; Universitat Gottingen, 2013).[6] Structure was solved using SIR-GUI methods (SHELXS-2013).[2] Full-matrix least-squares refinement on F^2 was carried out using *SHELXTL* suite of programs.[7] The crystallographic data and conditions for structure analysis are listed below. All the hydrogen atoms were located in difference Fourier and refined isotropically. Molecular diagrams were generated using ORTEP-

32[3] and Mercury programs.[4] Geometrical calculations were performed using SHELTL[7] suite and PLATON.[5]

References

1. Bruker (2006). APEX2, SAINT, and SADABS. Bruker AXS Inc.,Madison, Wisconsin, USA.
2. G. M. Sheldrick, *Acta Cryst*. 2008, **A64**, 112 – 122.
3. L. J. Farrugia, *J. Appl. Cryst*. 1997, **30**, 565.
4. C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P. A. Wood, *J. Appl. Crystallogr*., 2008, **41**, 466.
5. A. L. Spek, *J. Appl. Cryst*. 2003, **36**, 7– 13.
6. Bruker (2013). *APEX2*, *SAINT* and *SADABS*. Bruker AXS Inc., Madison, Wisconsin, USA.
7. SHELXTL Sofware Suite, Version 6.14; Bruker AXS, Madison, WI, 2000-2003.

Table S1: Crystallography data of polymorphs of **1**.

	Form I	**Form II**
Chemical formula	$C_{22}H_{24}N_4O_3ClF$	$C_{22}H_{24}N_4O_3ClF$
M_r	446.90	446.90
Temp. (K)	100(2)	297(2)
Crystal size	0.55 x 0.47 x 0.34	0.10 x 0.08 x 0.02
Crystal system	triclinic	triclinic
Space group	*P*-1	*P*-1
a/Å	8.8091(4)	7.1645(2)
b/Å	9.6702(4)	10.8025(3)
c/Å	12.4941(5)	14.3429(4)
α/°	93.700(2)	80.3020(10)
β/°	97.546(2)	75.6620(10)
γ/°	101.939(2)	89.2670(10)
V/Å^3	1027.65(8)	1059.63(5)
Z, D_{calc}/g cm^{-3}	2, 1.444	2, 1.401
μ/mm^{-1}	0.228	1.951
F(000)	468	468
θ max/°	26.00	34.15
Absor. Correction	multi-scan	multi-scan
T_{min}/T_{max}	0.885/0.926	0.929/0.962
Reflections collected	19219	22103
Unique reflections	4014	3826
Observed reflections	3758	3432
h, *k*, *l* (min, max)	(-10, 10), (-11, 11), (-15, 15)	(-8, 8), (-12, 12) (-17, 17)
R_{int}	0.0155	0.0339
Number of parameters	376	376
R_1_obs, R_1_all	0.0277, 0.0718	0.0328, 0.0859
wR_2_obs, wR_2_all	0.0297, 0.0738	0.0373, 0.0885
GoF	1.019	1.109
$\Delta\rho_{max}$,$\Delta\rho_{min}$/eÅ^{-3}	0.30, –0.23	0.38, –0.30
CCDC Nos.	990393	990394

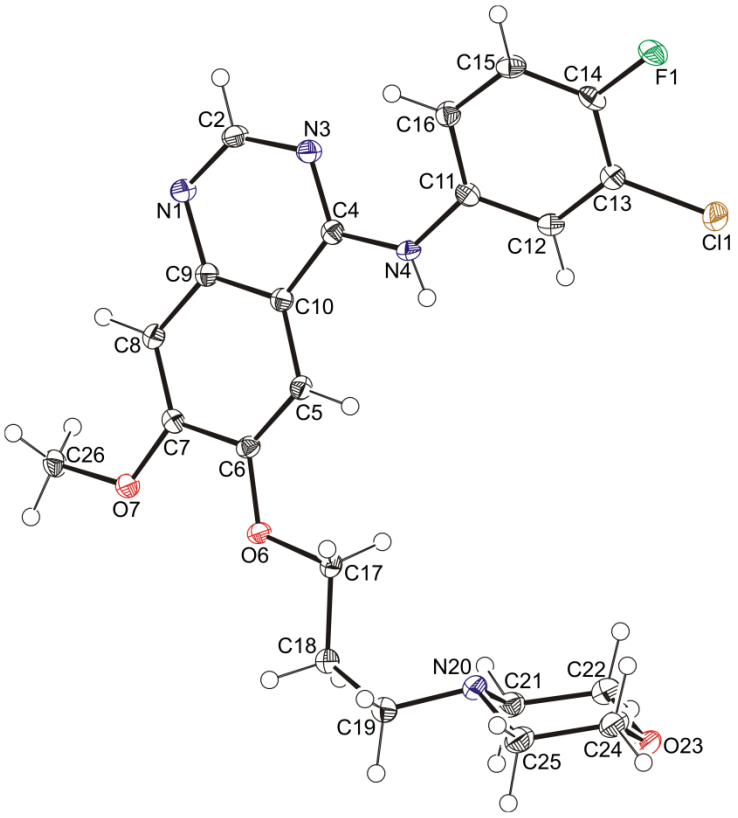


Figure S8. ORTEP of Form II crystals of **1** showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii.

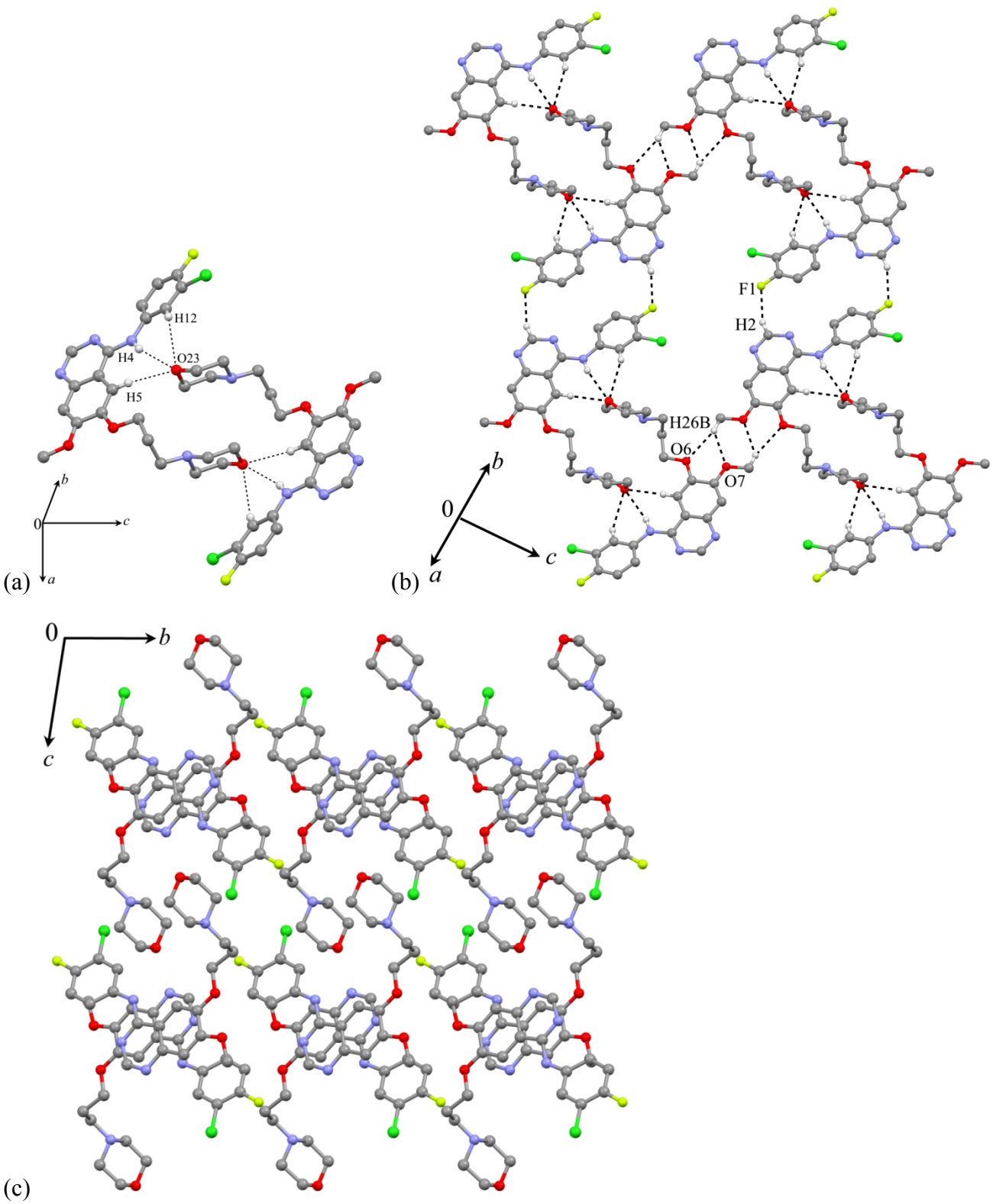


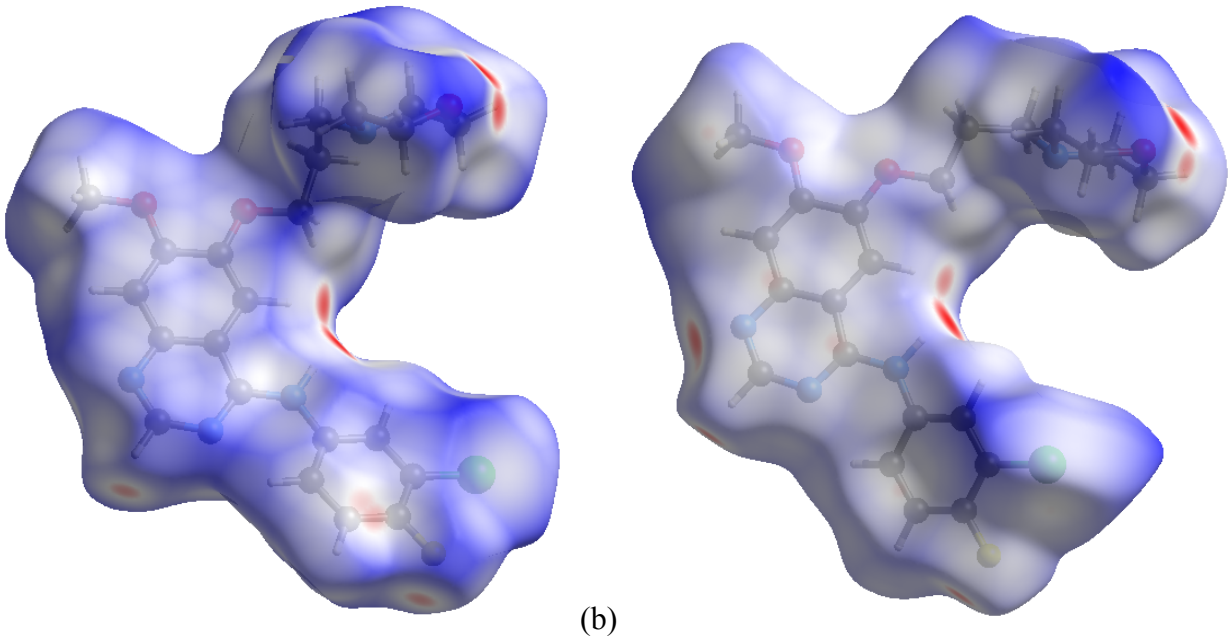
Figure S9. View of molecular packing in Form I crystals of **1**, (a) Dimeric association of molecules, (b) their aggregation to form 2D network and (c) stacking of the 2D assemblies along *a*-axis.

Table S2. Geomerical parameters for intermolecular interactions in polymorphs of **1**.

Interactions	D-H (Å)	H···A (Å)	D···A (Å)	D-H···A/α (°)	Symmetry codes
			Form I		
N4-H4···O23	0.848(16)	2.308(15)	3.0609(12)	148.2(13)	2-*x*,1-*y*,2-*z*
C5-H5···O23	0.944(15)	2.412(15)	3.3091(14)	158.6(12)	2-*x*,1-*y*,2-*z*
C12-H12···O23	0.962(15)	2.655(14)	3.2966(14)	124.5(10)	2-*x*,1-*y*,2-*z*
C2-H2··· F1	0.958(15)	2.496(16)	3.4220(14)	162.7(12)	1-*x*, 2-*y*, 1-*z*
C26-H26B···O6	0.964(15)	2.604(15)	3.5197(15)	158.7(11)	2-*x*, -*y*, 1-*z*
C26-H26B···O7	0.964(15)	2.512(15)	3.3124(15)	140.4(11)	2-*x*, -*y*, 1-*z*
C25-H25B···Cl1	0.986(15)	2.930(15)	3.3124(15)	151.1(11)	1-*x*, 1-*y*, 2-*z*
Cg2··· Cg2			4.3436(7)	0	1-*x*, 1-*y*, 1-*z*
Cg2··· Cg3			4.1011(7)	0.25(5)	2-*x*, 1-*y*, 1-*z*
Cg3··· Cg3			3.7564(7)	0	2-*x*, 1-*y*, 1-*z*
Cg3··· Cg4			3.8711(7)	16.17(6)	1-*x*, 1-*y*, 1-*z*
			Form II		
N4-H4···O23	0.804(19)	2.32(2)	3.1052(17)	166.9(17)	-*x*, 2-*y*, 2-*z*
C5-H5···O23	0.951(18)	2.514(18)	3.3980(18)	154.6(14)	-*x*, 2-*y*, 2-*z*
C12-H12···O23	0.93(2)	2.49(2)	3.281(2)	142.6(16)	-*x*, 2-*y*, 2-*z*
C2-H2··· F1	0.954(18)	2.458(19)	3.4048(18)	171.6(15)	-*x*, 1-*y*, 1-*z*
C26-H26B···O6	0.98(2)	2.75(2)	3.678(2)	160.1(15)	1-*x*, 3-*y*, 1-*z*
C26-H26B···O7	0.98(2)	2.60(2)	3.405(2)	140.4(14)	1-*x*, 3-*y*, 1-*z*
C19-H19A···Cl1	1.025(19)	2.852(19)	3.724(11)	3.6714(17)	1+*x*, 1+*y*, *z*
C22-H22A···N20	0.98(2)	2.70(2)	3.676(2)	170.4(15)	-*x*, 2-*y*, 2-*z*
C24-H24A···N1	0.97(2)	2.44(2)	3.401(2)	171.3(16)	*x*, *y*, 1+*z*
C26-H26A···Cg4	1.00(2)	2.67(2)	3.6592(16)	173.8(14)	-*x*, 2-*y*, 1-*z*
Cg2··· Cg2			3.5120(8)	0	-*x*, 2-*y*, 1-*z*
Cg2··· Cg3			3.6772(8)	1.82(7)	-*x*, 2-*y*, 1-*z*
Cg2··· Cg3			3.5713(8)	1.82(7)	1-*x*, 2-*y*, 1-*z*
Cg3··· Cg3			3.7073(5)	0	1-*x*, 2-*y*, 1-*z*

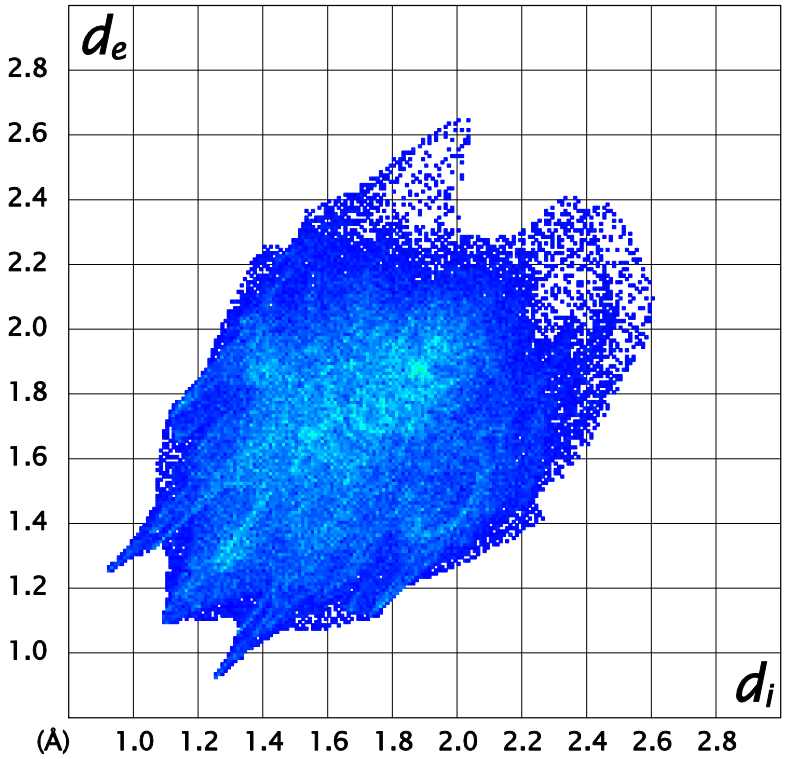
Cg is the centroid of the phenyl ring, Cg2 = N1 – C9, Cg3 = C5 – C10, Cg4 = C11 – C16; α is the dihedral angle between planes of phenyl rings.

Hirshfeld Surface Analysis

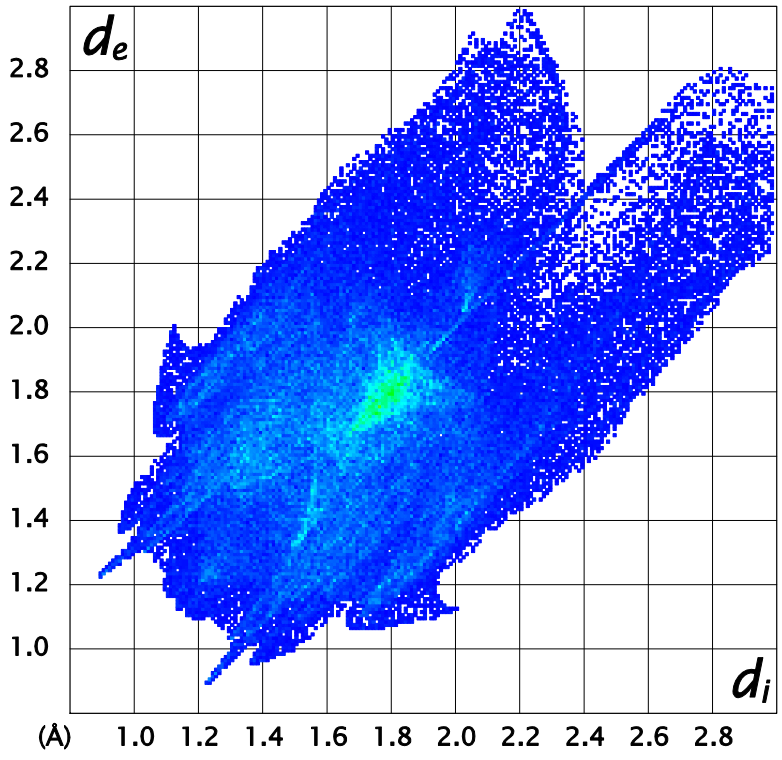


(a) (b)

Figure S10. Hirshfeld surfaces for polymorphs of **1**, (a) Form I and (b) Form II crystals.



(a)



(b)

Figure S11. Hirshfeld fingerprint plots for (a) Form I and (b) Form II crystals of **1**.

Solubility and Dissolution Rate Measurements

The solubility and dissolution rate measurements studies of polymorphs of gefitinib were performed in HCl solution of pH 3 using USP-certified Electrolab TDL-08 tablet dissolution tester at 37 °C with a constant stirring speed of 50 rpm. Aliquots (1 ml) were withdrawn at specific intervals of time and replenished with an equal amount of fresh pH solution so as to maintain the constant volume. These aliquots were analyzed by UV spectrophotometer (UV-1601 PC, Shimadzu Scientific Instrument) at wavelength 253 nm and compared with standard calibration curve. Comparative solubility and dissolution rate studies of polymorphs of **1** (gefitinib) revealed subtle difference in the dissolution profiles at pH 3 solution till a period of 180 minutes.

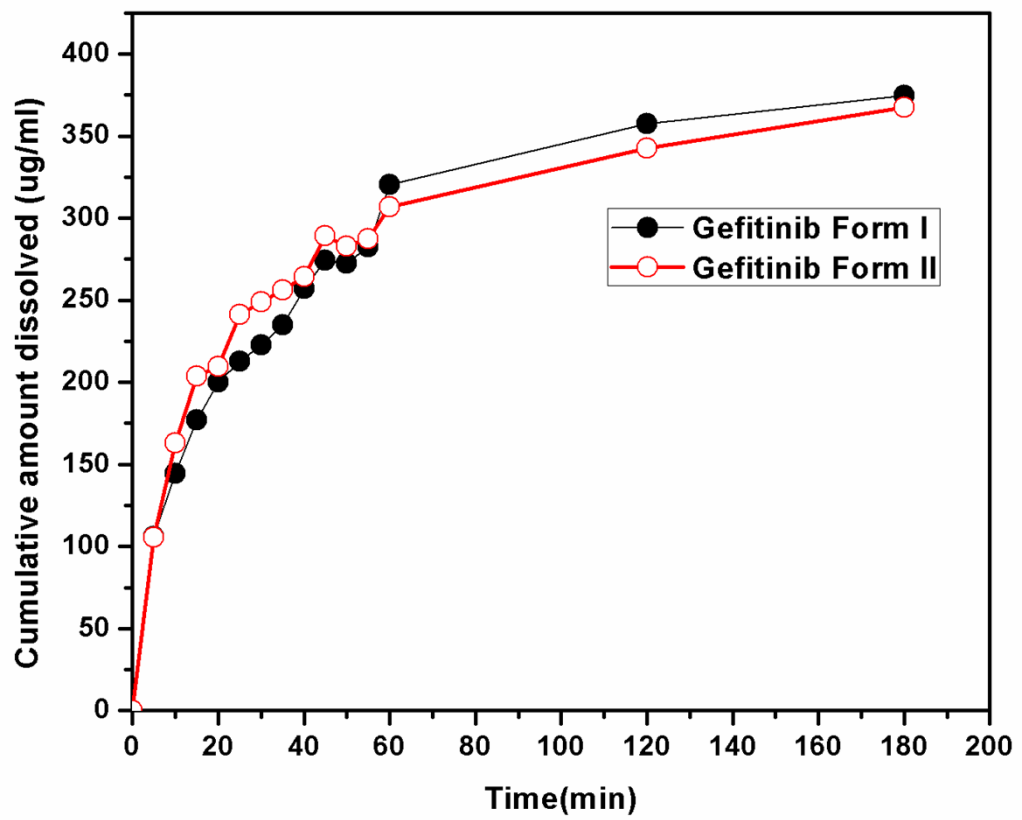


Figure S12. Dissolution rate measurement study of polymorphs of **1** in pH 3 solution.