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ARTICLE TYPE

Capturing a novel metastable polymorph of an anticancer drug gefitinib

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Gefitinib, a life extending anticancer drug exhibits solvent mediated conformational polymorphism to yield stable (Form I) and a novel metastable (Form II) polymorphs. Crystal structure analysis revealed 3D isostructurality in their molecular organization and the metastable polymorph undergoes crystal-to-crystal thermal phase transition to stable polymorph.

Active pharmaceutical ingredients (APIs) can exist in a variety of solid forms including polymorphs, hydrates, solvates, cocrystals and the properties such as, solubility, dissolution rate, bioavailability and stability which have the direct consequence on their therapeutic efficiency differ significantly for each polymorph.[1] Controlling polymorphism in APIs, a phenomenon that induces differences in the crystal structure of molecules because of their different arrangements/conformations is the subject of intense research due to different functionality and physicochemical properties associated with each polymorph.[2] Without adequate control, the polymorph can cause structural impurity in the final product as well as difficulty in process-ability due to different size and shape of solid material.[3] The most significant goal of pharmaceutical development is to obtain the thermodynamically stable form during industrial crystallization process to avoid formulation troubles and the threat of potential withdrawal of life saving drug from the market[4] due to the disappearance of its most stable form.[5] However the most stable form often suffers from poor solubility, low dissolution rate and inadequate bioavailability.[1] In such cases the metastable crystalline form of a drug[6] or its amorphous form[7] is preferred for better delivery.

Gefitinib (**1**), a synthetic aniliniquinazoline (Fig. 1a) is an orally administrated chemotherapy treatment for lung and breast cancers which inhibit the activity of the epidermal growth factor receptor (EGFR) of protein (tyrosine kinase) that transduces signals critical for cell proliferation.[8] It was approved for clinical use in 2003[9] and currently marketed over 64 countries for patients with advanced non-small cell lung cancer (NSCLC) who had received at least one previous chemotherapy regime.[10] Notably, in some countries it is used as first line treatment for patients with EGFR mutation for naive locally advanced or metastatic NSCLC.[11] However, the drug suffers from poor aqueous solubility. Surprisingly to our knowledge there are no investigation on the polymorphism of this drug, and the only crystal structures available in the CSD are that of solvent free (Form I) and trihydrate forms.[12] The trihydrate form is the most stable form in the presence of water while solvent free form converts to the trihydrate form in aqueous suspension.[12b] We started our research work with an aim to improve its solubility by exploring polymorphism and cocrystal/salt screening of this drug. In this contribution, we report the results of the crystallization trials of **1**, isolation and characterization of its new metastable polymorph (Form II) and its crystal structure correlation with the stable crystalline form (Form I).

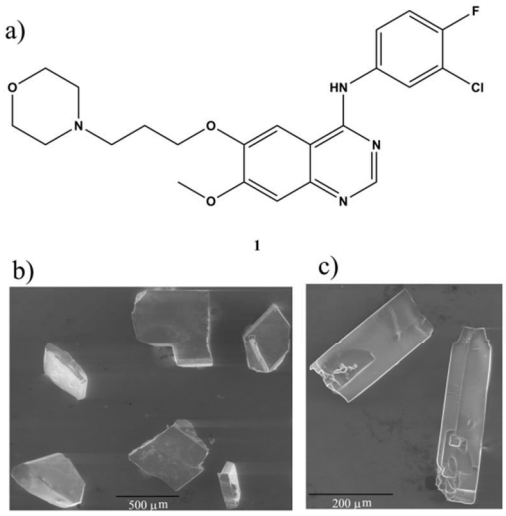

Fig. 1 (a) Structure of gefitinib **1**, SEM images of polymorphs of **1** (b) Form I and (c) Form II crystals.

Recrystallization of the commercial sample (for NMR, IR data see ESI, Fig. S1 and S2) was carried out at ambient conditions from almost all common organic solvents such as ethanol, acetone, ethyl acetate, acetonitrile, chloroform, dichloromethane, DMF, dioxane, nitromethane, *n*-propanol, nitrobenzene, *o*, *m* and *p*-xylene, etc. Consistently, we obtained Form I crystals (blocks) in all the trials (Fig. 1b and Fig. S3a, ESI).[12] Crystallization from methanol, DMSO, isopropanol and *n*-butanol yielded solvated crystals of gefitinib.[12b] However, rapid crystallization from the supersaturated solution of benzene and toluene yielded new Form II crystals (thin plates, Fig. 1c and Fig. S3b, ESI) and Form I crystals concomitantly. Different crystal shapes gave us the clue of polymorphism which latter on confirmed by single crystal XRD and PXRD studies. Form II crystals appeared from the solution within first few hours; while Form I crystals obtained after 1-2 days. This suggested that Form II crystals were perhaps obtained under kinetically controlled conditions, while Form I

plates were produced under thermodynamic conditions.[2] The yield of the Form I crystals was always much more compared to the Form II crystals, suggesting it's preference at the nucleation event. This strategy of successive crystallization from benzene and toluene solution was repeated many times, and on every occasion, similar results were obtained. Seeding Form II crystals in the saturated solution of **1** in benzene and toluene also produced more of these crystals initially, but not exclusively. This also rules out the possibility of solvent mediated polymorphic transformation between Form I and Form II crystals.

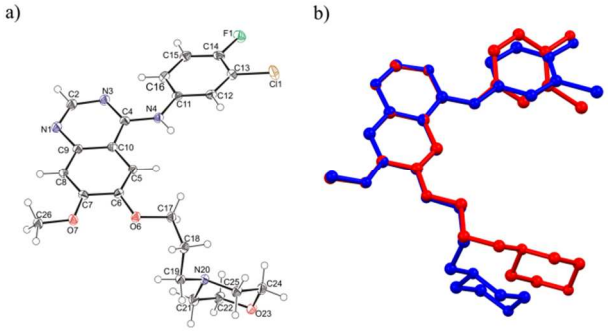

Fig. 2 (a) ORTEP of Form I crystals of **1** showing the atom-numbering scheme (Fig. S8 for ORTEP of Form II, ESI). Displacement ellipsoids are drawn at the 50% probability level and H atoms are shown as small spheres of arbitrary radii. (b) Structure overlay of Form I (blue) and Form II (red) crystals of **1**.

Differential scanning calorimetric (DSC) analysis (Fig. S4a, ESI) of Form I crystals showed a single sharp endotherm centred at 194.2 °C corresponding to its melting that is also in accordance with that recorded with a melting point apparatus. However, DSC profile of Form II crystals revealed a broad and small endothermic hump centered at 78.0 °C before the melting endotherm seen at 192.8 °C (Fig. S4b, ESI), indicating possible structural phase transition before melting. Interestingly, a repeat of the DSC experiment on the Form II crystals, cooled after heating beyond the transition temperature contained only a melting endotherm at 193.4 °C (Figs. S4c, d, ESI). PXRD pattern of the cooled Form II crystals, obtained after heating beyond the transition temperature (150 °C) matched with the PXRD profile of Form I crystals (Fig. S5, ESI), indicating conversion of Form II to Form I crystals at the transition temperature. Hot stage microscopic (HSM) studies revealed the inception of the fragmentation of Form II crystals around the transition temperature, 77 °C and continued up to 100 °C (Fig. S6, ESI). Each of the fragments observed under the polarizing microscope confirmed its single crystalline nature. The unit cell parameters determination of these fragments revealed it to be Form I crystals. Additionally, no weight loss was observed on heating to the melting of both polymorphs during thermogravimetric analysis confirming that both polymorphs did not contain any solvent (Fig. S7, ESI).

Crystal structure analysis shows Form II crystals also belong to triclinic, *P*-1 space group with one molecule in the asymmetric unit, as Form I crystals (Fig. 2a, Fig. S8, and Table S1, ESI). Both dimorphs are conformational polymorphs[13] displaying marked (*c.a.* 85° along O6-C17-C18-C19) and subtle (*c.a.* 8-9° along C4-N4-C11-C12) differences in the relative orientation of morpholine and halophenyl rings respectively (Fig. 2b). The intramolecular geometry of **1** in both polymorphs shows a 'molecular clip' type structure with morpholine and phenyl moieties constituting its pincers and the 4-aniliniquinazoline group as a rigid tether (Fig. 2a) holding these flexible moieties in *syn* orientation to create an open area of dimensions ~8 x ~7 Å^2 in Form I and Form II crystals respectively.[14]

A common structural feature observed in dimorphs of **1** is the centrosymmetric association of molecules through trifurcated hydrogen bonding interactions, N4-H4···O23, C5-H5···O23 and C12-H12···O23 generating a dimeric motif, however, with different geometries (Fig. 3a and Fig. S9a, Table S2, ESI). The morpholine moiety of the centrosymmetrically related molecules pierces into the open area of **1**, thus exhibiting 'self inclusion' phenomena. This centrosymmetric approach of molecules in Form II crystals also brings morpholine moieties in proximity to produce an additional C22-H22A···N20 interaction which is not seen in Form I crystals.

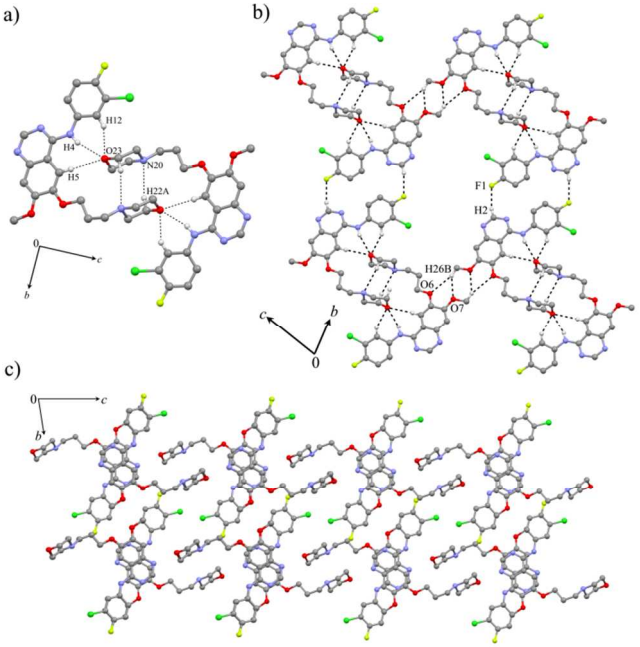

Fig. 3 View of molecular packing in Form II crystals of **1**, (a) dimeric association of molecules, (b) their aggregation to form 2D network and (c) stacking of the 2D assemblies along *a*-axis. Similar organization is observed in Form I crystals of **1** (Fig. S9, ESI).

The dimeric motifs in both forms are tightly held *via* centrosymmetric short and linear C2-H2···F1 interactions to generate a 1D isostructural layered assembly roughly along the *ac* and *bc* diagonals in Form I and Form II crystals respectively. The adjacent 1D layers are connected approximately along the *ab* diagonal *via* centrosymmetric bifurcated C-H···O interactions; short and non-linear C26-H26*B*···O6 and long and linear C26-H26*B*···O7 generating 2D isostructural network (Fig. 3b, Fig. S9b, ESI).[15] The geometrical parameters of these contacts (Table S2), suggests that the 1D isostructural layers are more tightly held in Form I crystals compared to Form II crystals.

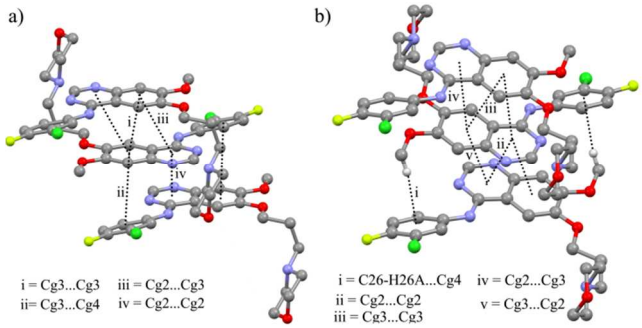

Fig. 4 Close view of aromatic π···π stacking patterns of molecules in (a) Form I, (b) Form II crystals of **1**.

Molecular packing viewed down the *a*-axis shows similar stacking of these 2D assemblies in both polymorphs through centrosymmetric π···π interactions,[16] revealing 3D isostructurality (if morpholine and halophenyl groups orientation is ignored, Fig. 3c and Fig. S9c, ESI). However, close inspection of the stacking pattern of molecules in both polymorphs shows a significant difference (Fig. 4a and b, Table S2, ESI). Molecules, in Form I crystals, shows parallel displaced stacking assembly[16] through π···π interactions, namely Cg3···Cg3, involving phenyl rings of the quinazoline moiety and Cg3···Cg4, engaging electron rich phenyl of quinazoline and an electron deficient π system of halophenyl. In contrast, molecules in Form II crystals, displays sandwich type stacking pattern[16] *via* Cg2···Cg2, Cg3···Cg3 and Cg2···Cg3 interactions. The marked difference between the two packings is found to be the differential engagement of halophenyl ring; in Form I it is engaged via π···π interactions while in Form II it is involved in C-H···π (C26-H26*A*···π) interaction.

The examination of the surroundings of the single molecule in dimorphs of **1** through Hirshfeld surface[17] (Fig. S10, ESI) clearly indicates the similar molecular environment for both polymorphs. But, Hirshfeld fingerprint images of both polymorphs markedly distinguish the difference in intermolecular interactions that stabilize the two structures (Fig. S11, ESI). The computation of packing energies[18] for the dimorphs of **1** revealed the values of -241.17 and -237.15 kJ mol^{-1} for Form I and Form II crystals respectively, indicating that Form I crystals are stable compared to Form II crystals. The values of crystal densities 1.444 g cm^{-3} (Form I) and 1.401 g cm^{-3} (Form II) are also consistent with the calculated packing energies. This also substantiates the formation Form I crystals exclusively in all crystallization experiments. Furthermore, the estimation of intermolecular potential revealed maximum value for Cg3···Cg4, and Cg3···Cg3 (-101.4 kJ mol^{-1} and -84.8 kJ mol^{-1}) in Form I crystals compared to Cg2···Cg2, Cg3···Cg3, Cg2···Cg3 and C26-H26*A*···π (Cg4) interactions (-89.1 to -92.4 kJ mol^{-1}) observed in Form II crystals. This suggests that the thermal transformation of Form II to Form I crystals is the reorganization of a metastable crystalline phase to a stable crystalline phase.

For the conversion of Form II to Form I crystals, molecules assembled *via* Cg2···Cg2, Cg3···Cg3, Cg2···Cg3 and C26-H26*A*···π interactions in Form II crystals (Fig. 4b) have to rearrange to establish Cg3···Cg3 and Cg3···Cg4 interactions observed in Form I crystals (Fig. 4a). This would involve the rotation and translation of quinazoline moieties towards each other by ~ 9° and ~0.40 – 0.50 Å respectively along the stack assembly with accompanying conformational changes in morpholine and dihalophenyl rings. The crystal-to-crystal transition could be the result of these concerted and highly cooperative movements of molecules to achieve tight association observed in Form I crystals. On the whole, the changes during the phase transformation are far too large (and thus irrevocable) and result in the fragmentation of the Form II crystals (Fig. S6, ESI).

Conclusions

In conclusion, we have obtained and characterized a new crystalline metastable polymorph of an anticancer drug, 'gefitinib' and optimized the process of its occurrence. Crystal structure of a new polymorph is grossly similar in 3D to a known form and hence has similar physicochemical properties such as solubility and dissolution rate; no significant difference between the solubility and the dissolution rate was found (Fig. S12, ESI). This suggests that the metastable form may not be a useful alternative crystal form; however, its contamination with the stable crystal form during drug development process may cause formulation problems. DSC, HSM and XRD analyses revealed crystal-to-crystal thermal phase transition of Form II to Form I crystals. Crystal structure analysis before and after the transition suggests a reasonable mechanism[15b] by which the differences in the stacking patterns in dimorphs is responsible for this phase transition. The 'molecular clip' like conformation of 'gefitinib' is currently being explored as receptor for the solvent inclusion and cocrystal/salt.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [^{1}H and ^{13}C NMR spectra, crystal photomicrographs, DSC, XRD data, molecular packings, interactions table, Hirshfeld surfaces and fingerprint plots, solubility data, etc.]. See DOI: 10.1039/b000000x/

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Table of contents entry

Gefitinib, an anticancer drug exhibited conformational polymorphism revealing 3D isostructurality in molecular organization with slight difference in their stacking pattern. The kinetic (red) polymorph undergoes crystal-to-crystal thermal phase transition to thermodynamic (blue) form.