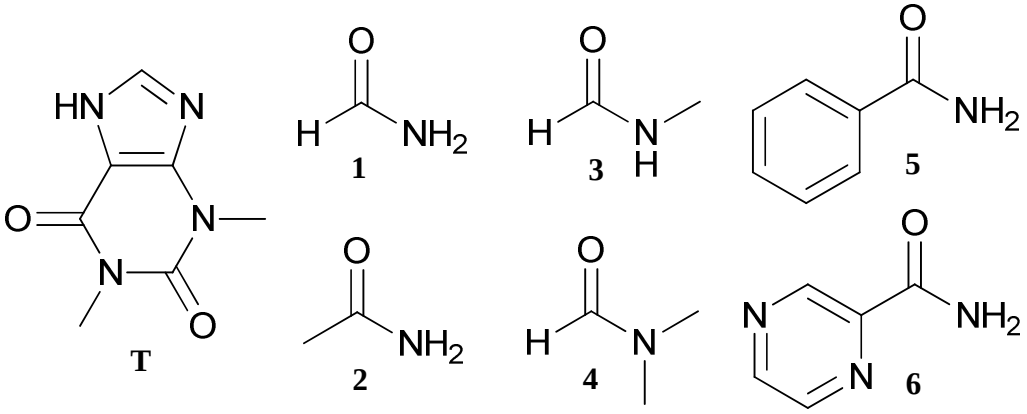
Investigation of an amide-pseudo amide hydrogen bonding motif within a series of theophylline:amide cocrystals

Mark D. Eddleston,[1] Mihails Arhangelskis,[1] László Fábián,[2] Graham J. Tizzard,[3] Simon J. Coles[3] and William Jones[1]*

1. Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom. 2. School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom. 3. National Crystallography Service, School of Chemistry, University of Southampton, Southampton, SO17 1BJ, United Kingdom.

The pharmaceutically active compound theophylline (**T**) was cocrystallised with the amides formamide (**1**), acetamide (**2**), N-methylformamide (**3**), N,N-dimethylformamide (**4**), benzamide (**5**) and pyrazinamide (**6**), with systems **T:1**, **T:5** and **T:6** displaying polymorphic behaviour. The cocrystals with formamide (**T:1**), acetamide (**T:2**) and benzamide (**T:5**), and one polymorph of the cocrystal with pyrazinamide (**T:6-I**), contain an $R_2^2(9)$ hydrogen bonding motif between the amide cocrystal formers and the HN-C-C=O moiety of the theophylline molecule (an amide-pseudo amide synthon). This motif was, however, absent from the other polymorph of the pyrazinamide cocrystal (**T:6-II**), and also from the N-methylformamide cocrystal (**T:3**) (and is not possible in the N,N-dimethylformamide cocrystal (**T:4**)). These observations are rationalised using hydrogen bond propensity calculations, although limitations of using such calculations for predicting cocrystallisation are noted. The amide-pseudo amide synthon is favoured when theophylline cocrystallises with both primary amides and with secondary amides which are locked in a cis configuration. On heating, all cocrystals were found to dissociate before melting due to loss of the amide, making stability to dissociation a more meaningful measure of cocrystal stability than melting point for these systems. On dissociation of the cocrystals, theophylline typically crystallises as the commonly observed polymorph Form II. In the case of the acetamide cocrystal (**T:2**), however, the rarely observed metastable polymorph, Form V, crystallises concomitantly with Form II suggesting that cocrystal dissociation on heating could be a strategy for generating novel polymorphic forms of compounds.



Mark Eddleston, Department of Chemistry, University of Cambridge, Cambridge, CB2 1EW, UK, tel: +44 1223336465, fax: +44 1223 762829, mde32@cam.ac.uk

Investigation of an amide-pseudo amide hydrogen bonding motif within a series of theophylline:amide cocrystals

Mark D. Eddleston,[1] Mihails Arhangelskis,[1] Laszlo Fábián,[2] Graham J. Tizzard,[3] Simon J. Coles[3] and William Jones[1]*

1. Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom.

2. School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom.

3. National Crystallography Service, School of Chemistry, University of Southampton, Southampton, SO17 1BJ, United Kingdom.

Cocrystals, Polymorphism, Stability, Hydrogen bonding, Crystal chemistry, Pharmaceutical.

The pharmaceutically active compound theophylline (**T**) was cocrystallised with the amides formamide (**1**), acetamide (**2**), N-methylformamide (**3**), N,N-dimethylformamide (**4**), benzamide (**5**) and pyrazinamide (**6**), with systems **T:1**, **T:5** and **T:6** displaying polymorphic behaviour. The cocrystals with formamide (**T:1**), acetamide (**T:2**) and benzamide (**T:5**), and one polymorph of the cocrystal with pyrazinamide (**T:6-I**), contain an $R_2^2(9)$ hydrogen bonding motif between the amide cocrystal formers and the HN-C-C=O moiety of the theophylline molecule (an amide-pseudo amide synthon). This motif was, however, absent from the other polymorph of the pyrazinamide cocrystal (**T:6-II**), and also from the N-methylformamide cocrystal (**T:3**) (and is not possible in the N,N-dimethylformamide cocrystal (**T:4**)). These observations are rationalised using hydrogen bond propensity calculations, although limitations of using such calculations for predicting cocrystallisation are noted. The amide-pseudo amide synthon is favoured when theophylline cocrystallises with both primary amides and with secondary amides which are locked in a cis configuration. On heating, all cocrystals were found to dissociate before melting due to loss of the amide, making stability to dissociation a more meaningful measure of cocrystal stability than melting point for these systems. On dissociation of the cocrystals, theophylline typically crystallises as the commonly observed polymorph Form II. In the case of the acetamide cocrystal (**T:2**), however, the rarely observed metastable polymorph, Form V, crystallises concomitantly with Form II suggesting that cocrystal dissociation on heating could be a strategy for generating novel polymorphic forms of compounds.

Introduction

In recent years, cocrystallisation has emerged as a strategy for improving the solid state properties of compounds,[1-3] and has received increasing interest in many sectors of chemistry including the pharmaceutical industry.[4,5] Cocrystals are crystal forms where two or more neutral molecules are present in the unit cell,[6,7] and can be prepared by a variety of approaches including solution crystallisation, solid state grinding, thermal methods, freeze-drying and slurrying.[8-13]

An important consideration with cocrystallisation is that not every pair of molecules has the propensity to form a cocrystal. In fact, identifying species (coformers) which will cocrystallise with a given compound can sometimes be an arduous process.[14] When screening for cocrystals, a typical first step is to consider the functional groups that are present in the compound of interest and to select coformers that have complementary groups which might be expected to form strong hydrogen bonding interactions.[15] Such a synthon based approach can be aided by using the Cambridge Structural Database (CSD) to identify interactions which form robustly in known crystal structures, and systematic surveys of these interactions, also referred to as supramolecular synthons,[16] have been conducted.[17,18] This has been taken further through the development of a hydrogen bond propensity tool which calculates, on the basis of previously reported crystal structures, the likelihood of each of interactions between the hydrogen bond donor and acceptor

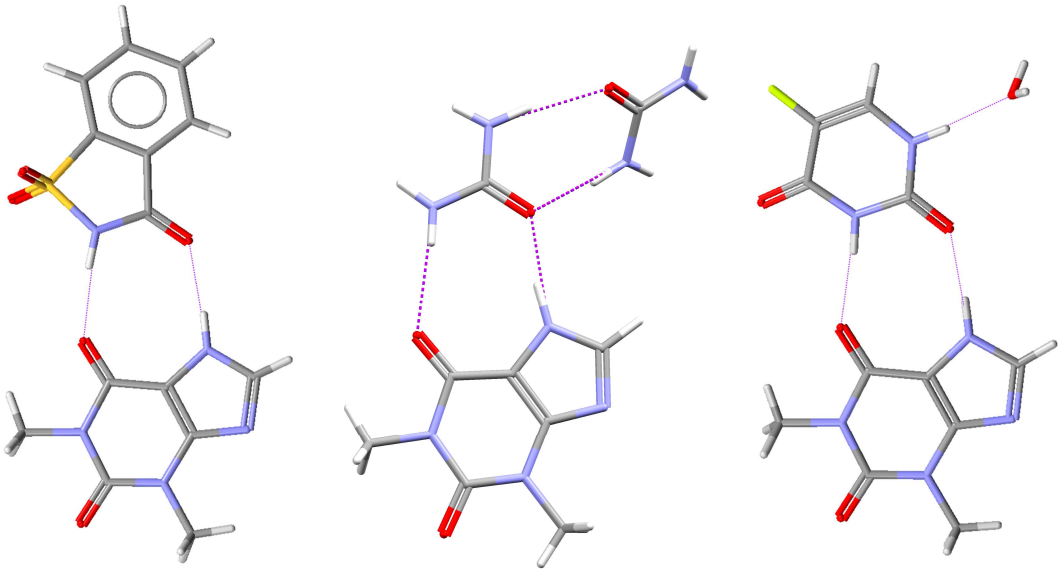
groups in a given molecule (or in multiple molecules), and can be used to predict whether two molecules will cocrystallise.[19,20] Cocrystallisation has also been predicted on the basis of parameters such as the sizes and shapes of coformers.[21]

While there has been much focus on understanding which compounds will form cocrystals, and optimum methods for preparing cocrystals, less attention has been paid to the equally important areas of cocrystal polymorphism and cocrystal stability.[22-25] During early studies into the cocrystallisation of pharmaceutical compounds, it was speculated that cocrystals may show less of a propensity for polymorphic behaviour as they would be expected to have fewer unsatisfied hydrogen bonding groups.[22,26] In fact, the number of reports of cocrystal polymorphism is similar to that for single component phases,[27,28] and any historical differences are more likely to be due to difficulties associated with screening for different polymorphic forms of cocrystals than to an inherent tendency for cocrystals to be monomorphic.[13,29] Cocrystal stability is not yet well understood, but studies have shown that cocrystals can dissociate spontaneously on heating or through partial dissolution of one of the coformers.[2,24,25,30-32]

Theophylline is a pharmaceutically active compound used as a treatment for asthma and COPD for which seven polymorphic forms have been reported.[33-38] Over 40 cocrystals of theophylline are present in the Cambridge Structural Database (version 5.36, see Supporting Information Table S1), three of which comprise coformers having

an amide functionality (a 1:1 cocrystal of theophylline and saccharin, a 1:1 cocrystal of theophylline and urea, and a 2:1 cocrystal monohydrate of theophylline and 5-fluorouracil).[39-41] In each case, there is an $R_2^2(9)$ hydrogen bonding motif between the HN-C-C=O moiety of the theophylline molecule and the amide group of the coformer (Figure 1). This interaction, which will be referred to here as an amide-pseudo amide motif, has not previously been considered as a synthon in supramolecular chemistry.[16]

a b c



d e

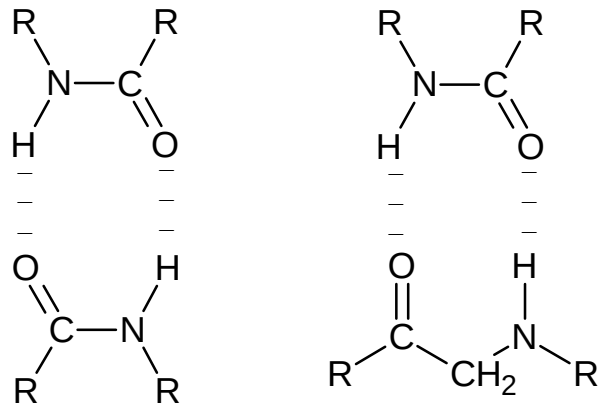


Figure 1. Amide-pseudo amide hydrogen bonding arrangements in the crystal structures of (a) a 1:1 theophylline:saccharin cocrystal (CSD ref XOBCUN[39]), (b) a 1:1 theophylline:urea cocrystal (CSD ref DUXZAX[40]) and (c) a 2:1 theophylline:5-fluorouracil cocrystal monohydrate (CSD ref ZAYLOA[41]). (d) A schematic of the commonly observed amide-amide synthon (graph set notation[42] $R_2^2(8)$). (e) A schematic of the amide-pseudo amide motif (graph set notation $R_2^2(9)$) which is present in the structures shown in (a-c).

In this study, the robustness of the amide-pseudo amide interaction is investigated by cocrystallising theophylline with a series of amides: formamide, acetamide, *N*-methylformamide, *N,N*-dimethylformamide (DMF), benzamide and pyrazinamide (Figure 2). It is noted that there is currently debate in the literature over how cocrystals are defined,[43] which includes whether or not crystal forms containing coformers which

are liquid at ‘room temperature’ should be classed as cocrystals or solvates. Here, solvates are regarded as a sub-set of cocrystals. Polymorphism and thermal stability are also examined within this series of cocrystals.

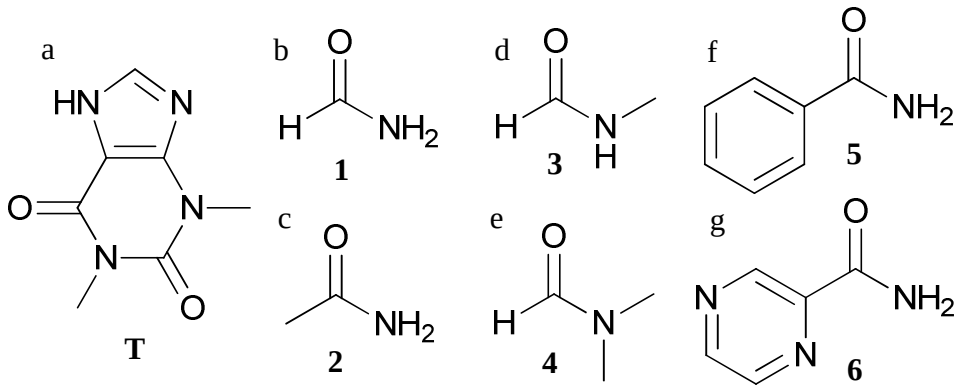


Figure 2. The molecular structures of (a) theophylline (**T**), (b) formamide (**1**), (c) acetamide (**2**), (d) *N*-methylformamide (**3**), (e) *N*,*N*-dimethylformamide (**4**), (f) benzamide (**5**) and (g) pyrazinamide (**6**).

Experimental

All chemicals were purchased from Sigma-Aldrich and used as received.

Cocrystals were prepared by solution crystallisation and liquid assisted grinding. Details are given for the theophylline:benzamide cocrystal (**T:5**) as an example:

Form I of the theophylline:benzamide cocrystal was prepared by adding 3.0 mg of theophylline and 2.2 mg of benzamide (1 mole equivalent) to a solution of chloroform pre-saturated at ambient temperature with theophylline and benzamide and dissolving the solid by heating. The resulting solution was allowed to cool slowly to ambient temperature to induce precipitation. A single crystal suitable for XRD analysis was generated by this method. The cocrystal was also prepared by grinding 150 mg of theophylline and 107.3 mg of benzamide (1 mole equivalent) with 30 µl of ethanol for 30 minutes at 30 Hz within a 10 cm^3 metal vial containing two 7 mm diameter metal balls using a Retsch MM200 ball mill.

Powder X-ray diffraction analysis was performed on a Philips X'Pert Diffractometer equipped with an X'celerator RTMS detector using CuKα radiation at a wavelength of 1.5406 Å. Data were collected between 3 and 50° 2θ at ambient temperature using a collection time of 5 minutes. Powders were pressed gently on a glass slide to give a level surface. PXRD overlays are plotted with an arbitrary intensity scale and were generated using X'Pert Highscore software. Measurements at non-ambient temperature were made using an Anton Paar TK450 heating stage (see Supporting Information).

Single crystal X-ray data were collected at 180 K (unless stated) on a Nonius Kappa CCD diffractometer equipped with an Oxford Cryosystems cooling device using MoKα radiation. The theophylline:acetamide cocrystal crystal structure was collected and solved by the EPSRC UK National Crystallographic Service at the University of Southampton[44] on a Bruker Nonius Instrument with KappaCCD area detector (ϕ scans and ω scans to fill asymmetric unit sphere).

The crystal structure of theophylline:pyrazinamide cocrystal Form II was determined from powder X-ray data. The powder pattern was indexed using the program DICVOL06.[45] The crystal structure was solved by a Simulated Annealing algorithm implemented in the program EXPO 2014.[46] Rietveld refinement[47] was performed using the program TOPAS Academic 4.1.[48] The background was modelled by a Chebyshev polynomial and the peak shape was modelled by a pseudo-Voigt function. Correction of preferred orientation by the March-Dollase[49] method was applied to the (111) crystallographic plane. Throughout the refinement, molecules were treated as rigid bodies, with the exception of a flexible parameter defined to permit rotation of the amide group of pyrazinamide molecule. The refined crystal structure was geometry-optimised using the plane-wave DFT code CASTEP 8.0.[50] The calculation was performed using the PBE[51] functional with G06[52] dispersion correction and norm-conserving pseudo-potentials.[53] The plane wave cutoff and k-point spacing were set to 700 eV and 0.03 $Å^{-1}$, respectively. The unit cell parameters were fixed during the optimisation. Molecular geometries extracted from the optimised structure were used in the final Rietveld

refinement. The covalent bonds to hydrogen atoms were shortened by 0.15 Å to account for the displacement of electron density towards the heavy atoms.

Hydrogen bond propensity calculations were performed using the Solid Form module available as part of Mercury v3.3 software from the Cambridge Crystallographic Data Centre (CCDC) with version 5.35 of the Cambridge Structural Database. The default options were used throughout (including functional group selection).

Differential scanning calorimetry (DSC) thermograms were recorded in a nitrogen atmosphere using a Mettler Toledo STARe DSC822e/700 calorimeter using a heating rate of 10 °C.min^{-1}. Endotherms are plotted as downward peaks. Samples were prepared in 40 µl aluminum pans which were sealed using a cold weld.

Results

Cocrystallisation between theophylline and formamide (**T** and **1**), and between theophylline and acetamide (**T** and **2**), was attempted experimentally by grinding (ethanol was added to the latter to facilitate conversion), yielding a new crystal form in each case.

Single crystals of these new phases suitable for X-ray structure determination were obtained from solution crystallisations and they were each determined to be of 1:1 stoichiometry (**T:1-I** and **T:2** respectively). In both structures, theophylline and amide molecules combine in a pairwise manner through amide-pseudo amide hydrogen bond dimer interactions. The dimers are themselves linked through hydrogen bonding between the NH_2 groups of the amide and the imidazole nitrogen atoms of theophylline to give hydrogen bonded chains (Figure 3a-b). The chains stack in an antiparallel manner to form layers, which in turn close pack to give the full 3-D arrangements. The most noteworthy difference between the two structures is a slight corrugation of the layers in the theophylline:acetamide cocrystal (Figure 3c-d).

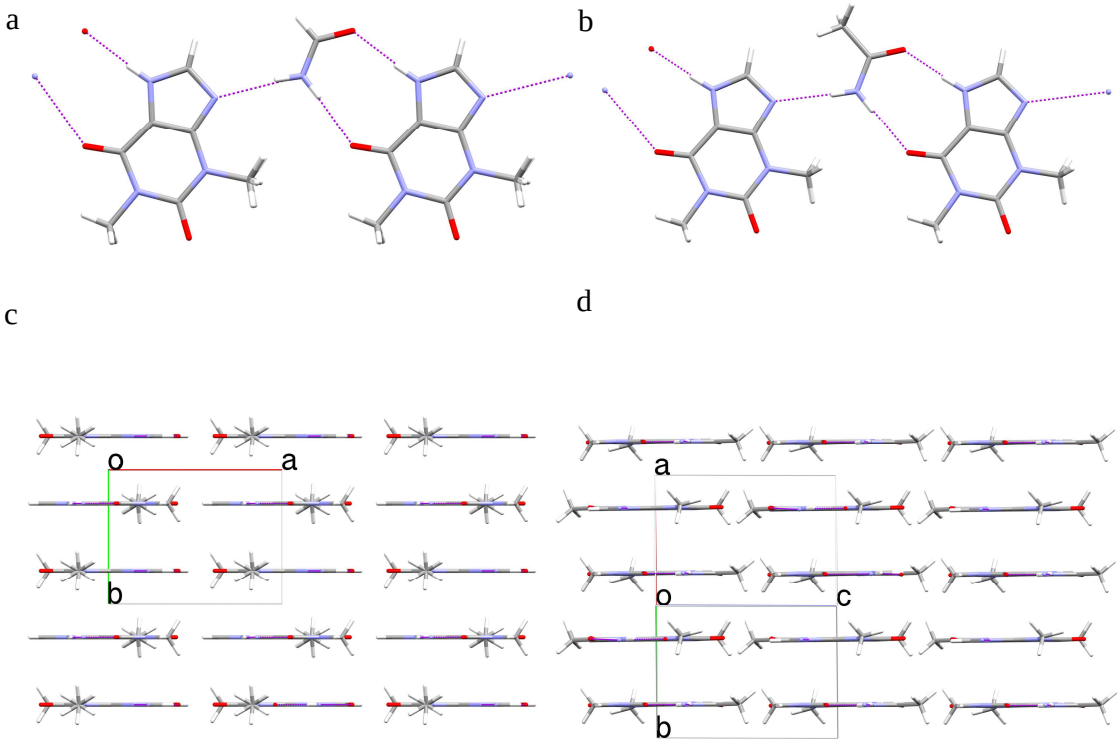


Figure 3. Hydrogen bonding arrangements in the crystal structures of (a) a 1:1 theophylline:formamide cocrystal (270 K) (**T:1-I**) and (b) a 1:1 theophylline:acetamide cocrystal (**T:2**). (c) Crystal packing in **T:1-I** viewed in the direction of the hydrogen bonded chains of theophylline and formamide molecules. (d) An equivalent view for the **T:2** structure.

It is noted that the existence of a theophylline:acetamide cocrystal has previously been postulated by Abourahma *et al* on the basis of powder X-ray diffraction data.[54] The full crystal structure of the 1:1 theophylline acetamide cocrystal reported here confirms this earlier observation.

The fact that theophylline cocrystallises with formamide and acetamide indicates that theophylline-amide interactions are favoured over amide-amide and theophylline-theophylline interactions for these pairs of molecules. In order to further probe this potential competition between homo and hetero interactions, cocrystallisation was attempted between theophylline and the bulkier amides benzamide (**5**) and pyrazinamide (**6**), and also with the amides *N*-methylformamide (**3**) and DMF (**4**), for which fewer theophylline-amide interactions are possible. Furthermore, pyrazinamide possesses two

aromatic nitrogen atoms which could potentially act as hydrogen bond acceptors. Cocrystallisation occurred with theophylline and each of the four amides, and the crystal structures were determined demonstrating a 1:1 theophylline to amide ratio in each case. The hydrogen bonding arrangements are shown in Figure 4.

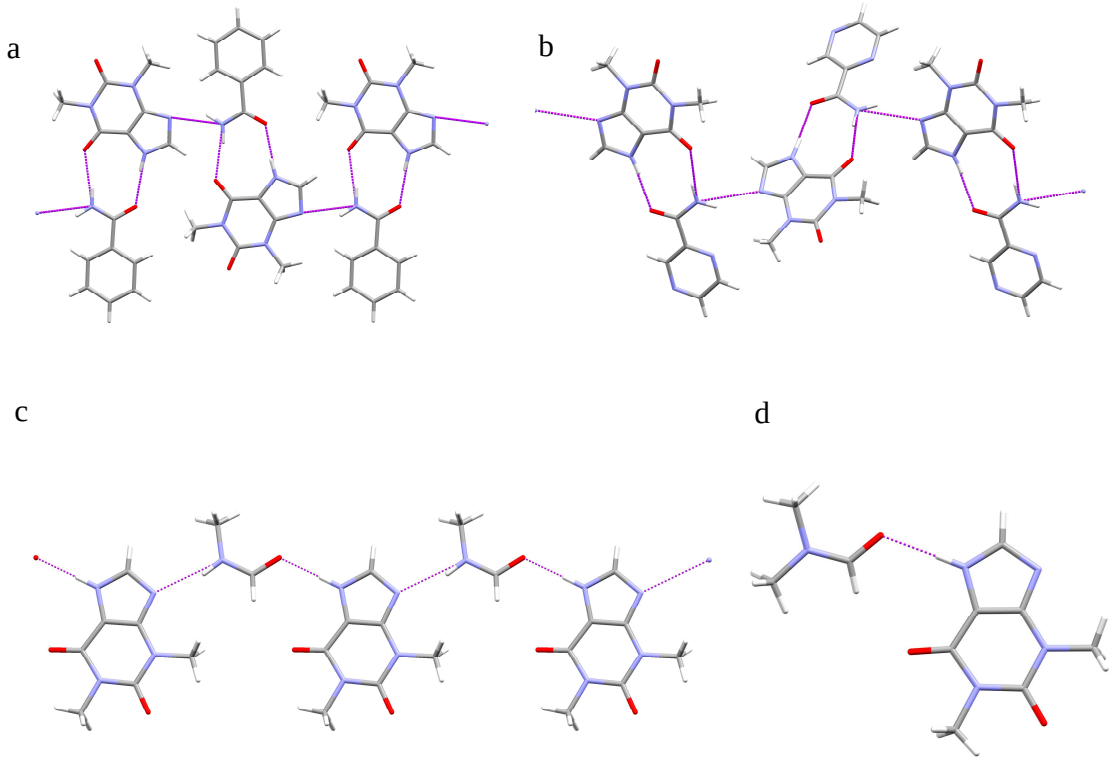


Figure 4. Hydrogen bonding arrangements in the crystal structures of (a) Form I of a theophylline:benzamide cocrystal (**T:5-I**), (b) Form I of a theophylline:pyrazinamide cocrystal (**T:6-I**), (c) a theophylline:*N*-methylformamide cocrystal (**T:3**) and (d) a theophylline:DMF cocrystal (**T:4**).

The amide-pseudo amide hydrogen bond dimer interaction is observed in **T:5-I** and **T:6-I**, with NH···N hydrogen bonds bridging these dimers to give hydrogen bonded chains of molecules as seen for the formamide and acetamide (**T:1** and **T:2**) cocrystals (though in **T:6-I** the hydrogen bonding no longer occurs in the same plane giving rise to a twisting of the chains). With both *N*-methylformamide (**3**) and DMF (**4**), although there is an interaction between the hydrogen bond donor NH group of theophylline and the amide carbonyl group, the full amide-pseudo amide interaction is not formed. In the case of DMF this is not surprising as the amide does not possess a hydrogen bond donor group. Interestingly, with *N*-methylformamide, the hydrogen bond donor NH group of the amide interacts with the imidazole nitrogen of a second theophylline molecule, rather than with theophylline's cyclic carbonyl group, to give hydrogen bonded chains instead of the expected discrete dimers. This observation can be rationalised by considering the energies of different conformations of the *N*-methylformamide molecule. The *cis*-form, which would be needed in order to form an amide-pseudo amide interaction in the cocrystal, has been calculated to be 0.872 kcal.mol^{-1} higher in energy than the *trans*-form (that shown in Figure 4c),[55] potentially making the formation of an amide-pseudo amide interaction energetically unfavourable.

Cocrystal Stability

A further observation is that the decreasing number of theophylline-amide hydrogen bonds in the cocrystal series with formamide (**T:1-I**), *N*-methylformamide (**T:3**) and DMF (**T:4**) has a pronounced influence on cocrystal stability. Each of these cocrystals was found to be unstable during storage under ambient conditions due to desolvation of the amide, but the relative rate of desolvation was found to be DMF >> *N*-methylformamide > formamide (the DMF cocrystal dissociates completely within an hour, whereas the formamide cocrystal loses solvent slowly over a period of several days as measured by PXRD (see Supporting Information Figures S1– S2)).

The thermal stability of the acetamide (**T:2**), benzamide (**T:5-I**) and pyrazinamide (**T:6-I**) cocrystals, which are stable under ambient conditions, was also investigated. The melting points of these cocrystals were determined by DSC analysis (in sealed pans) to be 169° C, 144° C and 205° C respectively (see Supporting Information Figures S3-S5). Interestingly, the trend in cocrystal melting points differs from that for the individual amide coformers (the melting points of acetamide, benzamide and pyrazinamide were measured by DSC to be 80° C, 125° C and 189° C respectively – Supporting Information Figures S6-S8). Similar observations have been made previously for cocrystals of the pharmaceutical ingredient diclofenac.[56]

When the three cocrystals, each prepared by grinding to give approximately equivalent particle sizes (as indicated by optical microscopy), were heated in an open system it was found that they undergo dissociation prior to melting. This dissociation is accompanied by sublimation of the amide coformer and crystallisation of the residual theophylline, typically as Form II, the most widely observed theophylline polymorph (see Supporting

Information Figures S9, S10 and S11). The onset of dissociation was 105° C for the acetamide cocrystal, 145° C for the pyrazinamide cocrystal and 165° C for the benzamide cocrystal (the loss of benzamide is rapid at this temperature). Importantly, the ranking of the cocrystals in terms of stability to thermal dissociation is different to that for melting temperatures, and because dissociation occurs at a lower temperature than melting it is perhaps the more relevant measure of thermal stability. Furthermore, the dissociation of the theophylline:amide cocrystals was also investigated at a constant temperature of 80° C, a temperature relevant from a pharmaceutical perspective as it may be reached during processes such as drying or tableting. The relative rate of cocrystal dissociation (as determined by measuring the intensities of the resulting theophylline reflections) was found to be acetamide > benzamide > pyrazinamide, this trend being inversely related to the melting points of the amide coformers (i.e. the higher the melting point of the coformer the slower the cocrystal dissociation).

Another noteworthy observation relating to the dissociation of the theophylline:acetamide cocrystal is that the loss of acetamide results in the concomitant crystallisation of two different polymorphic forms of theophylline, Forms II and V (see Supporting Information Figure S10). Form II is the most commonly observed theophylline polymorph, whereas Form V is a seldom observed crystal form that has been previously isolated during supercritical antisolvent crystallisations of theophylline,[35] and in trace amounts during crystallisations from methanol.[36] Here, thermal dissociation of the acetamide cocrystal has provided an alternative route to observing this unusual crystal form of theophylline, albeit as a minor phase in a mixture with Form II.

Cocrystal Polymorphism

Although no extensive crystal form screening was performed during this study, second polymorphic forms of the formamide (**T:1**), benzamide (**T:5**) and pyrazinamide (**T:6**) cocrystals were identified while investigating the cocrystallisation of theophylline with these amides.

The theophylline:formamide cocrystal (**T:1-I**) undergoes a reversible polymorphic conversion to a low temperature phase on cooling from room temperature to 180 K. The hydrogen bonding arrangement between theophylline and formamide molecules is maintained through this transition, enabling it to proceed in a single crystal to single crystal manner, but subtle changes to the crystal packing occur and give rise to a change of space group from $P2_1/m$ to *P-1*. The low temperature phase will be referred to as Form II of the cocrystal (**T:1-II**). An overlay of powder X-ray diffraction patterns of **T:1-I** and **T:1-II** is given in the Supporting Information (Figure S12).

A possible second polymorphic form of the theophylline:benzamide cocrystal (**T:5-II**) was isolated on grinding theophylline and benzamide in an equimolar ratio in the presence of nitromethane (as evidenced by the resulting sample having a PXRD trace that

was different to that of Form I of the cocrystal and to those of all of the known forms of theophylline and benzamide – it has not been possible to obtain a structure solution for this form to date and further work would be needed to unambiguously confirm that it is a cocrystal polymorph). Evidence that this new crystal form is a cocrystal polymorph, rather than a nitromethane solvate comes from the observation that grinding Form I of the cocrystal with nitromethane does not lead to a change in crystal form (even with several stoichiometric equivalents of solvent). The PXRD traces of the two polymorphs of the theophylline:benzamide cocrystal are shown in Figure 5.

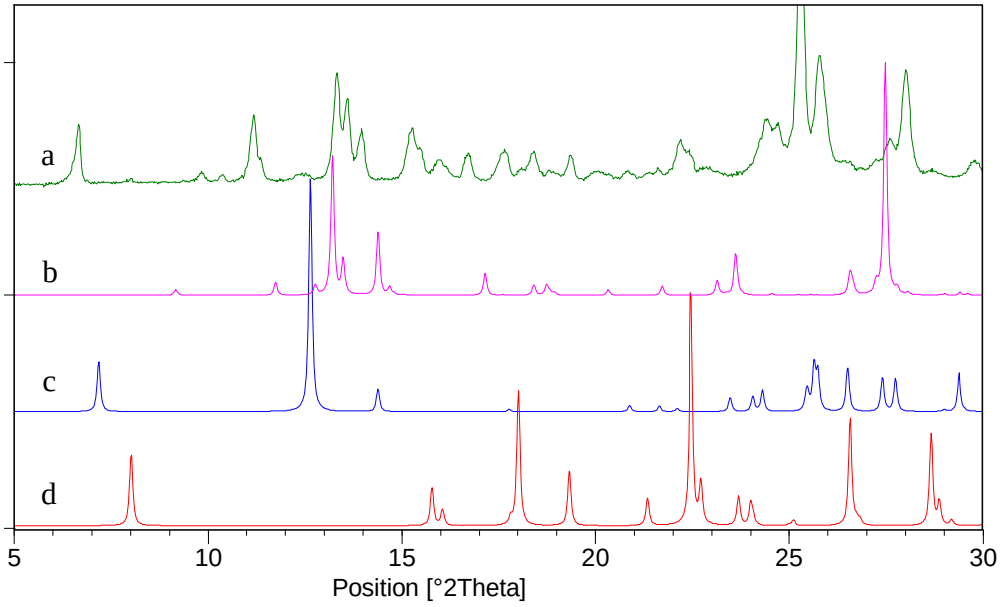


Figure 5. PXRD overlay of traces of two polymorphs of the 1:1 theophylline:benzamide cocrystal. (a) Experimental trace of **T:5-II** obtained by liquid assisted grinding with nitromethane. (b) Simulated trace of **T:5-I**. (c) Reference trace of Form II of theophylline

(simulated from CSD crystal structure BAPLOT01)[33]. (d) Reference trace of Form I of benzamide (simulated from CSD crystal structure BZAMID01).

Interestingly, Form II appears to display the characteristics of a disappearing polymorph. This form has been obtained only twice, on both occasions through grinding theophylline and benzamide in the presence of nitromethane, though the same grinding conditions more commonly lead to the formation of Form I of the cocrystal. It is believed that a seeding effect has an important influence on the polymorphic outcome of the experiment, and if Form I of the cocrystal is present initially, even in trace amounts, this will direct the cocrystallisation and Form I will be obtained from the grinding. Indeed, on the two occasions that Form II has been isolated there had been no work on the theophylline:benzamide cocrystal for several months prior to the experiment, meaning that seeds of Form I are likely to have been absent from the laboratory.

Two forms of the theophylline:pyrazinamide cocrystal were identified during this investigation, with the method of cocrystallisation dictating which form is obtained. Form I (**T:6-I** described above) can be generated by liquid assisted grinding in the presence of both polar (e.g. DMF) and non-polar (e.g. toluene) solvents. In contrast, solution crystallisation using both polar and non-polar solvents only yielded Form II of the cocrystal (**T:6-II**). Interestingly, in **T:6-II** theophylline and pyrazinamide molecules do not interact through amide-pseudo amide synthons, as in **T:6-I**, but instead form

homo-dimers that are linked by hydrogen bonding between the amide nitrogen atoms of pyrazinamide and the imidazole nitrogen atoms of theophylline to give chains (Figure 6). The fact that theophylline:amide dimers exist in one polymorph of the cocrystal, whereas theophylline:theophylline and amide:amide dimers are present in the other suggests that the respective hydrogen bonding arrangements are very similar in energy (experimentally observed polymorphs typically have lattice energies which differ by less than 10 kJ mol^{-1}).[57-60]

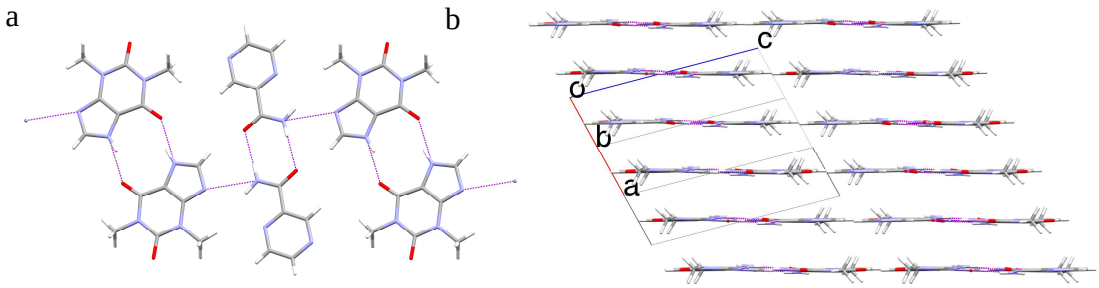


Figure 6. The crystal structure of Form II of the theophylline:pyrazinamide cocrystal (**T:6-II**). (a) Hydrogen bonding interactions between theophylline and pyrazinamide molecules. (b) Crystal packing in the cocrystal viewed in the direction of the hydrogen bonded chains of theophylline and pyrazinamide molecules. See Figure 4b for a comparison with Form I (**T:6-I**).

Crystallographic data for the reported theophylline:amide cocrystal structures are given in Table 1.

Table 1. Crystallographic data for the reported theophylline:amide cocrystals.

Amide coformer	Formamide (**T:1-I**)	Formamide (**T:1-II**)	*N*-methylform-amide (**T:3**)	DMF (**T:4**)
Stoichiometry	1:1	1:1	1:1	1:1
Crystal system	Monoclinic	Triclinic	Triclinic	Monoclinic
Space group	$P2_1/m$	*P-1*	*P-1*	$P2_1/c$
a (Å)	8.7314(3)	6.6058(13)	6.6316(3)	4.4183(1)
b (Å)	6.6582(3)	8.7163(17)	8.7905(4)	14.3872(6)
c (Å)	8.8996(4)	8.8843(18)	9.5955(4)	19.3622(9)
α (degrees)	90	81.34(3)	92.441(2)	90
β (degrees)	98.546(2)	87.63(3)	92.929(2)	93.41(3)
γ (degrees)	90	87.47(3)	90.609(2)	90
V (Å^3)	511.64(4)	504.90(17)	558.09(4)	1228.62(8)
Z	2	2	2	4
λ (Å)	0.71073	0.71073	0.71073	0.71073
θ range	3.55-27.56	3.55-27.56	3.65-30.11	3.53-26.07
Data/constraints/ parameters	1797/0/104	2267/0/149	3252/0/157	2403/0/167
ρ_{calc} (g.cm^{-3})	1.462	1.481	1.424	1.369
T (K)	270(2)	180(2)	180(2)	250(2)

R_1	0.0536	0.0570	0.1168	0.0545
wR_2	0.1319	0.1343	0.2978	0.1309

Amide coformer	Acetamide (**T:2**)	Benzamide (**T:5-I**)	Pyrazinamide (**T:6-I**)*	Pyrazinamide (**T:6-II**)
Stoichiometry	1:1	1:1	1:1	1:1
Crystal system	Triclinic	Monoclinic	Orthorhombic	Triclinic
Space group	*P-1*	*P21/c*	*Pna2$_1$*	*P-1*
a (Å)	7.6545(13)	7.5275(2)	13.455(2)	7.4800(2)
b (Å)	8.3489(14)	13.3891(4)	13.288(2)	7.6959(2)
c (Å)	8.9540(16)	13.8564(4)	7.6215(4)	12.7028(4)
α (degrees)	90.552(8)	90	90	86.113(2)
β (degrees)	91.339(11)	91.486(2)	90	75.930(2)
γ (degrees)	110.177(12)	90	90	68.995(2)
V (Å^3)	536.86(16)	1396.07(7)	1362.6(4)	662.02(3)
Z	2	4	4	2
λ (Å)	0.71073	0.71073	1.54056	0.71073
θ range	3.12-25.00	3.95-27.11	1.5-25.0	3.75-30.03
Data/constraints/ parameters	1869/0/157	3072/0/202	-/92/37	3825/0/201
ρ_{calc} (g.cm^{-3})	1.480	1.434	1.478	1.522
T (K)	120(2)	180(2)	Ambient	180(2)
R_1	0.0909	0.0477	-	0.0548
wR_2	0.2189	0.1125	-	0.1138

R_{wp}	-	-	0.0660	-
R_{exp}	-	-	0.0126	-

* This structure was solved using powder X-ray diffraction data.

Discussion

The Amide-Pseudo Amide Interaction

The $R_2^2(9)$ amide-pseudo amide motif is present in the formamide (both polymorphs), acetamide and benzamide cocrystals of theophylline, and in one of the polymorphs of the pyrazinamide cocrystal, but is absent from the *N*-methylformamide and DMF cocrystals (though it should be noted that there is no possibility of forming this interaction with DMF as this molecule has no hydrogen bond donor). From these observations it can be concluded that the amide-pseudo amide motif is a highly favourable interaction. On considering a wider set of theophylline:amide cocrystals, including both those reported here and the theophylline:amide cocrystals published in the CSD, it is evident that the amide-pseudo amide motif is seen with all of the primary amides (formamide, acetamide, benzamide, pyrazinamide and urea) and with secondary amides which are locked in a *cis* conformation, i.e. due to being part of a ring (saccharin and 5-fluorouracil). For other secondary amide coformers (*N*-methylformamide, paracetamol and sulfacetamide), where cocrystals with theophylline do not contain the amide-pseudo amide motif, it appears that adopting a *trans* geometry gives a greater energetic stabilisation than forming this interaction. The exception to this trend is the 2:1 theophylline:phenobarbital cocrystal, where, despite the *cis* arrangement of the amide moieties of

the conformer, the amide-pseudo amide interaction does not occur, probably because this would lead to the formation of discrete trimers of molecules rather than the observed extended hydrogen bonded chains.

Hydrogen bond propensities

In order to place the above observations into the wider context of known crystal structures hydrogen bond propensity calculations were performed for each of the pairs of molecules (theophylline + amide) which were found to cocrystallise in this study. These calculations take into account which functional groups are involved in hydrogen bonding interactions in the crystal structures of similar molecules present in the CSD, and were generated using the Solid Form module in the Mercury v3.3 software package. For example, the hydrogen bond donor and acceptor groups of theophylline (**T**) and the amides acetamide (**2**), DMF (**4**) and *N*-methylformamide (**3**) are labelled in Figure 7, and the resulting propensity values for the pairs of molecules theophylline/formamide and theophylline/acetamide are listed in Table 2.

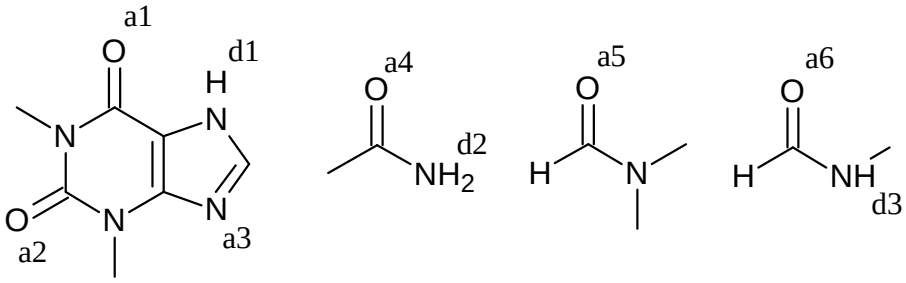


Figure 7. Hydrogen bond donor and acceptor groups of the molecules theophylline (**T**), acetamide (**2**), DMF (**4**) and *N*-methylformamide (**3**) labelled with reference to the hydrogen bond propensities listed in Table 2.

Table 2. Table showing calculated propensities for hydrogen bond formation between donor and acceptor groups of theophylline and the amides acetamide, DMF and *N*-methylformamide. The labelling of donor and acceptor groups is as shown in Figure 7 (**T** = theophylline group, **A** = amide group). Propensities are quoted on a scale from 0 to 1, with higher values indicating a greater likelihood of formation.

Theophylline and acetamide			Theophylline and DMF			Theophylline and *N*-methylformamide		
Donor	Acceptor	Propensity	Donor	Acceptor	Propensity	Donor	Acceptor	Propensity
d2 (**A**)	a4 (**A**)	0.90	d1 (**T**)	a5 (**A**)	0.74	d3 (**A**)	a6 (**A**)	0.73
d1 (**T**)	a4 (**A**)	0.74	d1 (**T**)	a3 (**T**)	0.50	d1 (**T**)	a6 (**A**)	0.64
d2 (**A**)	a1 (**T**)	0.74	d1 (**T**)	a1 (**T**)	0.48	d3 (**A**)	a1 (**T**)	0.63
d2 (**A**)	a3 (**T**)	0.73	d1 (**T**)	a2 (**T**)	0.23	d3 (**A**)	a3 (**T**)	0.53
d1 (**T**)	a1 (**T**)	0.46				d1 (**T**)	a1 (**T**)	0.52
d2 (**A**)	a2 (**T**)	0.44				d1 (**T**)	a3 (**T**)	0.43
d1 (**T**)	a3 (**T**)	0.43				d3 (**A**)	a2 (**T**)	0.31
d1 (**T**)	a2 (**T**)	0.19				d1 (**T**)	a2 (**T**)	0.23

For the pair of molecules theophylline and acetamide, the hydrogen bond calculated to have the highest likelihood of formation is that between the NH_2 and C=O moieties of acetamide (groups labelled d2 and a4 in Figure 7). Taken in isolation, this result would suggest that acetamide molecules are more likely to interact with each other, rather than with theophylline molecules, making cocrystallisation between theophylline and acetamide unlikely. When hydrogen bonding propensities relating to the NH donor group of theophylline (d1) are taken into consideration, however, it is evident that there is a much greater likelihood of this group interacting with the amide oxygen of acetamide (a4) than with an acceptor group from another theophylline molecule. In fact, the interactions that comprise an amide-pseudo amide interaction between theophylline and acetamide molecules (between groups d1 + a4 and d2 + a1) are significantly more likely to occur than any of the possible theophylline-theophylline interactions. This indicates there that there is competition as to whether it is the hydrogen bond donor group of acetamide (d2) or of theophylline (d1) that will interact with its preferred acceptor. The fact that theophylline and acetamide undergo cocrystallisation in practice suggests that overall theophylline-acetamide interactions are more favourable than the average of acetamide-acetamide and theophylline-theophylline interactions, but it would have been difficult to predict with confidence '*a priori*' whether cocrystallisation between theophylline and acetamide will occur without further calculations (such as determination of the relative lattice energies of coformers and the cocrystal through crystal structure prediction).

Propensity calculations for theophylline with formamide, benzamide and pyrazinamide are broadly similar to those for theophylline and acetamide (see Supporting Information Table S2),

which is unsurprising given the identical hydrogen bonding motifs seen in the four corresponding cocrystal structures.

As the molecule DMF does not possess a hydrogen bond donor group, the only donor for the theophylline/DMF system is the imidazole NH group of theophylline (d1). In the crystal structure, this group forms the interaction that has the highest calculated propensity, that to the amide oxygen of DMF (a5).

With *N*-methylformamide, the theophylline NH hydrogen bond donor group (d1) interacts with its most likely acceptor, the amide carbonyl oxygen (a6). The amide NH hydrogen bond donor group (d3) does not, however, interact with the theophylline carbonyl group (a1) to give an amide-pseudo amide motif, instead forming a hydrogen bond to the imidazole nitrogen of theophylline (a3). The amide NH group has a similar likelihood of interacting with the carbonyl group and the imidazole nitrogen (0.63 and 0.53 respectively), and the fact that an interaction with a1 requires a higher energy *cis* conformation of the amide (given that the d1 – a6 interaction is also present) explains why the interaction with a3 is seen experimentally.

Importantly, because formamide and *N*-methylformamide are liquids at room temperature, the most likely interaction, that between the NH and C=O groups of the amide (groups d2/d3 and a4/a6), is less relevant to a consideration of crystal forms. As theophylline-amide interactions are significantly more likely than theophylline-theophylline interactions, it would have been possible

to predict in advance that cocrystallisation would occur, as observed experimentally. Interestingly, when cocrystallisation between theophylline and *N*-methylbenzamide, an *N*-methyl substituted amide which is solid at room temperature, was attempted by liquid assisted grinding, no cocrystal formation occurred. Clearly, in this system there is competition between amide-amide and theophylline-amide interactions, and it appears that the amide-amide interactions are dominant.

Form II of the theophylline:pyrazinamide cocrystal is the only theophylline:amide cocrystal structure identified in this study where amide-amide hydrogen bonds, which have the highest calculated propensity, are actually observed. Moreover, this crystal form highlights a limitation of using hydrogen bond propensities to predict the likelihood that two compounds will cocrystallise. Even in a situation where the coformers form homosynthons, rather than heterosynthons, giving dimers or chains of the same molecule, there is still a possibility that cocrystallisation will occur if these units interact through secondary hydrogen bonds (as in the case of **T:6-II**), or favourable dispersive interactions. For this reason, other predictive tools (such as crystal structure prediction) will generally prove to be more robust for determining whether a pair of molecules will cocrystallise.

Conclusions

The robustness of the amide-pseudo amide interaction was probed by preparing a set of theophylline:amide cocrystals. This motif was noted to form reliably, both with primary amides and secondary amides locked in a cis geometry, indicating that should be treated as a plausible synthon for the purposes of crystal engineering. Hydrogen bond propensity calculations were useful for rationalising interactions in the theophylline:amide cocrystal structures, but would not have given '*a priori*' a clear indication of whether cocrystallisation would or would not occur in these systems. Furthermore, cocrystallisation was observed to occur in a system in which the two coformers did not interact through a strong hydrogen bond (theophylline:pyrazinamide, **T:6-II**), and it is noted that such a situation is not taken into account when using hydrogen bond propensities to predict cocrystal formation. In addition, there is an indication that it may be important to make a distinction between whether coformers are solid or liquid at room temperature when predicting the likelihood of cocrystal formation as less hydrogen bond competition would be expected for liquid coformers.

On heating in an open system, each of the theophylline:amide cocrystals isolated in this study dissociated through loss of the amide coformer prior to melting. Dissociation temperature is a more important measure of thermal stability for these cocrystals than melting point, and it is likely that such a situation will be common for cocrystals in general (where one or both of the coformers become volatile at a temperature below the melting point of the cocrystal).

Dissociation of the theophylline:acetamide cocrystal on heating yielded the rarely observed Form V of theophylline, demonstrating that cocrystallisation/thermal dissociation cycles could

be a route to preparing novel or unusual metastable polymorphic forms of compounds. The desolvation of solvates is a widely used method of exploring polymorphism of compounds,[61-63] but to the knowledge of the authors this is the first example to demonstrate that cocrystal formation/thermal dissociation may provide a method of exploring polymorphism of a compound by giving access to alternate crystallisation conditions.

ASSOCIATED CONTENT

Supporting Information. List of theophylline cocrystals published in the Cambridge Structural Database, thermal analysis of amides and theophylline:amide cocrystals and X-ray crystal structures of theophylline:amide cocrystals. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

Mark Eddleston, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK, tel: +44 1223336465, fax: +44 1223 762829, mde32@cam.ac.uk

ACKNOWLEDGMENT

The authors thank Dr J. E. Davies for single crystal X-ray crystal structure determinations and are grateful to the EU INTERREG IVA 2 Mers Seas Zeeën Cross-border Cooperation Programme for funding.

REFERENCES

(1) Remenar, J. F.; Morissette, S. L.; Peterson, M. L.; Moulton, B.; MacPhee, J. M.; Guzman, H. R.; Almarsson, Ö. *Journal of the American Chemical Society* **2003**, *125*, 8456.

(2) Trask, A. V.; Motherwell, W. D. S.; Jones, W. *Crystal Growth & Design* **2005**, *5*, 1013.

(3) Babu, N. J.; Sanphui, P.; Nangia, A. *Chemistry - An Asian Journal* **2012**, *7*, 2274.

(4) Shan, N.; Zaworotko, M. J. *Drug Discovery Today* **2008**, *13*, 440.

(5) Qiao, N.; Li, M.; Schlindwein, W.; Malek, N.; Davies, A.; Trappitt, G. *International Journal of Pharmaceutics* **2011**, *419*, 1.

(6) Caira, M. R. *Journal of Crystallographic and Spectroscopic Research* **1992**, *22*, 193.

(7) Almarsson, O.; Zaworotko, M. J. *Chemical Communications* **2004**, 1889.

(8) Rodriguez-Hornedo, N.; Nehm, S. J.; Seefeldt, K. F.; Pagan-Torres, Y.; Falkiewicz, C. J. *Molecular Pharmaceutics* **2006**, *3*, 362.

(9) Shan, N.; Toda, F.; Jones, W. *Chemical Communications* **2002**, 2372.

(10) Davis, R. E.; Lorimer, K. A.; Wilkowski, M. A.; Rivers, J. H.; Wheeler, K. A.; Bowers, J. *ACA Transactions* **2004**, *39*, 41.

(11) Eddleston, M. D.; Patel, B.; Day, G. M.; Jones, W. *Crystal Growth & Design* **2013**, *13*, 4599.

(12) Zhang, G. G. Z.; Henry, R. F.; Borchardt, T. B.; Lou, X. *Journal of Pharmaceutical Sciences* **2007**, *96*, 990.

(13) Eddleston, M. D.; Sivachelvam, S.; Jones, W. *CrystEngComm* **2013**, *15*, 175.

(14) Karki, S.; Friscic, T.; Fabian, L.; Jones, W. *CrystEngComm* **2010**, *12*, 4038.

(15) Etter, M. C. *Journal of Physical Chemistry* **1991**, *95*, 4601.

(16) Desiraju, G. R. *Angewandte Chemie, International Edition in English* **1995**, *34*, 2311.

(17) Shattock, T. R.; Arora, K. K.; Vishweshwar, P.; Zaworotko, M. J. *Crystal Growth & Design* **2008**, *8*, 4533.

(18) Bucar, D.-K.; Henry, R. F.; Lou, X.; Duerst, R. W.; Borchardt, T. B.; MacGillivray, L. R.; Zhang, G. G. Z. *Molecular Pharmaceutics* **2007**, *4*, 339.

(19) Galek, P. T. A.; Fabian, L.; Motherwell, W. D. S.; Allen, F. H.; Feeder, N. *Acta Crystallographica, Section B: Structural Science* **2007**, *B63*, 768.

(20) Delori, A.; Galek, P. T. A.; Pidcock, E.; Jones, W. *Chemistry - A European Journal* **2012**, *18*, 6835.

(21) Fabian, L. *Crystal Growth & Design* **2009**, *9*, 1436.

(22) Aitipamula, S.; Chow, P. S.; Tan, R. B. H. *CrystEngComm* **2014**, *16*, 3451.

(23) Friščić, T.; Fábián, L.; Burley, J. C.; Jones, W.; Motherwell, W. D. S. *Chemical Communications* **2006**, 5009.

(24) Eddleston, M. D.; Lloyd, G. O.; Jones, W. *Chemical Communications* **2012**, *48*, 8075.

(25) Eddleston, M. D.; Thakuria, R.; Aldous, B. J.; Jones, W. *J. Pharm. Sci.* **2014**, *103*, 2859.

(26) Vishweshwar, P.; McMahon, J. A.; Peterson, M. L.; Hickey, M. B.; Shattock, T. R.; Zaworotko, M. J. *Chemical Communications* **2005**, 4601.

(27) Clarke, H. D.; Arora, K. K.; Bass, H.; Kavuru, P.; Ong, T. T.; Pujari, T.; Wojtas, L.; Zaworotko, M. J. *Crystal Growth & Design* **2010**, *10*, 2152.

(28) Aitipamula, S.; Chow, P. S.; Tan, R. B. H. *Crystal Growth & Design* **2010**, *10*, 2229.

(29) Porter III, W. W.; Elie, S. C.; Matzger, A. J. *Crystal Growth & Design* **2008**, *8*, 14.

(30) Trask, A. V.; Motherwell, W. D. S.; Jones, W. *International Journal of Pharmaceutics* **2006**, *320*, 114.

(31) Eddleston, M. D.; Madusanka, N.; Jones, W. *J. Pharm. Sci.* **2014**, *103*, 2865.

(32) I. Sarcevica, L. O., K. P. Nartowski, Y. Z. Khimyak, A. N. Round, L. Fabian *Molecular Pharmaceutics* **2015**, *12*, 2981.

(33) Ebisuzaki, Y.; Boyle, P. D.; Smith, J. A. *Acta Crystallographica, Section C: Crystal Structure Communications* **1997**, *C53*, 777.

(34) Seton, L.; Khamar, D.; Bradshaw, I. J.; Hutcheon, G. A. *Crystal Growth & Design* **2010**, *10*, 3879.

(35) Roy, C.; Vega-Gonzalez, A.; Subra-Paternault, P. *International Journal of Pharmaceutics* **2007**, *343*, 79.

(36) Eddleston, M. D.; Bithell, E. G.; Jones, W. *Journal of Pharmaceutical Sciences* **2010**, *99*, 4072.

(37) Eddleston, M. D.; Hejczyk, K. E.; Bithell, E. G.; Day, G. M.; Jones, W. *Chemistry - A European Journal* **2013**, *19*, 7883.

(38) Eddleston, M. D.; Hejczyk, K. E.; Cassidy, A. M. C.; Thompson, H. P. G.; Day, G. M.; Jones, W. *Crystal Growth & Design* **2015**, *15*, 2514.

(39) Lu, E.; Rodriguez-Hornedo, N.; Suryanarayanan, R. *CrystEngComm* **2008**, *10*, 665.

(40) Wiedenfeld, H.; Knoch, F. *Archiv der Pharmazie (Weinheim, Germany)* **1986**, *319*, 654.

(41) Zaitu, S.; Miwa, Y.; Taga, T. *Acta Crystallographica, Section C: Crystal Structure Communications* **1995**, *C51*, 1857.

(42) Etter, M. C.; MacDonald, J. C.; Bernstein, J. *Acta Crystallographica, Section B: Structural Science* **1990**, *B46*, 256.

(43) Aitipamula, S.; Banerjee, R.; Bansal, A. K.; Biradha, K.; Cheney, M. L.; Choudhury, A. R.; Desiraju, G. R.; Dikundwar, A. G.; Dubey, R.; Duggirala, N.; Ghogale, P. P.; Ghosh, S.; Goswami, P. K.; Goud, N. R.; Jetti, R. R. K. R.; Karpinski, P.; Kaushik, P.; Kumar, D.; Kumar, V.; Moulton, B.; Mukherjee, A.; Mukherjee, G.; Myerson, A. S.; Puri, V.; Ramanan, A.; Rajamannar, T.; Reddy, C. M.; Rodriguez-Hornedo, N.; Rogers, R. D.; Row, T. N. G.; Sanphui, P.; Shan, N.; Shete, G.; Singh, A.; Sun, C. Q. C.; Swift, J. A.; Thaimattam, R.; Thakur, T. S.; Thaper, R. K.; Thomas, S. P.; Tothadi, S.; Vangala, V. R.; Variankaval, N.; Vishweshwar, P.; Weyna, D. R.; Zaworotko, M. J. *Crystal Growth & Design* **2012**, *12*, 2147.

(44) Coles, S. J.; Gale, P. A. *Chemical Science* **2012**, *3*, 683.

(45) Boultif, A.; Louër, D. *J. Appl. Cryst.* **2004**, *37*, 724.

(46) Altomare, A.; Cuocci, C.; Giacovazzo, C.; Moliterni, A.; Rizzi, R.; N., C.; A., F. *J. Appl. Cryst.* **2013**, *46*, 1231.

(47) Rietveld, H. M. *Acta Cryst.* **1967**, *22*, 151.

(48) Coelho, A. A. **2007**, TOPAS Academic. Version 4.1.
(49) Dollase, W. A. *J. Appl. Cryst.* **1986**, *19*, 267.
(50) Clark, S. J.; Segall, M. D.; Pickard, C. J.; Hasnip, P. J.; Probert, M. I. J.; Refson, K.; Payne, M. C. *Z. Kristallogr.* **2005**, *220*, 567.
(51) Perdew, J. P.; Burke, K.; Ernzerhof, M. *Phys. Rev. Lett.* **1996**, *77*, 3865.
(52) Grimme, S. *J. Comput. Chem.* **2006**, *27*, 1787.
(53) Rappe, A. M.; Rabe, K. M.; Kaxiras, E.; Joannopoulos, J. D. *Phys. Rev. B* **1990**, *41*, 1227.
(54) Abourahma, H.; Urban, J. M.; Morozowich, N.; Chan, B. *CrystEngComm* **2012**, *14*, 6163.
(55) Shin, S.; Kurawaki, A.; Hamada, Y.; Shinya, K.; Ohno, K.; Tohara, A.; Sato, M. *Journal of Molecular Structure* **2006**, *791*, 30.
(56) Aakeröy, C. B.; Grommet, A. B.; Desper, J. *Pharmaceutics* **2011**, *3*, 601.
(57) Bernstein, J. *Polymorphism in Molecular Crystals*; Oxford University Press: Oxford, U.K., 2002.
(58) Beyer, T.; Day, G. M.; Price, S. L. *Journal of the American Chemical Society* **2001**, *123*, 5086.
(59) Day, G. M.; Motherwell, W. D. S.; Jones, W. *Crystal Growth & Design* **2005**, *5*, 1023.
(60) Gavezzotti, A.; Filippini, G. *Journal of the American Chemical Society* **1995**, *117*, 12299.
(61) Matsuo, K.; Matsuoka, M. *Crystal Growth & Design* **2007**, *7*, 411.
(62) Suitchmezian, V.; Jess, I.; Näther, C. *International Journal of Pharmaceutics* **2006**, *323*, 101.
(63) Bhattacharya, S.; Saha, B. K. *Crystal Growth & Design* **2013**, *13*, 606.

For table of contents use only

An amide-pseudo amide interaction was found to be a robust synthon within a series of theophylline:amide cocrystals. Polymorphism and thermal stability within this cocrystal series is also described.