

Assessing the likelihood of polymorphism through hydrogen bond capabilities

Aim

To examine the possible donor/acceptor pairings of functional groups with a view to assessing the likelihood of polymorphism.

Introduction

Polymorphism, the ability for a molecule to crystallise in more than one form, has been an issue of great importance to the pharmaceutical industry for many decades now. The infamous case of Ritonavir¹, where the appearance of a previously unknown polymorphic form threatened the supply of the drug, has brought into sharp focus the need to have a good understanding of the energy landscape of the active pharmaceutical ingredient.

Hydrogen bonding has become a crucial part of the crystal engineering strategy with synthons, or recognisable, robust intermolecular interaction motifs, being at the forefront. Since the seminal work of Etter *et al*² and the observation that strong hydrogen bond donors are commonly matched with strong acceptors, it has been recognised that considering the strengths of hydrogen bond donors and acceptors is a valuable design tool. Thus examining the hydrogen bonding capability of a molecule may provide information relevant to assessing the likelihood of polymorphism. For example, competing but equally likely hydrogen bonding outcomes may indicate a risk of more than one solid form.

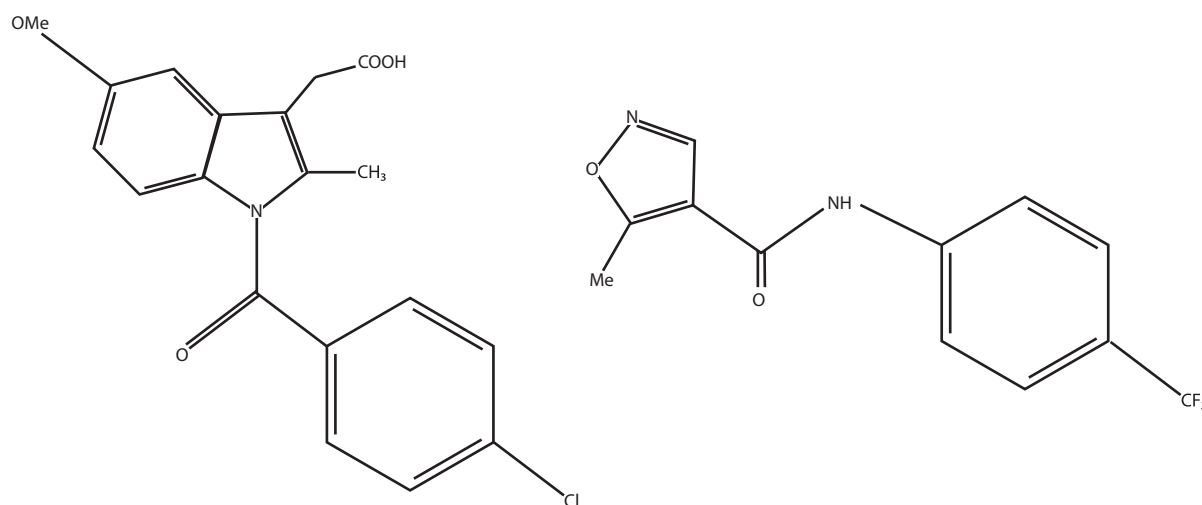


Figure 1 - Chemical Structures of Indomethacin (left) and Lefluomide (right)

To demonstrate, we look at two known drug molecules, Indomethacin (Refcode INDMET³), a non-steroidal anti-inflammatory drug and Leflunomide (Refcode VIFQIL⁴), used to treat rheumatoid arthritis, with a view to assessing the hydrogen bond capabilities of the molecules and possible outcomes for the solid forms.

Method

The first step is to identify the hydrogen donors and acceptors within the molecule. In most cases, this is straightforward and can be done manually. However, in some cases, such as the isoxazole ring of Leflunomide, it is not necessarily obvious which heteroatom is more likely to accept hydrogen bonds, if indeed there is a preference. IsoStar,⁵ a knowledge base of intermolecular interactions found in the Cambridge Structural Database, is very useful for answering this question. By looking at the hydrogen bond interactions of isoxazole rings (Figure 2) it is clear that it is the aromatic nitrogen of isoxazole that is the primary site for H-bond acceptance.

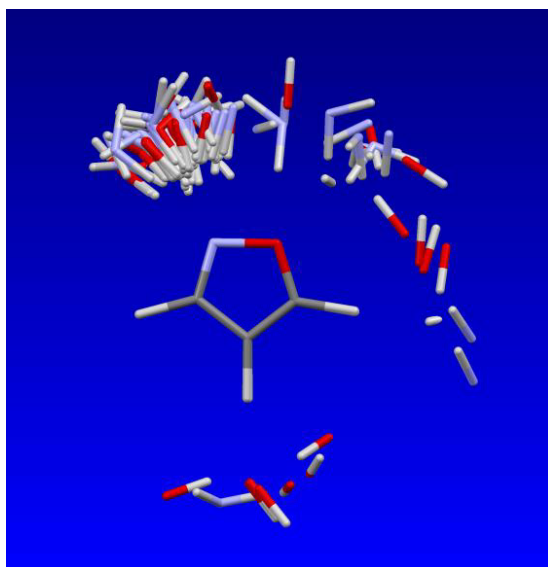


Figure 2 – Hydrogen bond interactions to Isoxazole as viewed in IsoStar. It is clear that the N atom of the ring is the stronger acceptor site.

Once the functional groups are identified, donor/acceptor interactions are investigated using the Motif tool^{6,7} in the Materials module of Mercury. Within the tool, it is possible to sketch functional groups, or choose them from a predefined list, or load previously saved groups. Intermolecular interactions, or motifs, are then defined between the donor-acceptor pairs simply by sketching a connection between the interacting atoms. These “sketched” interactions then form the basis of the queries submitted to the database. The significant information returned from motif searches performed in the Materials module is not just the number of instances of the motif found, but the frequency of occurrence observed for the motif: that is, the ratio of the number of molecules which contain the two functional groups, and which show the motif, to the number of molecules which contain the two functional groups. In other words, the frequency of occurrence represents how often the interaction is observed when it is possible. A frequency of occurrence of a few percent indicates that the

interaction is rarely observed and is perhaps weak. Strong interactions, such as an amide chain motif have frequencies of occurrence of around 25-30%.

Results

Donor and acceptor functional groups have been identified in Indomethacin, and, for the purposes of this study, are as follows: there is a single donor group, the OH of the carboxylic acid group, and four acceptor groups: C=O in carboxylic acid, the OH of carboxylic acid, the C=O group of the tertiary amide, and the methoxy oxygen. In Leflunomide, again, there is a single donor, the NH of a secondary amide, and two acceptors: the carbonyl of the secondary amide and the heterocyclic, isoxazole group.

The frequencies of occurrence found for the possible pairings of the donor group in Indomethacin with the possible acceptor groups are given in Table 1.

Doner	Acceptor	Frequency of Occurrence	Number of observations
Carbox OH	Tertiary Amide C=O	35.3	343
Carbox OH	Carbox OH	1.5	214
Carbox OH	Carbox C=O	23.7	3366
Carbox OH	Methoxy O	3.2	22

Table 1 – Possible hydrogen bond interactions and observed frequencies of occurrence for Indomethacin.

It can be seen that the donor-acceptor interactions between the carboxylic acid OH group and acceptor groups of ether and carboxylic acid OH have low frequencies of occurrence and hence we would not expect to observe them in a solid form of Indomethacin. Conversely, the self association of the carboxylic acid group and the heterosynthon of carboxylic OH to tertiary amide show high frequencies of occurrence, and are likely to be observed. Since there is only one donor in Indomethacin and two good acceptors, there may be a risk of polymorphism: it is impossible to satisfy both strong donor/ acceptor pairings in a $Z' = 1$ system. Therefore there are a number of plausible outcomes: the donor is paired with "Acceptor1" or the donor is paired with "Acceptor2", or a third possibility is a $Z' > 1$ system where both donor/acceptor pairings are present. In fact what is observed for Indomethacin is a stable $Z' = 1$ polymorph displaying only the carboxylic acid dimer interaction, and a metastable polymorph⁸ (the predominant form at high humidity), where $Z' = 3$, and which displays both the carboxylic acid dimer interaction and the carboxylic acid- tertiary amide interaction (Figure 3).

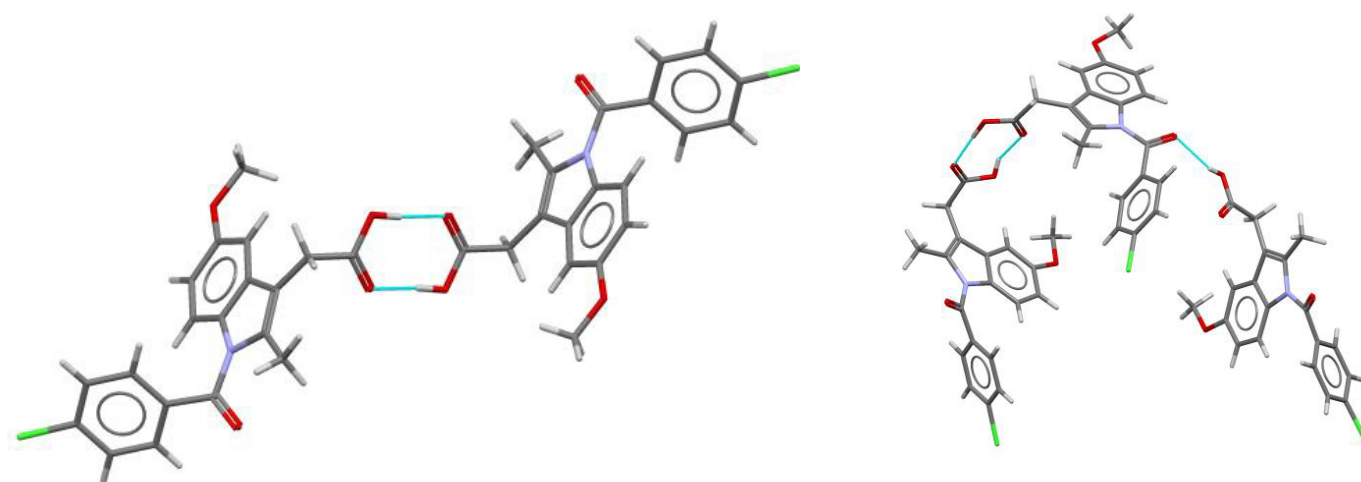


Figure 3 – The two forms of Indomethacin. The stable form on the left and the metastable form ($Z'=3$) on the right.

The situation is similar in Leflunomide. There is a single donor, the NH group from a secondary amide, and two acceptor sites; the amide C=O or the nitrogen of the isoxazole ring. Searches for these motifs were constructed using the Motif tool in the Materials module, and it was found that both the trans-amide homosynthon and the amide-isoxazole heterosynthon had high frequencies of occurrence (Table 2).

Donor	Acceptor	Frequency of Occurrence (%)	Number of observations
Amide NH	Amide C=O	25.3	4921
Amide NH	Isoxazole N	21.4	39

Table 2 – Possible hydrogen bond interactions and observed frequencies of occurrence for Leflunomide.

Once again, since there is only a single donor and two strong acceptors, there are a number of possible outcomes which may indicate a risk of polymorphism. In the case of Leflunomide, Form I is the thermodynamically stable form (below 400K) has $Z'=2$ and displays both the amide-amide interaction and the amide to isoxazole interaction. Form II, stable above 400K is a $Z' = 1$ structure and shows only the amide-isoxazole interaction.

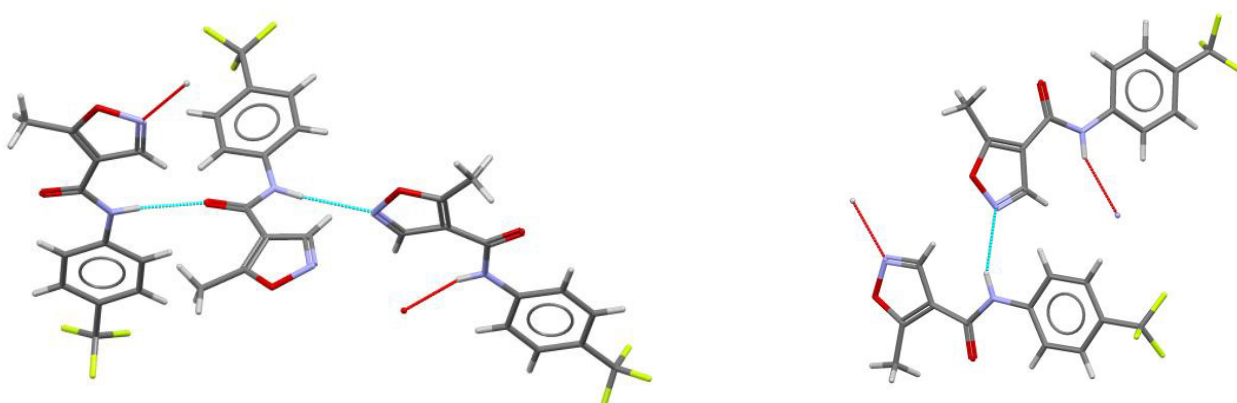


Figure 4 – The two forms of Leflunomide. The stable form ($Z'=2$) on the left and the metastable form on the right.

Therefore, from the above examples, it appears that strong and competing hydrogen bonding outcomes may indicate a risk of polymorphism. The corollary, that non-competitive outcomes in hydrogen bonding indicate a low risk of polymorphism may also be true. Aspirin, another system with a single donor group but with two acceptor groups (Figure 5) has only recently been shown to be polymorphic⁹ despite many years as a best-selling drug, and presumably thousands of crystallisations. The meta-stable form of Aspirin also shows the same carboxylic acid motif as the stable form and no polymorphic form, where the alternate carboxylic acid to ester(C=O) interaction has been found to date. The frequencies of occurrence determined support this observation: the carboxylic acid – carboxylic acid interaction has a high frequency of occurrence (23.7%) whilst the ester C=O interaction with the hydroxyl group of the carboxylic acid only occurs in 8.5% of cases. Therefore in systems where there is no competition between acceptors, polymorphism in hydrogen bonding may not be likely.

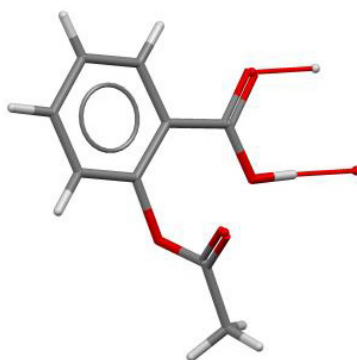


Figure 5 - Aspirin

Conclusions

A method for assessing the likelihood of occurrence of hydrogen bonds and an application to evaluating the risk of polymorphism has been described. The Materials module of Mercury makes searching for motifs quick and easy and perhaps most significantly, returns a frequency of occurrence value which allows a judgement to be made about how common or unusual is an interaction. In the examples studied here, both Indomethacin and Leflunomide have been shown to have strong, competing acceptor groups for a single donor group and both molecules exhibit polymorphism. For these systems, the additional flexibility inherent in Z'^{>1} structures has been utilised to allow both strong hydrogen bond acceptors to be satisfied.

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