

**Co-crystal Engineering of *N*-heterocyclic Active Pharmaceutical
Ingredients with Nitrobenzoic Acid Derivatives**

by

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Abstract

The pharmaceutical industry continues to face challenges with properties of drugs like poor solubility, limited bioavailability and insufficient stability of active pharmaceutical ingredients (APIs), which collectively hinder drug development. Co-crystallization has been shown to be an effective strategy to improve these physicochemical properties of drugs without altering their chemical properties. In this study, fifteen novel crystalline structures were obtained using both solution-based crystallization (slow evaporation) and mechanochemistry methods. A series of *N*-heterocyclic APIs were co-crystallized with selected nitro-substituted aromatic co-formers, enabling the evaluation of multiple co-former families across diverse APIs. 3,5-Dinitrosalicylic acid (DNSA) formed crystalline phases with seven APIs, nitroterephthalic acid (NTPA) with six APIs, and 2-(nitrophenyl)methanol with two APIs.

The crystalline structures formed included neutral co-crystals, salt co-crystals, hydrated forms and solvates, reflecting the structural diversity of outcomes possible with carefully selected co-formers and varied crystallization techniques. Notably, an unexpected esterification product was structurally identified in one of the crystalline systems, highlighting the potential for solid-state or solution-mediated transformations during crystallization. Structural characterization was performed using single-crystal X-ray diffraction, with structure solution and refinement carried out using SHELXT and SHELXL respectively. Findings on some of the crystalline structures were supported by complementary analytical techniques, including Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, and Hirshfeld surface analysis.

Overall, the findings of this study provide valuable insight into the structural diversity in co-crystallization, supramolecular interaction driving successful co-crystallization and how different crystallization methods affect co-crystallization. Furthermore, the study introduces a series of novel co-crystals which contribute to the knowledge of pharmaceutical crystal engineering and provides a strong foundation for future research aimed at improving drug performance.

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Glossary

Term	Definition
Co-crystal	Co-crystals are crystalline materials consisting of two or more different molecular compounds in a defined stoichiometric ratio held together by non-covalent interactions.
Salt	A crystalline material formed by proton transfer between an acidic and basic functional group, generally guided by the pKa rule.
Synthon	Synthons are recurring hydrogen-bonding patterns between functional groups.
Homosynthon	A synthon formed between identical functional groups, such as amide–amide interactions.
Heterosynthon	A synthon formed between different but complementary functional groups, such as carboxylic acid–pyridine interactions.
GRAS	Generally Recognized As Safe; a designation assigned to substances considered safe for use in food and pharmaceutical applications.
API	Active Pharmaceutical Ingredient; biologically active component of a drug responsible for its therapeutic effect.
ΔpK_a	The difference between the pKa of the protonated base and that of the acid, used to assess the likelihood of salt or co-crystal formation.
SCXRD	Single-crystal X-ray diffraction, a technique used to determine the three-dimensional arrangement of atoms in a crystal.

List of abbreviations

API – Active Pharmaceutical Ingredient

BSC- Biopharmaceutical Classification System

CBZ – Carbamazepine

CCDC – Cambridge Crystallographic Data Centre

CSD – Cambridge Structural Database

DMSO- Dimethyl sulfoxide

DNSA- 3,5-Dinitrosalicylic acid

FDA- United States Food and Drug Administration

FTIR- Fourier Transform Infrared

GRAS- Generally Regarded As Safe

INA- Isonicotinamide

ISN- Isoniazid

NIC- Nicotinamide

NMR- Nuclear Magnetic Resonance

NTPA- Nitroterephthalic acid

SCXRD – Single Crystal X-ray Diffraction

Chapter 1: Introduction and Aims

1.1 Introduction

1.1.1 Active Pharmaceutical Ingredients (APIs)

Pharmaceutical drugs are generally made up of two components, an active pharmaceutical ingredient (API) which is the biologically active component of a drug and pharmacologically inactive excipients. The API is responsible for the therapeutic effect of the drug, including curing, mitigation and treatment of diseases (Kumar et al., 2022). The other component known as excipients are pharmacologically inactive substances used for non-medical purposes, such as holding the tablet together and stabilizing the API against degradation (Kumar et al., 2022). The drug efficacy largely depends on its physicochemical properties (Datta & Grant, 2004) such as solubility and bioavailability (Devhare & Kore, 2016) among others.

Drug compounds characterized by limited aqueous solubility are increasingly prevalent in the research and development portfolios of pharmaceuticals (Lipinski, 2000). These compounds present several difficulties during pharmaceutical development. They may exhibit slow dissolution in bodily fluids, inadequate and irregular systemic exposure, and subsequently reduced therapeutic effectiveness, especially when administered orally (Blagden et al., 2007). Several approaches have been explored towards overcoming such challenges, including salt formation (Agharkar et al., 1976), polymorphism (Llinas and Goodman 2008), and co-crystal preparation (Dutt et al., 2020) among others.

1.1.2 Pharmaceutical co-crystal engineering

Pharmaceutical co-crystals are crystalline materials consisting of two or more different molecular compounds in a defined stoichiometric ratio, with at least one of them being an API (Wong et al., 2021). Pharmaceutical co-crystals play a significant role in addressing the evolving challenges in the pharmaceutical industry (Liu et al., 2019). Co-crystallization can be used to modify important properties of APIs such as bioavailability, dissolution rate (Aitipamula, 2012), solubility, hygroscopicity, and thermal stability (Steed, 2013) which influences the drug's pharmacokinetics. An example of a pharmaceutical drug where co-crystallization has proven to be a success is ipragliflozin, a sodium-glucose co-transporter-2 inhibitor used in the treatment of type 2 diabetes. On its own, ipragliflozin tends to convert into a non-stoichiometric hydrate under certain moisture

and thermal conditions, which may compromise the drug's stability. The co-crystal form provides superior stability, good reproducibility, and better handling properties compared to ipragliflozin alone. It does not change the drug's clinical efficacy or safety but optimizes its physicochemical properties (Almansa et al., 2019). Other examples of successful co-crystals and the improved properties are given in Table 1.

Table 1: Successful co-crystals drugs with their improved properties

Components (API + co-formers)	API uses	Observation	References
Valsartan sodium + Sacubitril sodium	Heart failure	Enhanced stability and solubility	Bolla & Nangia (2016)
Escitalopram oxalate + Oxalic acid	Depression and anxiety	Stable, easy-to-manufacture co-crystal form of antidepressant	Harrison et al., 2007
Valproate sodium + Valproic acid	Seizure disorders, mood stabilization	Improved dissolution, better pharmacokinetic control over its original form	Petruševski et al., 2008
lamivudine-zidovudine	treatment of HIV infection	Improved therapeutic efficacy	Bhatt et al., 2009

1.1.3 Supramolecular interactions in pharmaceutical co-crystals

Co-crystals are generally held together by non-ionic and non-covalent bonds (Aakeröy & Beatty, 2001), such as hydrogen bonding, π - π stacking, and van der Waals forces (Rodrigues et al., 2018). Figure 1 shows a co-crystal formed between isonicotinamide (API) and 2-(nitrophenyl)methanol (co-former) a co-crystal produced as part of this investigation. The co-crystal is stabilized through hydrogen bonding between the pyridine nitrogen of isonicotinamide and the hydroxyl group of the co-former, representing a heterosynthon. This example highlights how directional intermolecular interactions govern the assembly of pharmaceutical co-crystals.

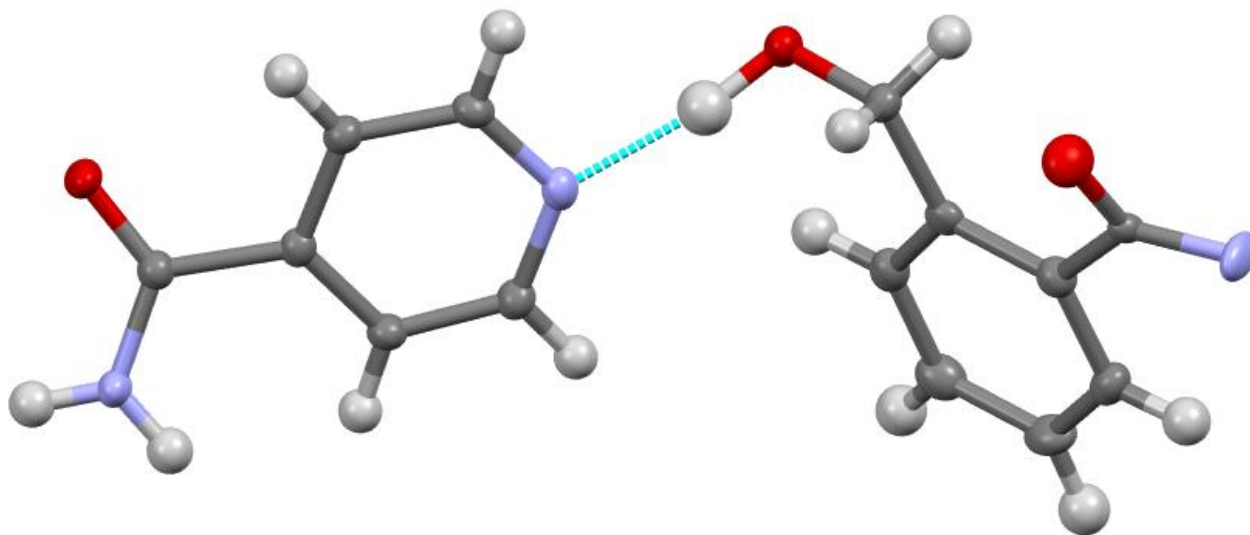


Figure 1: O-H...N hydrogen bonding interaction between Isonicotinamide and 2-(nitrophenyl)methanol. Hydrogen atoms are shown as spheres.

Among these interactions, hydrogen bonding is one of the most important due to its high directionality and the ability of its energy to be influenced by the chemical environment. Therefore, the design of a pharmaceutical co-crystal is based on the identification of specific hydrogen-bond donor and acceptor groups in APIs to select a complementary interacting molecule that can act as an efficient co-former (Cvetkovski et al., 2016). Hydrogen bonding rules have been developed based on Cambridge Structural Database (CSD) analyses to predict favorable hydrogen-bond formation and crystal structures (Haynes et al., 2004); the rules are as follows:

- 1) All good proton donors available in the molecule will be used for hydrogen bond formation in the crystalline structure of the compound.
- (2) All good acceptors will be used for hydrogen bond formation, when hydrogen bond donors are present.
- (3) Intramolecular hydrogen bonds of six-member rings preferably form over intermolecular hydrogen bonds.
- (4) The best donor and the best acceptor will preferably form hydrogen bonds with one another (Etter, 1990).

Strong hydrogen bonds are N–H...O, O–H...O, N–H...N, and O–H...N, while weak hydrogen bonds include C–H...O–N and C–H...O=C (Martínez-Alejo et al., 2014)

1.1.4 Multicomponent crystals

Multicomponent crystals include co-crystals, salts, solvates, salt co-crystals, salt solvates and co-crystal solvates (Boycov et al., 2022), most of these multicomponent crystals were observed in this study. Each of the different solid forms has merits and limitations, for example, salts may improve the solubility of a drug, but proton transfer may lead to supersaturation risks, potentially impacting exposure of the API by reducing the amount of drug absorbed into the systemic circulation (Neusaenger et al., 2023). The physicochemical characteristics of the molecules under research may determine how successful these methods are.

Salts are solid forms of drugs that have a major role in improving the properties of APIs (Swapna & Nangia, 2017). Salt formation requires the presence of an ionizable functional group in the compound, which limits their applicability (Stahl & Wermuth, 2011). A salt results when proton transfer occurs between the acidic and basic functional groups, generally guided by the pKa rule. The pKa rule suggests that salts are likely to form if the pKa difference between the proton donor and proton acceptor is greater than 3 (Loya et al., 2019). Unlike salts, co-crystals retain the neutral form of the API (Anjana et al., 2013) without proton transfer, while still enhancing physical properties (Diniz et al., 2018), making them ideal for non-ionizable compounds (Aitipamula et al., 2012).

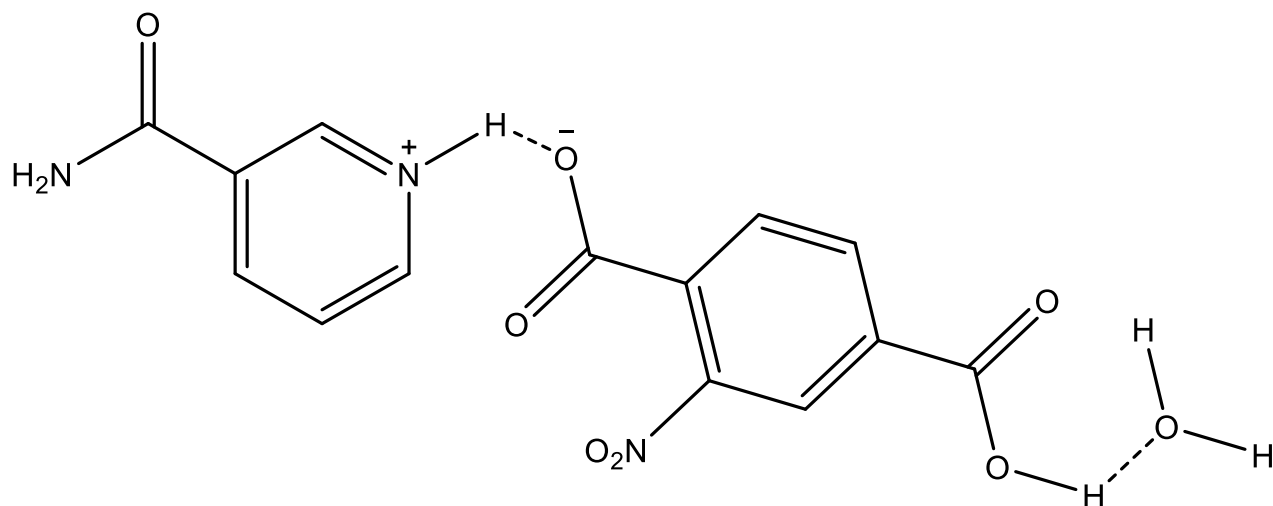


Figure 2: Crystal structure of a co-crystal hydrate salt, obtained in this study

Hydrates form through the incorporation of water into the crystal lattice, (Grothe et al., 2016). The disadvantage of hydrates over co-crystals is that the presence of the water molecule in the crystalline lattice may alter the solubility and hygroscopic behavior of the molecule (Guo et al., 2021). In this study, some co-crystallization reaction resulted in the formation of salts and hydrates rather than co-crystals. Evidence from literature suggests that co-crystallization reactions can result in the formation of salts when proton transfer occurs (Banerjee et al., 2005).

1.1.5 Co-former selection

Co-former selection for an API is the most important aspect in the design and screening of co-crystals. Selection of co-formers is commonly guided by knowledge-based methods including hydrogen-bonding propensity, synthon engineering, supramolecular compatibility using CSD, pKa based models, among others. It is also important to choose a co-former that can improve the physicochemical properties of the API. The chosen co-formers should belong to compounds commonly regarded as GRAS (Generally Regarded as Safe) (Martinez et al., 2013).

For successful crystal development, understanding and exploitation of the inter-molecular interaction occurring between the API and the co-former to generate robust supra-molecular synthons is key (Shattock et al., 2008). Targeted synthons help rationally design co-crystals with desirable solid-state properties.

Synthons are recurring hydrogen-bonding patterns between functional groups and exist in two distinct categories. Hydrogen-bonding pattern between similar functional groups, for example, two carboxylic acid groups, are called homosynthons. Patterns between different functional groups are called a heterosynthon. Synthons that form predictable and frequently found hydrogen bonding patterns are called robust synthons. (Shattock et al., 2008).

The most widely used supramolecular synthons in crystal engineering are shown in Figure 3. Common homosynthons are formed between (a) carboxylic acid dimers and (b) amide dimers, while heterosynthons are formed between (c) carboxylic acid and amide groups, (d) carboxylic acid and aromatic nitrogen. This is the backbone of many successful co-crystals in crystal engineering, they form strong hydrogen bonds and often result in the formation of $R_2^2(8)$ ring motifs (Buddhadev et al., 2020). Lastly, (e) hydroxyl and aromatic nitrogen/pyridine, this interaction is observed in over 99% of relevant structures, demonstrating exceptional reliability for molecular recognition in co-crystal design (Bis et al., 2007).

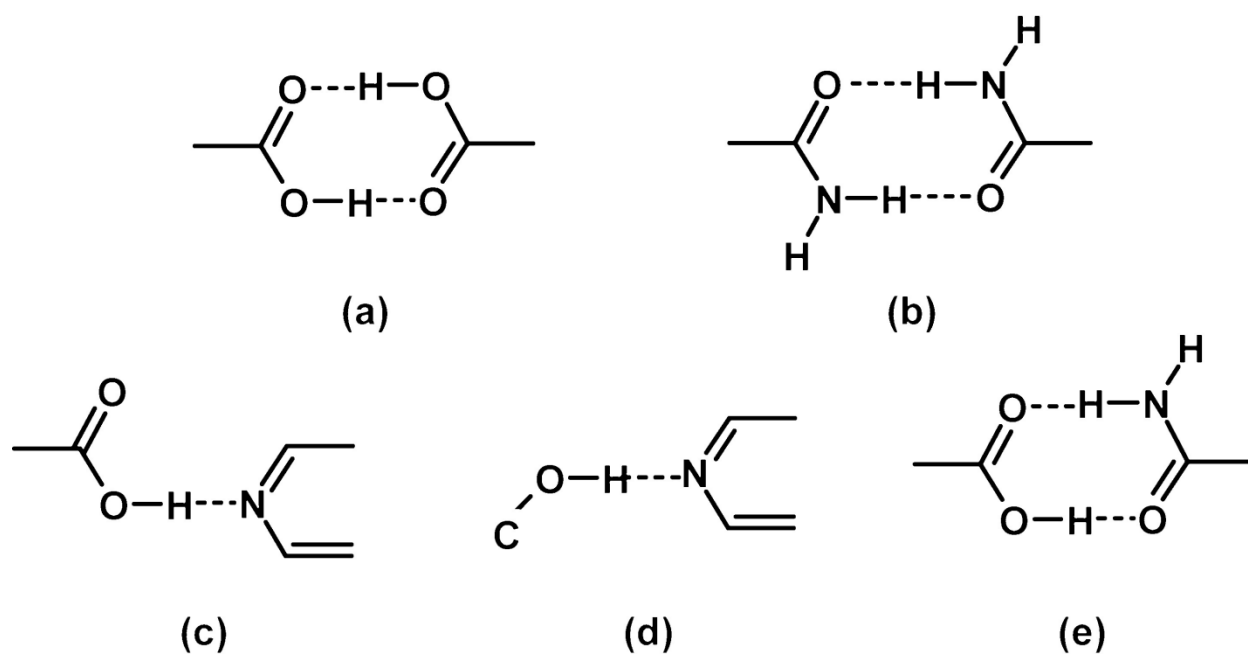


Figure 3: Examples of homosynthons (a,b) and heterosynthon (c,d,e)

1.1.6 *N*-heterocyclic APIs

N-heterocyclic APIs which are the focus of this study represent a diverse class of compounds that are of great significance in the pharmaceutical industry. Many pyrimidine and pyrazine derivatives are used as medicines for a variety of diseases (Venugopala & Kamat, 2024). Not only are they of importance in the pharmaceutical industry, *N*-heterocyclic APIs are characterized by versatile functional groups which can participate in diverse intermolecular interactions making them favorable candidates in co-crystal development since the functional groups present in the API and the co-former play a major role in co-crystallization (Ancheria, 2019). From a hydrogen bonding point of view, *N*-heterocyclic compounds contain lone pairs of electrons on the hetero-atoms which can interact with hydrogen atoms from other molecules forming a strong hydrogen bond.

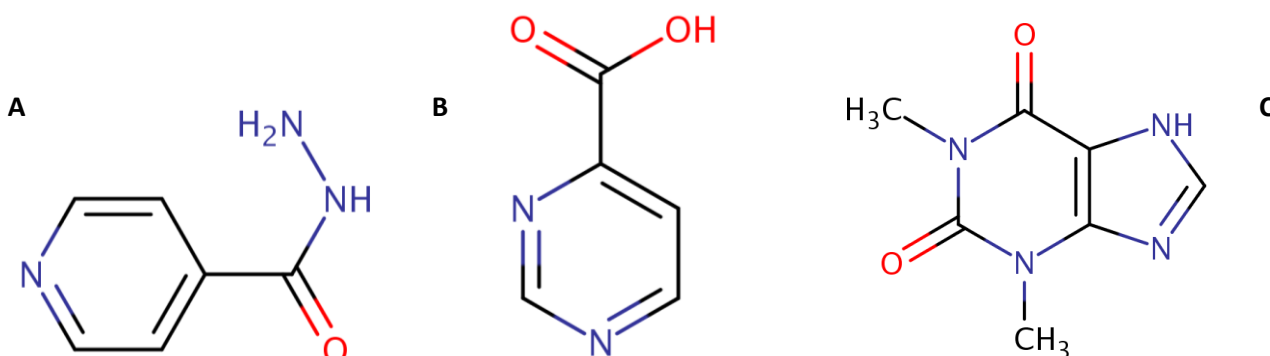


Figure 4: Examples of *N*-heterocyclic APIs with different functional groups; a) Isoniazid, b) pyrazinecarboxylic acid, c) Theophylline

1.1.7 Co-crystal screening

Co-crystal screening methods include solution-based techniques and mechanochemistry, among others. Slow evaporation method, a common solution-based procedure, involves dissolving the reactants in a suitable solvent followed by slow evaporation, resulting in nucleation and growth of co-crystals from solutions. An advantage of this method when compared to mechanochemistry is that it gives the reaction enough time for the compounds to arrange into the most thermodynamically stable crystalline structure (Chow et al., 2012). However, the method is time consuming, and the solvent choice has significant impact on the success of the reaction (Charpentier et al., 2022).

Mechanochemistry, on the other hand, occurs between solid reactants without a solvent or with a small amount of solvent. This technique uses mechanical energy to drive a chemical reaction. Mechanochemistry has several advantages compared to other screening methods. Other conventional techniques require solvents, which might not be appropriate for every synthesis. In this sense, mechanochemistry is advantageous for insoluble solid-to-solid reactions, reactions that are sensitive to solvents, or which solvents interfere, and reactions that can be made safer by not requiring the use of hazardous solvents (Lei et al., 2015).

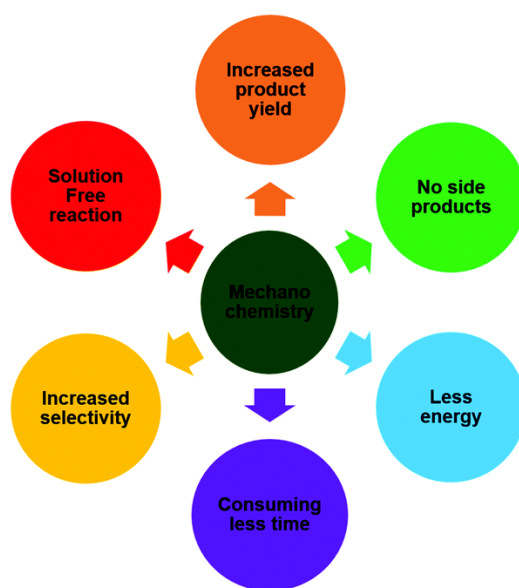


Figure 5: Advantages of mechanochemistry over solution-based method (Mateti et al., 2021)

Over the past two decades, there has been a notable shift in pharmaceutical research from utilizing salt formation to exploring co-crystals as a promising strategy for improving the pharmaceutical properties of drugs. However, despite the growing interest in co-crystallization and the pharmaceutical uses of *N*-heterocyclic APIs, there remains a need for future research to investigate the molecular interactions governing co-crystal formulation and optimize co-crystal formulation for pharmaceutical applications. This study aims to address this gap in knowledge by investigating the intermolecular interaction between selected *N*-heterocyclic APIs and nitrobenzoic acid derivatives co-formers and their application to co-crystal formation and properties. By exploring molecular interactions in co-crystallization, this research seeks to advance our understanding of

N-heterocyclic APIs and pave the way for the development of co-crystal APIs with improved bioavailability and therapeutic efficacy.

1.2 Problem statement

Poor solubility and bioavailability of APIs pose a significant challenge in the pharmaceutical industry, often leading to the necessity for increased dosage size to achieve therapeutic efficacy (Bhalani et al., 2022). However, escalating dosages may increase the risk of toxicity, particularly with drugs exhibiting toxicity at higher concentrations. Additionally, co-crystallization encounters challenges due to the unpredictability of crystal formation. Current methodologies lack reliable predictive capability to determine whether a co-crystal will form under specific conditions, hindering the efficient development of desired co-crystals (Deng et al., 2023). Furthermore, there is limited understanding regarding the conditions favoring co-crystal formation, impeding progress in this critical area of pharmaceutical research and development (Wong et al., 2021).

1.3 Aims and objectives

Aims:

To synthesize a series of co-crystals of *N*-heterocyclic active pharmaceutical ingredients (APIs) with nitrobenzoic acid co-formers and to investigate molecular interactions driving successful co-crystal formation between these APIs and co-formers, with a specific emphasis on hydrogen bonding, π - π stacking and van der Waals interactions.

Objectives:

1. To synthesize a series of co-crystals of selected *N*-heterocyclic APIs with suitable co-formers using solvent-mediated crystallization and mechanochemistry and to compare the products of the supramolecular synthesis using the two methods.
2. To evaluate the influence of synthesis and crystallization conditions such as temperature, solvent choice and crystallization time on the formation and yield of co-crystals, aiming to optimize the process for efficient co-crystal formation
3. To determine the structure of the synthesized co-crystals using single crystal X-ray diffraction (SCXRD)
4. To refine the structures using Olex2 and X-seed software, and to perform a structural study of the results.

1.4 Significance of the study

N-heterocyclic APIs are widely used in the treatment of various diseases, including cancer, infectious diseases, and central nervous system disorders. Inadequate formulation strategies may

lead to reduced drug efficacy, compromised patient safety, and increased healthcare costs due to the need for a higher dose or alternative treatments. Co-crystallization has the potential to revolutionize drug development by offering solutions to improve physicochemical properties of drugs. This study aims to provide comprehensive understanding of the intermolecular interactions between *N*-heterocyclic APIs and nitrobenzoic acid derivative co-formers leading to successful co-crystal formation. This study also hopes to address the unpredictable nature of co-crystal formation and the lack of reliable methodologies for predicting co-crystal outcomes which hinder efficient drug development processes. By shedding light on the factors influencing co-crystal formation and identifying conditions conducive to successful co-crystal synthesis, this study aims to fill critical knowledge gaps and contribute to improvements in the pharmaceutical industry.

Chapter 2: Literature review

2.1 Introduction

Co-crystallization is a widely applied strategy for modifying the physicochemical properties of APIs (Ngilirabanga, 2021) wherein the intermolecular interaction between the API and the co-former plays a crucial role (Singh et al., 2023). The application of co-crystal engineering to *N*-heterocyclic APIs presents a promising strategy for optimizing drug formulation and potentially improving their therapeutic efficacy considering that they are among the most prominent structures of pharmaceuticals (Desiraju & Parshall, 1989).

This literature review aims to explore the current state of knowledge in co-crystal engineering focusing on *N*-heterocyclic APIs. It investigates the factors that influence successful co-crystallization, understanding the supramolecular interaction between the *N*-heterocyclic APIs and nitrobenzoic acid derivatives co-formers. Additionally, it studies the behavior of the three successful co-formers used in this study and analyzes the behavior, therapeutic uses and formulation challenges of the APIs being studied. By studying existing literature on synthesis methodologies and characterization techniques, this review aims to provide insight into the role of intermolecular interactions in the design and development of pharmaceutical co-crystals.

2.2 Crystallization conditions

Successful co-crystallization is influenced by several factors; this literature review provides insight into method dependent outcomes during the crystallization process.

2.2.1 Method of crystallization

Many studies have shown that liquid assisted grinding methods tend to be faster than slow evaporation techniques; however slow evaporation often yields higher quality single crystals suitable for SCXRD analysis, as gradual solvent removal allows the system to approach thermodynamic equilibrium. Weyna et al. (2009) reported that many co-crystals obtained via solution methods could also be prepared using the solvent drop grinding (SDG), highlighting SDG as a cost-effective and environmentally favorable technique. The same study indicated that the mechanochemistry method often yields microcrystalline powder material often requiring powder X-ray diffraction for validation unlike slow evaporation method. This study aligns with the

findings of the present study, mechanochemistry yielded microcrystals where selecting a single crystal was hard, while slow evaporation yielded large, clear crystals with well-defined edges.

Haskins et al. (2021) compared slurry and SDG approaches and reported high success rates for both methods, with slurry exhibiting slightly higher success under certain conditions. The author concluded that supersaturation plays an important role in the success of both methods. Haskins et al. (2021) also highlighted that solution crystallization is the traditional method for preparing co-crystals but can require trial and error experiments because the solid form obtained is sensitive to concentration and solvent selection.

2.2.2 Solvent choice

Solvent choice plays a crucial role in determining the success of co-crystallization. Solvent affects factors like solubility of the materials, crystal growth rate, polymorphic outcome and molecular interactions. Zhao et al. (2022) demonstrated this using CL-20/HMX solvent models with quantum chemistry and molecular dynamics, finding ethyl acetate optimal due to weaker solvent-cocrystal binding and stronger hydrogen bonds between components. It was concluded that solvent polarity directly affects hydrogen bonding strength between co-formers, impacting the co-crystallization rate and crystal morphology. Less polar solvents like ethyl acetate promote stronger hydrogen bonds and more efficient co-crystal formation than polar solvents such as ethanol (Zhao et al., 2022).

Mixed-solvent systems can be used to modulate solvent polarity and crystallization pathways. Competitive interactions between the solvents may steer crystallization toward hydrates, solvates, or pure co-crystals, making mixed solvent systems like water–alcohol mixtures particularly important (Munshi et., 2016). For example, methanol or ethanol can behave as both rivals for functional groups and stabilizers of co-crystal assemblies, whereas water significantly encourages the formation of hydrates because of its strong hydrogen-bonding ability (Rager & Hilfiker, 2010).

Crystallization studies have shown that solvents used for crystallization may appear in the crystal lattice as solvates. Infantes et al., 2007 analyzed entries in the CSD to evaluate solvent inclusion in crystalline materials. Trends in crystal formation showed water as the most common solvent incorporated into crystal lattice. This is because of the size of water molecules and strong hydrogen bonding capacity. Other solvents like methanol, ethanol and acetonitrile appeared less frequently

but tend to form solvents when the molecular packing can accommodate their steric and hydrogen bond preferences (Goerbitz & Hersleth, 2000).

2.3 Co-crystallization leading to salt and hydrate

The resulting crystalline structure of co-crystallization experiment is not always predictable. As observed in this study, co-crystallization can result in the formation of salt co-crystals or hydrates instead of pure co-crystals. This may be due to several factors, such as the nature of the API and the co-former and crystallization conditions. Childs et al., 2007, reported that the formation of salt or co-crystal in acid-base complexes with similar pKa values is often unpredictable and a continuum exists between the two extremes. Several studies show that salts and hydrates may also emerge during co-crystallization and significantly influence the API's physicochemical properties (Karagianni et al., 2018). In a study by Childs et al., 2007, twenty crystalline structures containing theophylline and guest molecules with pKa values less than three were prepared, resulting in thirteen cocrystals, five salts, and two complexes with mixed ionization states based on InfraRed spectroscopy and SCXRD data.

2.3.1 The ΔpK_a Rule for Predicting Salt and Co-crystal Formation

The formation of either salt or co-crystals between an acid and a base can be predicted using the pKa rule by Cruz-Cabeza et al., (2022). This rule serves as a guideline for predicting the likelihood of salt or co-crystal formation. According to Cruz-Cabeza et al. (2022), multi components crystals with $\Delta pK_a > 4$ leads to salt formation, while $\Delta pK_a < -1$ generally favour co-crystal formation. ΔpK_a between -1 and 4, may lead to either a salts or a co-crystal, depending on factors such as solvent choice and crystallization conditions.

2.4 Pharmaceutical co-crystals of selected APIs

N-heterocyclic APIs are the primary focus of this study due to their structural versatility and well-established hydrogen bonding capabilities, which make them highly suitable candidates for co-crystal engineering. In addition to these, structurally distinct model compounds, namely carbamazepine, acetaminophen, and benzhydrazide, were also investigated. These compounds were selected to introduce functional group diversity, including amide and hydrazide moieties, and

to enable comparative evaluation of supramolecular interactions beyond *N*-heterocyclic systems. Some of the selected APIs are discussed below

2.4.1 Theophylline

Theophylline is a prescribed drug for the treatment of asthma and chronic obstructive pulmonary disease (Barnes & Peter, 2013). It is known to exist as a crystalline monohydrate and four anhydrous polymorphs (Form I-IV) (Khamar et al., 2011). Theophylline is characterized by low aqueous solubility which affects drug bioavailability in the patients hence the drug is administered orally and intravenous as aminophylline, an ethylenediamine salt (Weinberger & Hendeles, 1996). Literature studies show that extensive co-crystal reviews have reported improved physicochemical properties of the drug (Childs et al., 2007) including aqueous solubility and bioavailability.

CSD analyses show to date there are no structures of theophylline and nitrobenzoic acid derivatives reported. Reported studies on theophylline include a study done by McTague & Rasmuson, (2021) where a drug-drug co-crystal composed of theophylline and salicylic acid was successfully synthesized and characterized in chloroform solution.

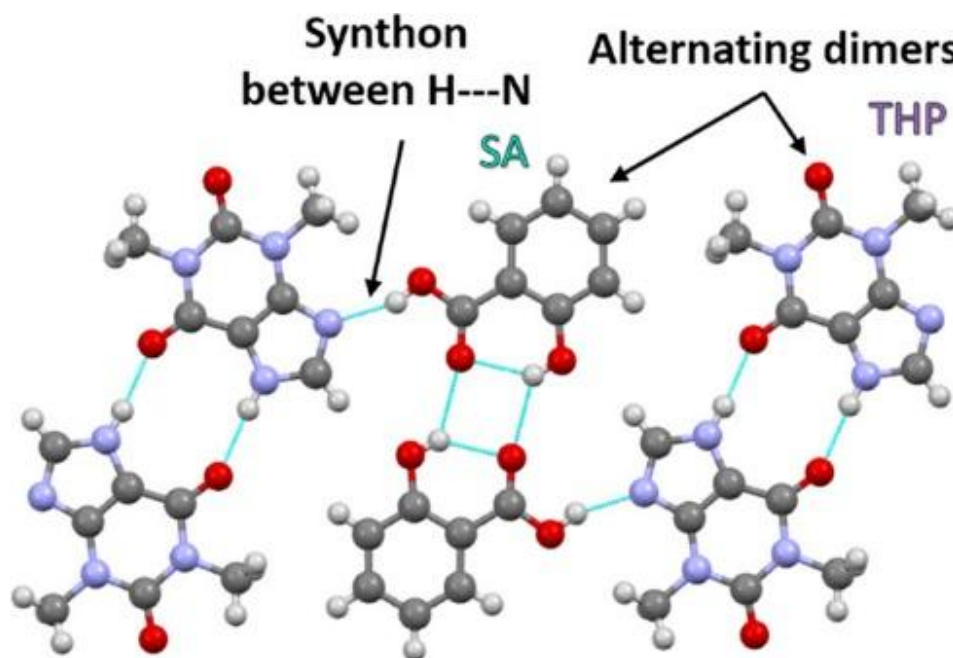


Figure 6: Co-crystal of theophylline and salicylic acid showing packing and synthons observed in the co-crystal [76]

Theophylline contains a variety of functional groups that can participate in hydrogen bonding. It has two carbonyl groups which are good hydrogen bond acceptors, an imide group that can act as a hydrogen bond donor, pyridine nitrogen that can also act as a hydrogen bond acceptor and methyl substituted nitrogen.

2.4.2 Carbamazepine

Carbamazepine (CBZ) is an anticonvulsant and analgesic drug widely prescribed for the management of seizures and the treatment of trigeminal neuralgia-related pain. It was first approved by the United States Food and Drug Administration (FDA) in 1965 (Tolou-Ghamari *et al.*, 2013). Beyond its anti-epileptic use, CBZ is also employed in the treatment of bipolar disorder, representing the first anticonvulsant used clinically for this purpose (Chen & Lin, 2012).

A major limitation of CBZ is its very low aqueous solubility (Li *et al.*, 2020), resulting in the need for patients to take very large doses to achieve therapeutic effect (Du *et al.*, 2018). Consequently, the compound has been extensively investigated for co-crystallization with different methods and co-formers to enhance its solubility and stability. Co-crystals of CBZ have demonstrated improved solubility compared with the commercially available product Tegretol. CBZ co-crystals with saccharin (SAC) and nicotinamide (NIC) have previously been studied, with the CBZ-SAC crystal being more soluble than the commercially available product CBZ.

The success of CBZ co-crystallization largely stems from its strong propensity to form hydrogen bonds, which are the primary driving forces behind the formation of stable co-crystals (Cvetkovski *et al.*, 2016). Specifically, the amide group in CBZ readily participates in hydrogen bonding with co-formers containing amide, acid, or hydrazide groups (Katzhendler *et al.*, 1998). Furthermore, its ability to generate characteristic $R_2^2(8)$ ring motifs (Herboth & Lyubartsev, 2024) provides additional lattice stabilization, making it an excellent candidate for co-crystal design.

CSD analysis done in July 2025 shows 41 carbamazepine co-crystals on the CSD. Of these, 29 involve aromatic co-formers. In almost all cases, hydrogen bonding occurs *via* the amide oxygen acceptor of the carbamazepine and the co-former. Notable exceptions are QABHIO (Schneider-Rauber *et al.*, 2020) and HORWIX (Pawłędzio *et al.*, 2019), where hydrogen bonding involves the amide N–H donor, and FAYXOV (Drozd *et al.*, 2017) where hydrogen bonding involves both the

oxygen acceptor and the N–H donor, forming an $R_2^2(8)$ ring motif. Only one reported carbamazepine co-crystal has been identified where the co-former contains a nitro- group, namely HORWIX (Figure 7). In this structure, hydrogen bonding occurs between the amide N–H donor of carbamazepine and the nitro group of the co-former (3,5-dinitrobenzoic acid).

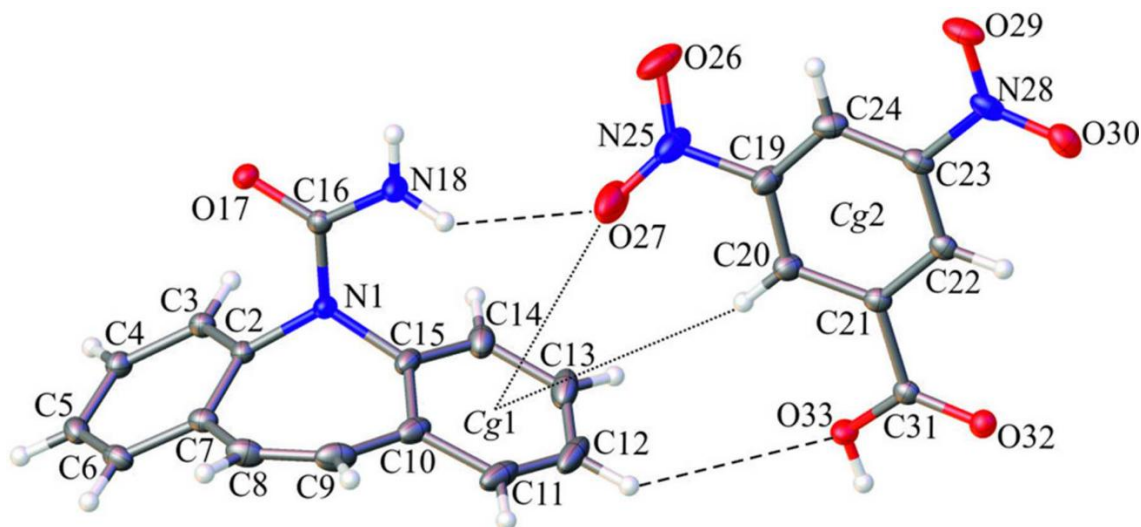


Figure 7: Co-crystal of carbamazepine and 3,5-Dinitrobenzoic acid synthesized [82]

2.4.3 Niacinamide

Niacinamide is a form of vitamin B3 that is used in the pharmaceutical industry for the treatment and prevention of pellagra and is also widely used in cosmetic formulations (Boo, 2012). It is an isomer of isonicotinamide, with the amide group in para position to the pyridine nitrogen. Niacinamide is highly soluble and has been used frequently as a co-former to improve APIs with low solubility. However, as an API it faces stability issues under certain conditions.

CSD study shows that about half of niacinamide's structures with nitrobenzoic acid derivatives are protonated at the pyridine nitrogen resulting in the formation of $N^+H\cdots O$ unlike isonicotinamide where neutral $N\cdots H-O$ hydrogen bond is common. Koman et al., (2003) synthesized niacinamide with 3,5-dinitrosalicylic acid which resulted in formation of nicotinamidium, 3,5-dinitrosalicylate (HUYXII). Notably, the hydrogen bonding in the nitrobenzoic acid co-formers favors the carboxyl or hydroxyl group rather than the nitro group.

In contrast, neutral niacinamide co-crystals are typically stabilized by amide–amide homosynthons $R_2^2(8)$ or, less frequently, amide–pyridine heterosynthons. More studies done on

niacinamide include, Bairagi et al., (2019) co-crystallized nicotinamide (NIC) with 2-chloro-5-nitrobenzoic acid (2C5NBA), a GRAS compound which is an ideal candidate for hydrogen bonding due to the presence of hydrogen bond donors and acceptors functional groups (Hutchins, 2018). Using a 1:1 stoichiometric ratio, nicotinamide·2-chloro-5-nitrobenzoic acid co-crystal was formed via slow evaporation method. The API and co-former were dissolved in an ethanol-hexane solvent mixture and left to evaporate slowly. After a few days, colorless crystals were obtained. Upon characterization and structure refinement, it was concluded that a co-crystal was formed between NIC and NIT which was held together by a strong hydrogen bond (2C5NBA) O—H $\cdots$ N (NIC). Additionally, (NIC) N—H $\cdots$ O (2C5NBA) and (NIC) C—H $\cdots$ O (2C5NBA) were also observed, N—H $\cdots$ O dimer hydrogen bonds of NIC. Weak π - π interactions stabilizing the molecular assembly of the co-crystal were also observed.

2.4.4 Isoniazid

Isoniazid (ISN) is primarily used for the treatment and prevention of tuberculosis (TB), both as monotherapy for latent infection and in combination with other medicines to treat active TB (Scheepers & Lemmerer, 2022). ISN is classified as a borderline biopharmaceutical classification system (BCS) Class I or III drug due to its low permeability (Becker et al., 2007). This drug is also characterized by poor stability which makes it a candidate for co-crystallization.

The presence of the pyridine nitrogen and hydrazide functional groups contributes to the success of the compound in co-crystallization. A CSD survey on this compound shows several modified isoniazid derivatives and several solid form compounds that have been successfully synthesized which include salts, co-crystals and hydrates. Only two reported CSD structures involve pure isoniazid co-crystals with nitrobenzoic acid compounds. These include ACUDIO structure synthesized by Diniz et al., (2018).

Diniz et al., 2018 successfully co-crystallized isoniazid with gentisic acid via slow evaporation. The resulting hydrogen bond formed between the pyridine nitrogen of the API and the hydroxyl group of the co-former (N $\cdots$ H—O). The layers were held together mainly by van der Waals interactions: both the isoniazid and the gentisic acid molecules formed segregated π - π stacks with like molecules of adjacent layers. It was concluded that isoniazid forms co-crystals with hydroxyl derivatives of benzoic acids (gentisic acid) due to complementary nature of the functional groups of isoniazid and gentisic acid (Mashhadi et al., 2021).

Lemmerer et al., 2012 co-crystallized isoniazid with dicarboxylic acid using slow evaporation method. Methanol and acetone solution were used as solvents. The co-crystal formed in methanol solution showed a two-dimensional sheet structure that makes use of all three H bond donors on the carbohydrazide functional group. When co-crystallized with the same dicarboxylic acids, the ensuing decreased hydrogen bonding functionality results in a novel supramolecular structure.

2.4.5 Isonicotinamide

Isonicotinamide (INA) is a monomer used in the synthesis of quaternized polyurethanes (PUs), which exhibit bactericidal and bacteriostatic activities against specific bacteria such as *Staphylococcus aureus* and *Escherichia coli* (Francolini & Piozzi 2016). INA contains two primary hydrogen-bonding moieties, the pyridine nitrogen which is a good hydrogen bond acceptor and the amide group which is a good hydrogen bond acceptor (carboxyl oxygen) and a good hydrogen bond donor (amine H atoms). The pyridine nitrogen is a better acceptor compared to the carboxyl Oxygen. This dual functionality makes INA an extensively studied molecule in the field of crystallography (Tothadi & Desiraju 2012).

CSD studies show that almost all co-crystal structure where INA is co-crystallized with aromatic nitro compounds form the expected $N\cdots O-H$ hydrogen bond where the pyridine nitrogen in INA is preferred as a hydrogen bond acceptor over the amide group. Example structures from CSD include TUQTUX (Sarkar & Biradha, 2006). Two synthons are proven to dominate the supramolecular chemistry of INA, amide-amide homosynthon resulting in $R_2^2(8)$ ring motifs and amide-pyridine heterosynthon. However, Tothadi & Desiraju (2012) reported an exception where co-crystallization of INA with 3,5-Dinitrobenzoic acid resulted in an unusual $N-H\cdots N$ hydrogen-bonded pattern. CSD analyses also demonstrated that INA could adopt various solid forms including salts where the pyridine nitrogen becomes protonated ERAHEN (Singh & Dubey, 2024).

2.4.6 Benzhydrazide

Benzhydrazide is an aromatic hydrazide derivative that is of great importance in medicinal chemistry (Teixeira et al., 2025). It serves as a precursor for the preparation of Schiff bases, hydrazones, oxadiazoles, pyrazoles, and other biologically active compounds exhibiting antimicrobial, antitubercular, anti-inflammatory, anticancer, and antioxidant activities (Sharma D. & Sharma A., 2024).

Benzhydrazide is also characterized by good hydrogen-bond donor and acceptor capabilities. The presence of the carbohydrazide (CONHNH₂) functional group makes it a suitable candidate for co-crystallization studies. Despite its propensity for hydrogen bonding, a CSD search conducted in June (2026) revealed no reported co-crystal structures containing benzhydrazide. Therefore, the multicomponent crystals reported in this study provide valuable insight into the intermolecular interactions driving successful co-crystallization involving the benzhydrazide moiety.

2.4.7 Acetaminophen

Acetaminophen, also known as paracetamol, is a widely used analgesic and antipyretic API for treating minor pain and fever (Srivastava et al., 2016). Acetaminophen has gained interest in crystal engineering studies due to its poor compressibility and tabletability, which pose challenges in the pharmaceutical industry (Trzybinski et al., 2016). Multicomponent crystals have been extensively studied to try and modify and improve the physicochemical properties of the API (Bučar et al., 2015).

Muzioł and Bronikowska (2024) investigated the intermolecular forces driving the formation of paracetamol multicomponent crystals through the preparation of three co-crystals comprising paracetamol with naphthalene, quinoline, and acridine. Their structural analyses revealed that the crystal packing consisted of alternating layers of paracetamol and coformer molecules stabilized by various supramolecular ring motifs. Hirshfeld surface and energy framework analyses demonstrated that strong N–H $\cdots$ O=C and O–H $\cdots$ O/N hydrogen bonds play a dominant role in stabilizing the crystal network, while dispersive interactions become increasingly significant in tail-to-tail arrangements of paracetamol molecules and in π – π stacking interactions involving aromatic coformers.

2.4.8 Summary of other reported co-crystals

Some reported co-crystals are summarized in the table below, highlighting preparation methods and key supramolecular synthon for each. This summary facilitates comparison of recurring intermolecular interaction patterns and crystallization strategies.

Table 2: Summary of reported co-crystals

API	Co-former	Preparation method(s)	Key supermolecular synthon	References
Theophylline	Oxalic acid	Slow evaporation	O-H $\cdots$ N (Acid-amide), N-H $\cdots$ O	Zhang et al., 2012
Theophylline	Pyrazinecarboxylic acid	Slow evaporation	O-H $\cdots$ N, N-H $\cdots$ O	Raish et al., 2025
Acetaminophen	Trimethyl glycine	Slow evaporation	N-H $\cdots$ O, O-H $\cdots$ O; zwitterion assisted	Sarkar et al., 2020
Benzhydrazide	5-Aminoisophthalic acid	Slow evaporation	N-H $\cdots$ O, O-H $\cdots$ O framework	Bourletidis How et al., 2025

2.5 Nitrobenzoic acid derivatives co-formers

2.5.1 General introduction

Derivatives of nitrobenzoic acid are increasingly recognized as useful co-formers in co-crystallization and drug formulations. This class of co-formers contains carboxylic acid and nitro functional groups that can act as both hydrogen bond donors and acceptors, while also displaying π - π stacking and nitro aromatic interactions. The two subchapters below discuss some of the co-formers chosen for this study.

2.5.2 Nitroterephthalic acid (NTPA)

Nitroterephthalic acid has the potential to act as a good co-former by forming strong hydrogen bonds and other interactions with API molecules thus leading to successful co-crystallization. Carboxylic acid groups can act as good hydrogen bond donors and acceptors while the nitro group can also act as a hydrogen bond acceptor. For example, terephthalic acid, closely related structurally to nitroterephthalic acid, has been successfully used as a co-former. Cruz et al., 2020

co-crystallized Itraconazole, an antifungal drug characterized by very low solubility with terephthalic acid.

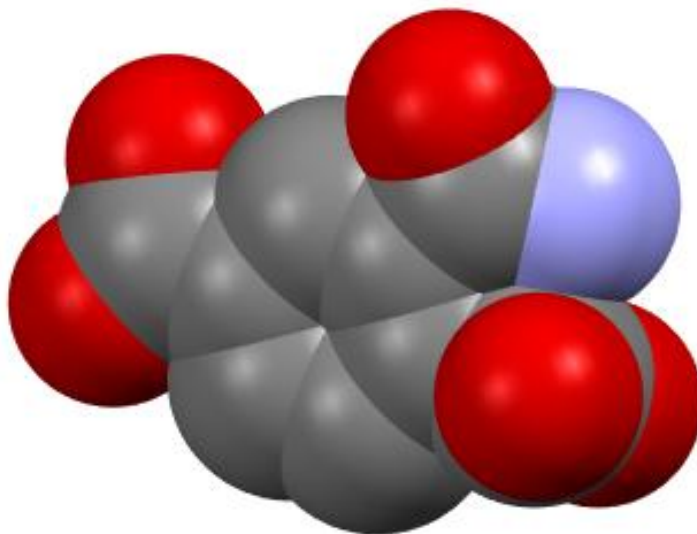


Figure 8: Space fill diagram for Nitroterephthalic acid co-former

2.5.3 3,5-Dinitrosalicylic acid (DNSA)

DNSA is an aromatic carboxylic acid containing both a carboxylic acid group and nitro groups, making it a suitable candidate as a co-former. The presence of these functional groups makes this compound a hydrogen bond donor through the hydroxyl group and a good hydrogen bond acceptor through both the nitro groups and the carboxylic acid group. Although CSD search shows no crystals of *N*-heterocyclic APIs with DNSA, several studies have reported successful co-crystals of DNSA as a co-former with other compounds. Shi et al., (2023) successfully co-crystallized levetiracetam, an API used for partial onset seizure with DNSA. Characterization of this structure showed hydrogen bonds are the main interaction contributing to structural stabilization.

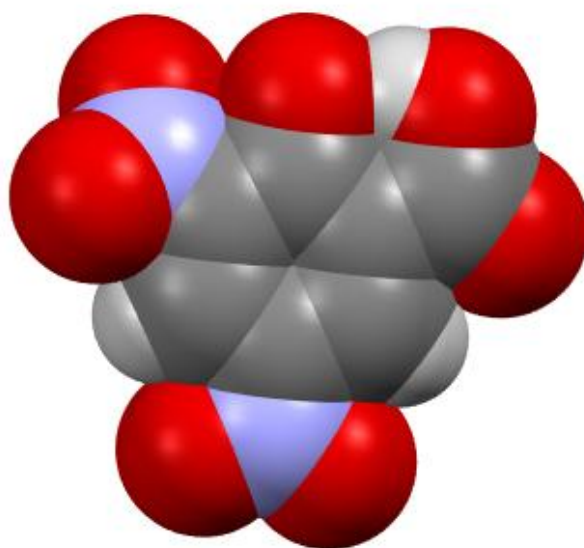


Figure 9: Space fill diagram for 3,5-Dinitrosalicylic acid co-former

Despite the progress made on crystal engineering and the success in developing *N*-heterocyclic co-crystals, there is a notable scarcity of research on co-crystallization of *N*-heterocyclic APIs compared to other classes of drugs despite their significance in the pharmaceutical industry. Nitrobenzoic acid derivatives have also been under-utilized as co-formers in co-crystallization. Therefore, there is a need to expand the *N*-heterocyclic co-crystal landscape using a variety of nitrobenzoic acid derivatives relatively unexplored. This study aims to explore the specific bond interactions and molecular mechanisms that influence co-crystal formation in *N*-heterocyclic APIs and nitrobenzoic acid derivatives co-formers. Understanding these interactions at a deeper level may lead to new crystalline forms exhibiting improved physicochemical properties.

Chapter 3: Methodology

3.1 Materials

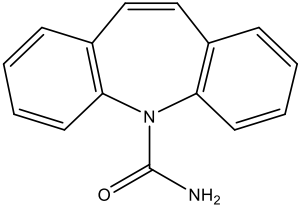
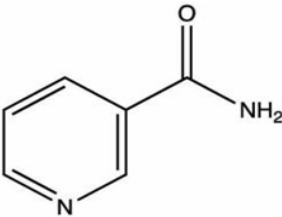
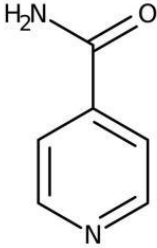
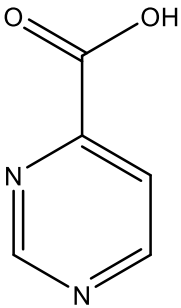
Analytical grade APIs and nitrobenzoic acid derivative co-formers were purchased from commercial suppliers and used without further purification. The APIs used are; Carbamazepine ($\geq 98.0\%$, Sigma-Aldrich), Niacinamide ($\geq 99.0\%$, Sigma-Aldrich), Benzhydrazide ($\geq 97.0\%$, Sigma-Aldrich), Nicotinic hydrazide ($\geq 98.0\%$, Sigma-Aldrich), Isonicotinamide ($\geq 98.0\%$, Sigma-Aldrich), Theophylline ($\geq 99.0\%$, Sigma-Aldrich), Acetaminophen ($\geq 99.0\%$, Sigma-Aldrich), Pyrazinecarboxylic acid ($\geq 98.0\%$, Sigma-Aldrich) and 3-Amino-2-pyrazinecarboxylic acid ($\geq 99.0\%$, Sigma-Aldrich).

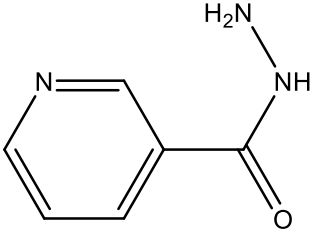
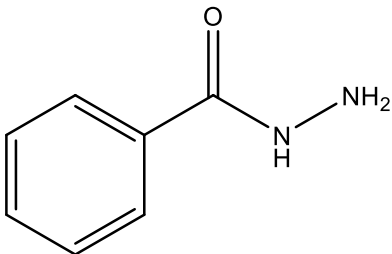
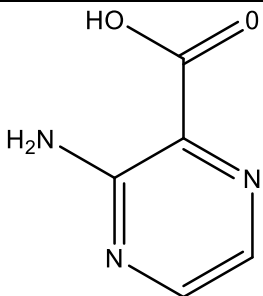
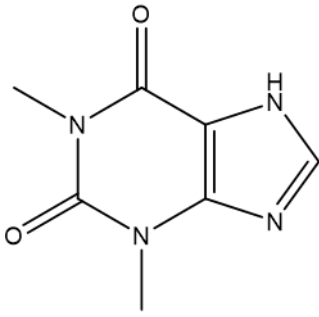
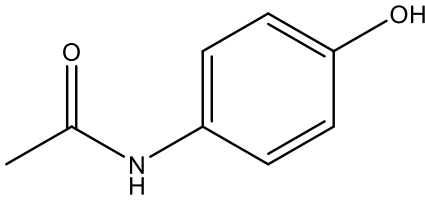
2-Nitroterephthalic acid ($\geq 98.0\%$, Sigma-Aldrich), 2-(nitrophenyl)methanol ($\geq 99.0\%$, Sigma-Aldrich) and 3,5-Dinitrosalicylic acid (DNSA, $\geq 98.0\%$, Sigma-Aldrich) were used as co-formers.

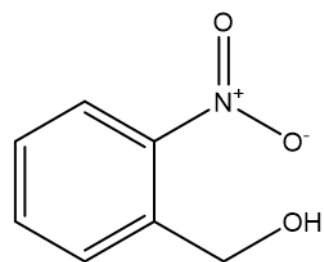
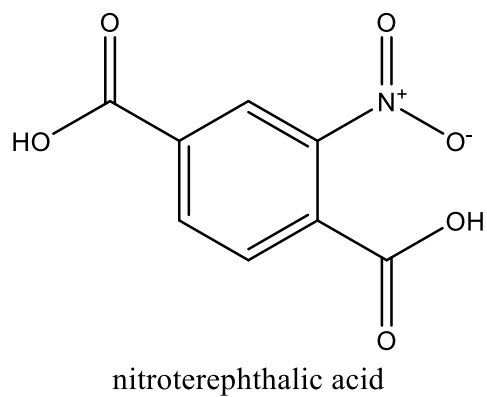
Methanol (HPLC grade, $\geq 99.9\%$, Sigma-Aldrich), Ethanol (absolute, $\geq 99.8\%$, Sigma-Aldrich), Acetone (HPLC grade, $\geq 99.8\%$, Sigma-Aldrich), Chloroform ($\geq 99.0\%$, Sigma-Aldrich), DMSO ($\geq 99.9\%$, Sigma-Aldrich, Cat. D8418) were used as preferred solvents. All materials were used as received.

Table 3 and figure 10 show selected APIs and co-formers, their structures and physicochemical properties.

Table 3: Structures and physicochemical properties of selected APIs

API	Reason for selection	Structure
Carbamazepine	Very low solubility; classic BCS II	
Niacinamide	H-bond donors/acceptors; forms strong acid...pyridine synthons	
Isonicotinamide	H-bond donors/acceptors; forms strong acid...pyridine synthons	
Pyrazinecarboxylic acid	Can form acid...base or OH...N networks	

Nicotinic acid hydrazide	H-bond donor/acceptor sites; good partner for acid...pyridine / amide networks	
Benzhydrazide	Presence of the hydrazide group which is a good hydrogen bond donor and acceptor	
3-amino-2-pyrazinecarboxylic acid	Moderate solubility: amino group provides additional H-bond donor for synthon formation	
Theophylline	H-bond donors and acceptor	
Acetaminophen	Moderate solubility; strong phenolic OH donor	



2-(nitrophenyl)methanol

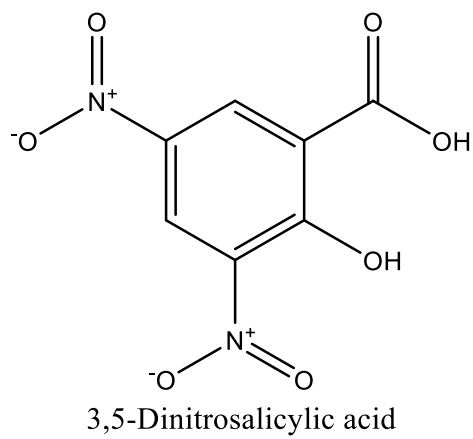


Figure 10: Selected co-formers for co-crystallization

3.2 General strategy for co-crystal screening

A total of 110 crystallization trials were conducted to maximize the probability of successful co-crystal formation by systematically varying experimental parameters. The goal was to identify solvent and methods that favour co-crystal formation of *N*-heterocyclic APIs, a method suitable and crystallization condition favouring co-crystal formation.

3.3 Co-crystallization method

3.3.1 Slow evaporation method

A stoichiometric ratio of 1:1, mostly observed in successful co-crystallization was used. Approximately 50 mg- 100 mg of each API and equimolar of each co-former were weighed into a 50 ml polytop vial, about 3 ml of the different solvent chosen were used to dissolve the mixture. These solvents were chosen based on their ability to dissolve both components, and to provide a range of polarities and hydrogen-bonding characteristics.

The mixture was placed on a magnetic stirrer, stirred until everything was dissolved, stirrer hotplate and or DMSO was applied for other samples that did not dissolve in the solvents chosen to facilitate complete dissolution.

The solution was then left to crystallize at room temperature on a flat surface with no mechanical disturbance with the vial lid slightly opened to allow slow evaporation of the solvent. The samples were monitored for any crystal formation within a few hours to about 5 days. Figure 11 shows some of the samples during the slow evaporation process.



Figure 11: Some samples during slow evaporation process

The table show some of the samples prepared using slow evaporation methods, where different solvents and different crystallization conditions were employed. Some of the trials resulted in the formation of co-crystals, while others yielded only starting materials or no crystals at all. Starting materials refer to the pure individual components used in the experiment, whereas co-crystals are defined as crystalline solids in which the two different components are held together in a well-defined stoichiometric ratio. The identities of the co-crystals and starting materials were confirmed using SCXRD. Each of the APIs was tested with different co-formers, using various solvents and at different temperatures. Most experiments were conducted at room temperature, while some were heated to 70 °C for approximately 2 minutes to ensure complete dissolution of the reagents

Table 4: Summary of successful and unsuccessful co-crystallization outcomes of slow evaporation. Successful attempts are highlighted in color

Co-crystal ID	API	Co-former	Solvent	Condition	Outcome
LM001	Carbamazepine	Nitroterephthalic acid	Methanol	Room temperature	Starting material
LM002	Niacinamide	Nitroterephthalic acid	Methanol, few drops DMSO	Room temperature	No-crystals
LM003	Benzhydrazide	Nitroterephthalic acid	Methanol	Room temperature	Starting material
LM004	Nicotinic hydrazide	Nitroterephthalic acid	Methanol +DMSO drops	Room temperature	Co-crystal
LM049	Isonicotinamide	2-(nitrophenyl)methanol	Methanol	Room temperature	Co-crystal
LM050	Carbamazepine	2-(nitrophenyl)methanol	Methanol	Room temperature	Co-crystal
LM051	Benzhydrazide	Nitroterephthalic acid	1:1 methanol, water	Heated to about 70 °C	Co-crystal
LM052	Carbamazepine	Nitroterephthalic acid	1:1 methanol, water	Heated	Co-crystal
LM053	Niacinamide	Nitroterephthalic acid	1:1 methanol, water	Heated to about 70 °C	Co-crystals

LM054	Nicotinic hydrazide	Nitroterephthalic acid	1:1 methanol, water	Heated	Co-crystal
LM055	3-Amino-2- pyrazinecarboxylic acid	Nitroterephthalic acid	1:1 methanol, water	Heated	Starting material
LM056	3-Amino-2- pyrazinecarboxylic acid	Nitroterephthalic acid	Acetone, DMSO drops	Room temperature	Starting material
LM089	Carbamazepine	3,5-dinitrosalicylic acid	Methanol	Room temperature	Co-crystal
LM090	Isonicotinamide	3,5-dinitrosalicylic acid	Methanol	Room temperature	Co-crystal
LM091	Nicotinic hydrazide	3,5-dinitrosalicylic acid	Methanol, DMSO drops	Room temperature	Co-crystal
LM092	Niacinamide	3,5-dinitrosalicylic acid	Methanol, DMSO drops	Room temperature	Co-crystal
LM094	Benzhydrazide	3,5-dinitrosalicylic acid	Methanol	Room temperature	Co-crystal
LM095	Pyrazinecarboxylic acid	2-nitroterephthalic acid	Methanol	Heated to about 70	Co-crystal
LM102	Theophylline	3,5-dinitrosalicylic acid	Methanol	Room temperature	Co-crystal
LM104	Theophylline	Nitroterephthalic acid	Methanol	Room temperature	Starting material
LM108	Acetaminophen	3,5-dinitrosalicylic acid	Methanol	Room temperature	Co-crystal

3.3.2 Ball milling method (Mechanochemistry)

A stoichiometric ratio of 1:1 was also used for this method. About 100mg of each API and equal moles of the co-former were weighed into a grinding jar. A small amount of methanol solvent was added into the mixture, about 1ml. Stainless steel balls (5 mm diameter, 3 balls per jar) were put into the jar and sealed. The jar was then mounted in the ball mill and milled at 25 Hz for 40 minutes. After grinding most of the samples would appear as a thick solution and upon drying, they produced a powder with no crystals. Some of the samples were further dissolved using methanol and DMSO, and some were exposed to prolonged heating after grinding. Table 5 shows some of the samples made using ball milling method with only one sample resulting in a co-crystal.

Table 5: Summary of successful and unsuccessful co-crystallization outcomes of ball milling. Successful attempts are highlighted in color

Co-crystal ID	API	Co-former	Solvent	Condition	Outcome
LM070	Isonicotinamide	Nitroterephthalic acid	Methanol	25hz/40 minutes	Powder
LM071	3-Amino-2-pyridinecarboxylic acid	Nitroterephthalic acid	Methanol	25hz/40 minutes	Powder
LM101	Isonicotinamide	Nitroterephthalic acid	Methanol	25hz/ 40 minutes	Co-crystal

3.4 Characterization and structure refinement

Single crystal X-ray diffraction (SCXRD) was employed as a characterization technique for all crystals synthesized in this study, providing unambiguous determination of their molecular structures, protonation states, and supramolecular architectures. For LM095C, however, additional characterization techniques, including FTIR, ¹H NMR and ¹³C NMR spectroscopy, were done owing to the unexpected formation of the esterified product, methyl pyrazine-2-carboxylate-2-nitroterephthalic acid. These complementary analyses were performed to confirm the occurrence of esterification, verify the chemical identity of the isolated phase, and provide supporting evidence for the presence of the methyl ester functional group

For SCXRD analysis, a suitable single crystal was selected using a microscope with polarizing attachment. The selected crystal was mounted on mitigen mount and placed on a diffractometer and analysed using Bruker Apex II CCD area diffractometer with monochromated Mo K α ($\lambda = 0.71073$) at a temperature of -173.15°C (100 K). A combination of Olex 2 (Dolomanov et al., 2009) and X-seed (Barbour, 2001) were used for solving and refining the crystal structures wherein both programs made use of SHELXT (Sheldrick, 2015a) for solving crystal structures and SHELXL (Sheldrick, 2015b) for refining structures. Mercury (Macrae et al., 2008) was used for analysing the packing within the lattices of each molecule, how the molecules interact with each other outside the unit cell. Ortep diagram was used to generate diagrams of asymmetric unit of each structure and showing interaction of the different molecule within the unit cell. Crystal explorer (Spackman et al., 2021) was used for Hirshfeld surface analyses for one of the structures.

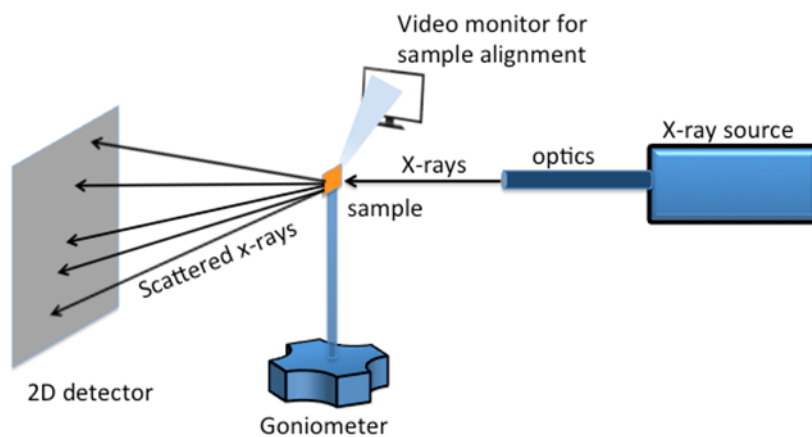


Figure 12: Schematic representation of the Single Crystal X-ray diffractometer used in the study [55]

Chapter 4: Results and discussion

4.1 Comparison of co-crystallization methods and conditions

4.1.1 Co-crystallization methods

Of the two methods used, slow evaporation and ball milling (mechanochemistry), slow evaporation yielded a higher success rate, producing fourteen of the fifteen successful crystalline structures while ball milling yielded only one co-crystal. Slow evaporation yielded co-crystals, salts and hydrates and the one crystalline structure yielded using mechanochemistry was a pure co-crystal. The slow evaporation method produced mostly transparent, clear and large crystals which may be attributed to gradual solvent evaporation which promotes slow nucleation and allows molecules enough time to arrange into well-ordered lattices.

Ball milling produced mostly polycrystalline samples. The crystals produced with ball milling tend to have an unclear morphology, irregular shape with no defined edges making it very difficult to select a single crystal for characterization.

Co-crystals prepared by ball milling exhibit high defect density due to rapid, high-energy collisions, which favor kinetically trapped structures over well-ordered crystals. The two images in figure 13 show representative crystals obtained via slow evaporation (A) and ball milling (B). Crystal A is transparent, big in size with well-defined edges whereas in B the morphology of the crystals is unclear.

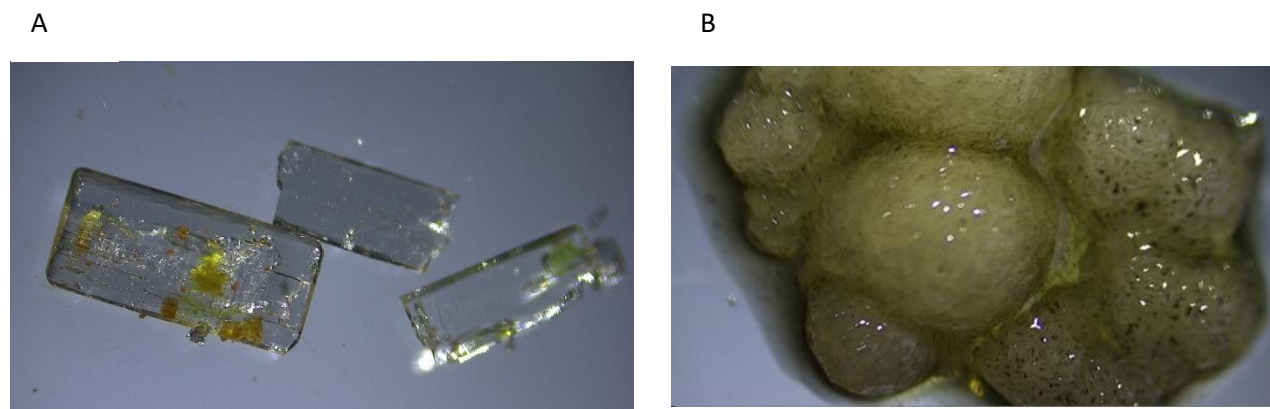


Figure 13: Comparison of crystal size (A) obtained from slow evaporation and (B) mechanochemistry

In the slow evaporation method, different samples formed crystals at different rates, some samples took a few hours to grow crystals and others took a maximum of five days to grow crystals. Crystals that were left for longer tend to be bigger than crystals that formed within a few hours. Extended crystallization times reduce nucleation rate relative to growth rate. This favors the formation of fewer, but larger and well-ordered crystals. Rapid crystallization (shorter times) produces smaller crystals because many nuclei form quickly, competing for solute and limiting growth. Figure 14 shows crystal (A) left to evaporates for five days and crystal (B) left to evaporate for a about six hrs.

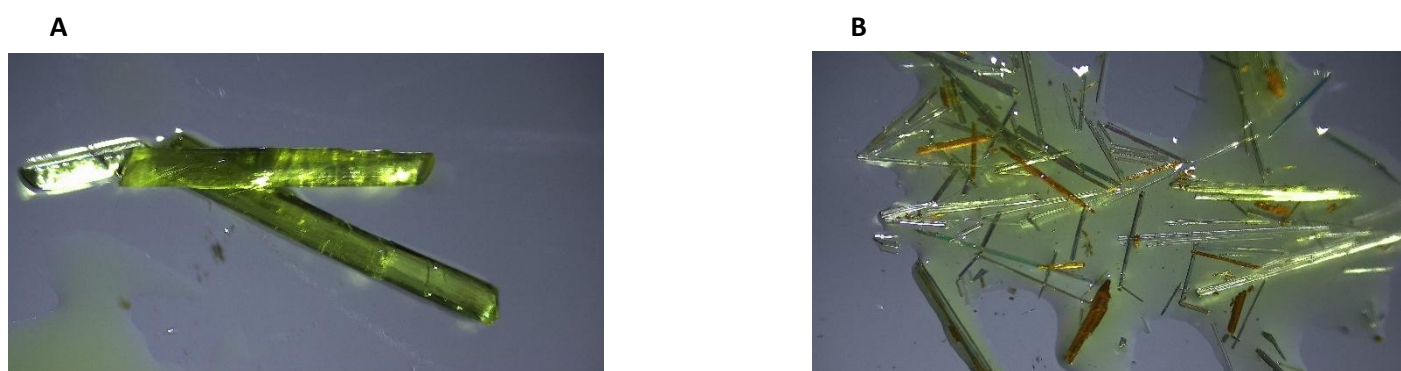


Figure 14: Comparison of the effect of evaporation time on crystal size

4.1.2 Effect of solvent choice and crystallization conditions

The effect of different solvent was also investigated in this study and the results showed that solvent choice significantly influences the crystallization outcome. Methanol was the most used solvent and generally yielded most of the co-crystals.

This can be attributed to its intermediate polarity, which allows both the API and co-former to dissolve adequately without excessive solvation. Methanol also has a good balance of hydrogen-bond donor and acceptor properties, facilitating the formation of strong and directional intermolecular interactions between the two components. Additionally, its moderate volatility allows controlled evaporation, promoting the growth of well-formed single crystals suitable for SCXRD analysis.

In one case where methanol was used as a solvent, it was incorporated into the co-crystal lattice resulting in a salt solvate, a behavior commonly observed with small polar solvents (Chadha &

Bhalla, 2014). Figure 15 shows the structure of nicotinic hydrazide. 3,5-dinitrosalicylic co-crystals salt with methanol present in the crystal lattice.

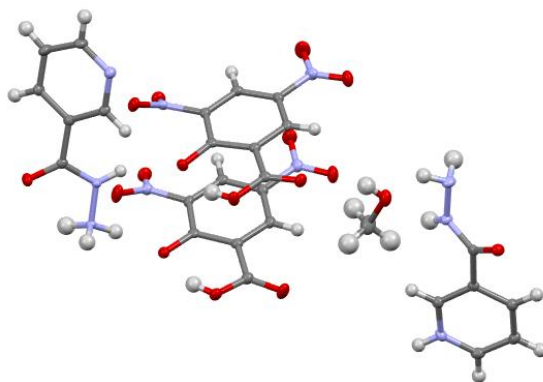


Figure 15: Asymmetric unit for LM091 showing methanol solvent incorporated into the crystal lattice

In cases where methanol did not fully dissolve the reagents, a few drops of DMSO were added to enhance solubility since DMSO is highly polar and a strong hydrogen-bond acceptor. The resulting mixed-solvent system promoted co-crystal formation, and in most cases DMSO was not incorporated into the final crystal structures, showing that the interaction of the API and co-former were favoured over solvent inclusion. Only one structure showed DMSO incorporation, likely due to favourable hydrogen-bonding interactions within the lattice.

In contrast, DMSO used as a sole solvent did not yield crystalline products but resulted in oiling out, which may be attributed to its high boiling point and strong solvation ability that hindered controlled supersaturation and nucleation. Acetone was also unsuccessful, likely due to its rapid evaporation rate, which induced fast supersaturation and inhibited ordered crystal growth. Ethanol similarly failed to produce co-crystals, possibly due to differences in polarity and hydrogen-bonding characteristics that did not sufficiently promote heteromolecular assembly.



Figure 16: Some of DMSO samples oiling out

Mixed solvent systems were also explored, particularly methanol–water and ethanol–water combinations. Methanol and water yielded co-crystal hydrates, salt, and salt co-crystals while ethanol and water resulted in starting material. The complementary polarity and hydrogen-bonding characteristics of the methanol and water system enable balanced solvation of both API and co-former, facilitating heteromolecular nucleation. Conversely, the ethanol and water system may preferentially stabilize one component in solution, thereby reducing the probability of heteromolecular assembly and favoring recovery of the starting materials.

4.2 A series of Co-crystals of 2-nitroterephthalic acid

In this section we present a series of novel crystalline structures obtained from co-crystallizing 2-nitroterephthalic acid with 4 *N*-heterocyclic APIs and two other APIs (benzhydrazide and carbamazepine). A diversity of crystalline structures was observed in this series, two salt co-crystals, two pure co-crystal, one salt hydrate and one co-crystal hydrate. Table 6 below summarizes the predicted and observed solid forms predicted based on the ΔpK_a values.

$\Delta pK_a = pK_a(\text{base}) - pK_a(\text{acid})$. According to the ΔpK_a rule proposed by Cruz-Cabeza et al. (2022), values of $\Delta pK_a < -1$ generally favor co-crystal formation, values of $\Delta pK_a > 4$ favor salt formation, whereas systems with ΔpK_a values between -1 and 4 lie within the salt–co-crystal continuum, where the outcome may depend on factors such as solvent choice and crystallization conditions. The observed forms were confirmed by SCXRD analyses.

The chapter further highlights resulting supramolecular synthons, how 2-nitroterephthalic acid interacts with structurally diverse *N*-Heterocyclic APIs, hydrogen bonding patterns and crystal packing. All six crystals were synthesized using the slow evaporation method.

Table 6: summary of predicted and observed solid forms

API	pKa value (API)	pKa value (NTPA)	ΔpKa	Expected outcome	Observed outcome
Benzhydrazide	12.45	1.73	10.72	Salt	Salt
Niacinamide	3.63	1.73	1.90	Borderline	Salt
Nicotinic hydrazide	11.43	1.73	9.70	Salt	Salt
Pyrazinecarboxylic acid	2.92	1.73	1.19	Borderline	Co-crystal
Isonicotinamide	3.45	1.73	1.72	Borderline	Co-crystal

Table 7 Co-crystal structure of 6 different APIs with 2-nitroterephthalic acid (NTPA)

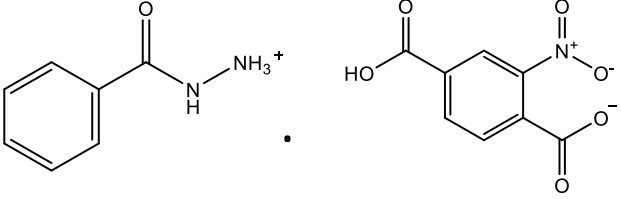
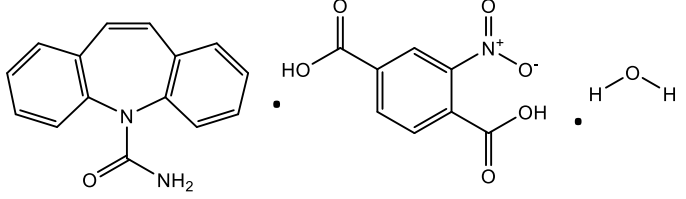
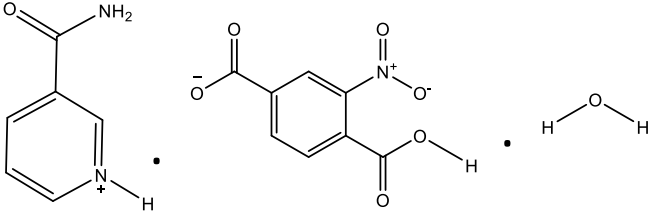
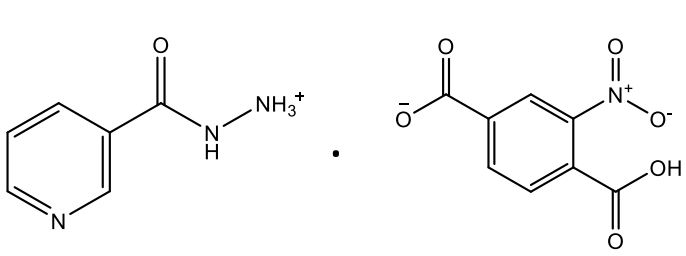
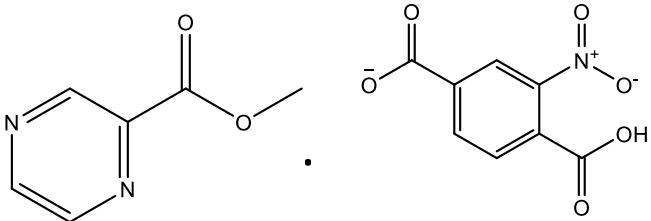
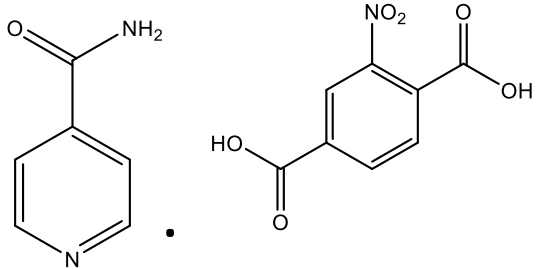
 Sample name: LM051 Co-crystal name: Benzhydrazide · NTPA salt co-crystal CCDC number: 2482400	 Sample name: LM052d Co-crystal name: Carbamazepine · NTPA co-crystal hydrate CCDC number: 2471640
 Sample name: LM053d Co-crystal name: Niacinamide · NTPA salt hydrate CCDC number: 2471621	 Sample name: LM054D Co-crystal name: Nicotinic hydrazide · NTPA Salt co-crystal CCDC number: 2471737
 Sample name: LM095c Co-crystal name: Methyl pyrazine-2-carboxylate · NTPA CCDC: 2449438	 Sample name: LM101 Co-crystal name: Isonicotinamide · NTPA CCDC number: 2503459

Table 8: Crystal data and structure refinement for 2-Nitrorerephthalic acid series of co-crystals

Identification code	LM051C	LM052D	LM053D
Empirical formula	C ₁₅ H ₁₃ N ₃ O ₇	C ₂₃ H ₁₉ N ₃ O ₈	C ₁₄ H ₁₃ N ₃ O ₈
Formula weight	347.28	465.41	351.27
Temperature/K	100.00	100.00	131.00
Crystal system	Orthorhombic	Monoclinic	Monoclinic
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i> /Å	4.6380(2)	11.3581(4)	14.7277(4)
<i>b</i> /Å	12.7148(5)	7.4373(2)	6.7676(2)
<i>c</i> /Å	25.5689(9)	24.6629(7)	15.1906(4)
α /°	90	90	90
β /°	90	90.6880(10)	95.6690(10)
γ /°	90	90	90
Volume/Å ³	1507.83(10)	2083.21(11)	1506.66(7)
<i>Z</i>	4	4	4
$\rho_{\text{calc}}/\text{cm}^3$	1.530	1.484	1.549
μ/mm^{-1}	0.124	0.114	0.130
<i>F</i> (000)	720.0	968.0	728.0
Crystal size/mm ³	0.062 × 0.098 × 0.378	0.092 × 0.164 × 0.243	0.156 × 0.176 × 0.299
Radiation	MoK α (λ = 0.71073)	MoK α (λ = 0.71073)	MoK α (λ = 0.71073)
2 Θ range for data collection/°	4.518 to 54.982	4.846 to 55.17	5.39 to 55.27
Index ranges	-5 ≤ <i>h</i> ≤ 6, -16 ≤ <i>k</i> ≤ 16, -33 ≤ <i>l</i> ≤ 32	-14 ≤ <i>h</i> ≤ 14, -9 ≤ <i>k</i> ≤ 9, -32 ≤ <i>l</i> ≤ 31	-19 ≤ <i>h</i> ≤ 19, -8 ≤ <i>k</i> ≤ 8, -19 ≤ <i>l</i> ≤ 19
Reflections collected	20940	52400	43505
Independent reflections	3440 [<i>R</i> _{int} = 0.0559, <i>R</i> _{sigma} = 0.0423]	4809 [<i>R</i> _{int} = 0.0405, <i>R</i> _{sigma} = 0.0216]	3483 [<i>R</i> _{int} = 0.0363, <i>R</i> _{sigma} = 0.0200]
Data/restraints/parameters	3440/0/236	4809/0/312	3483/0/232
Goodness-of-fit on <i>F</i> ²	1.087	1.035	1.069
Final <i>R</i> indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0360, <i>wR</i> ₂ = 0.0856	<i>R</i> ₁ = 0.0358, <i>wR</i> ₂ = 0.0903	<i>R</i> ₁ = 0.0336, <i>wR</i> ₂ = 0.0938
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0419, <i>wR</i> ₂ = 0.0886	<i>R</i> ₁ = 0.0451, <i>wR</i> ₂ = 0.0965	<i>R</i> ₁ = 0.0374, <i>wR</i> ₂ = 0.0976
Largest diff. peak/hole / e Å ⁻³	0.33/-0.21	0.34/-0.28	0.35/-0.29

Identification code	25_LM_LM054D_P	25_LM_LM095C_p	25_LM_LM101_p
Empirical formula	C ₁₄ H ₁₂ N ₄ O ₇	C ₂₃ H ₁₇ N ₂ O ₁₀	C ₁₆ H ₁₄ N ₃ O ₈ S
Formula weight	348.28	481.399	408.36
Temperature/K	100.00	100.00	100.00
Crystal system	Monoclinic	Orthorhombic	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>c</i>	<i>Pccn</i>	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i> /Å	8.2054(4)	22.2664(5)	6.8199(2)
<i>b</i> /Å	15.1092(6)	13.0176(3)	30.1281(10)
<i>c</i> /Å	11.9418(5)	14.5637(3)	9.1017(3)
α /°	90	90	90
β /°	98.411(2)	90	109.8680(10)
γ /°	90	90	90
Volume/Å ³	1464.58(11)	4221.36(16)	1758.82(10)
<i>Z</i>	4	8	4
$\rho_{\text{calc}}/\text{cm}^3$	1.579	1.515	1.542
μ/mm^{-1}	0.130	0.121	0.237
<i>F</i> (000)	720.0	1993.7	844.0
Crystal size/mm ³	0.102 × 0.149 × 0.322	0.226 × 0.223 × 0.132	0.102 × 0.110 × 0.308
Radiation	MoK α (λ = 0.71073)	Mo K α (λ = 0.71073)	MoK α (λ = 0.71073)
2 θ range for data collection/°	4.376 to 55.134	3.66 to 55	4.948 to 54.986
Index ranges	-10 ≤ <i>h</i> ≤ 10, -19 ≤ <i>k</i> ≤ 19, -15 ≤ <i>l</i> ≤ 15	-27 ≤ <i>h</i> ≤ 28, -16 ≤ <i>k</i> ≤ 15, -18 ≤ <i>l</i> ≤ 18	-8 ≤ <i>h</i> ≤ 8, -39 ≤ <i>k</i> ≤ 39, -11 ≤ <i>l</i> ≤ 11
Reflections collected	42702	79441	55732
Independent reflections	3379 [<i>R</i> _{int} = 0.0582, <i>R</i> _{sigma} = 0.0288]	4855 [<i>R</i> _{int} = 0.0397, <i>R</i> _{sigma} = 0.0184]	4021 [<i>R</i> _{int} = 0.0675, <i>R</i> _{sigma} = 0.0342]
Data/restraints/parameters	3379/0/234	4855/0/334	4021/0/264
Goodness-of-fit on <i>F</i> ²	1.035	1.062	1.022
Final <i>R</i> indexes [<i>I</i> > 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0368, <i>wR</i> ₂ = 0.0928	<i>R</i> ₁ = 0.0373, <i>wR</i> ₂ = 0.1088	<i>R</i> ₁ = 0.0845, <i>wR</i> ₂ = 0.2115
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0495, <i>wR</i> ₂ = 0.1009	<i>R</i> ₁ = 0.0462, <i>wR</i> ₂ = 0.1179	<i>R</i> ₁ = 0.1010, <i>wR</i> ₂ = 0.2252
Largest diff. peak/hole / e Å ⁻³	0.37/-0.23	0.44/-0.26	1.43/-1.03

4.2.1 Benzhydrazide · 2-Nitroterephthalic acid salt co-crystal (LM051)

Colourless plate-like crystals were afforded after two days through slow evaporation method. A single suitable crystal was selected for SCXRD analysis. Figure 17 shows the morphology of the crystals under the microscope.

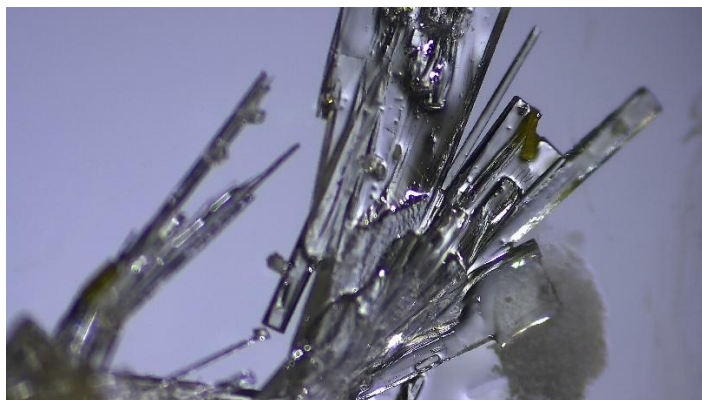


Figure 17: Optical image of benzhydrazide · 2-Nitroterephthalic acid (LM051) at 100×

The asymmetric unit of benzhydrazide · nitroterephthalic acid contains one molecule of protonated benzhydrazide cation and one molecule of deprotonated nitroterephthalic acid anion. Proton transfer occurs from the carboxylic acid group of nitroterephthalic acid (the COOH group ortho to the nitro substituent) to the terminal amino group of benzhydrazide, resulting in an NH_3^+ moiety. This co-crystal crystallizes in the $P2_12_12_1$ space group.

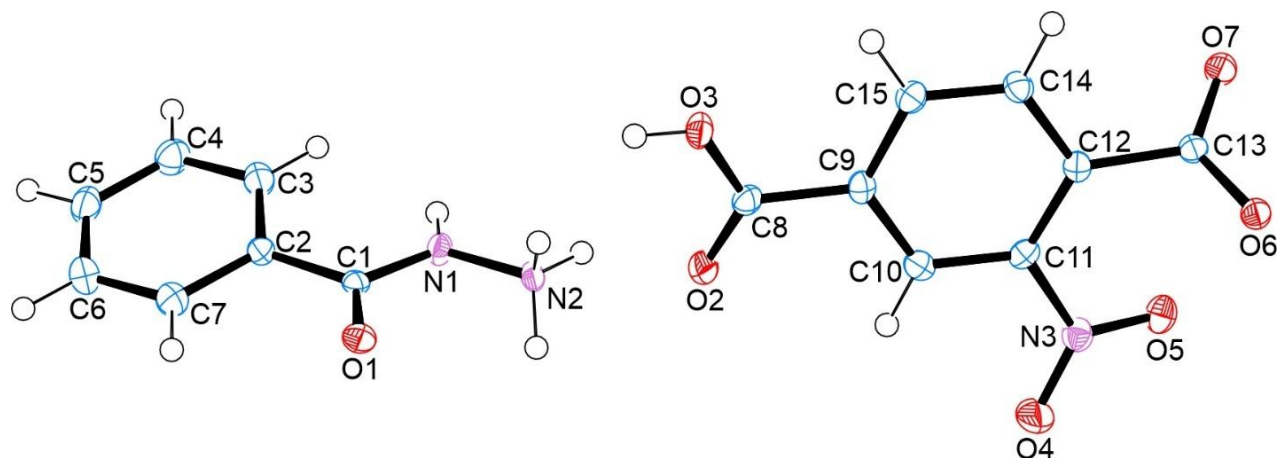


Figure 18: The asymmetric unit of Benzhydrazide · NTPA with ellipsoids at 50% probability

Within the unit cell, the deprotonated carboxylate group of nitroterephthalic acid engages in bifurcated hydrogen bonding, simultaneously interacting with the protonated NH_3^+ group of benzhydrazide and the hydroxyl group of a neighboring, un-ionized carboxylic acid.

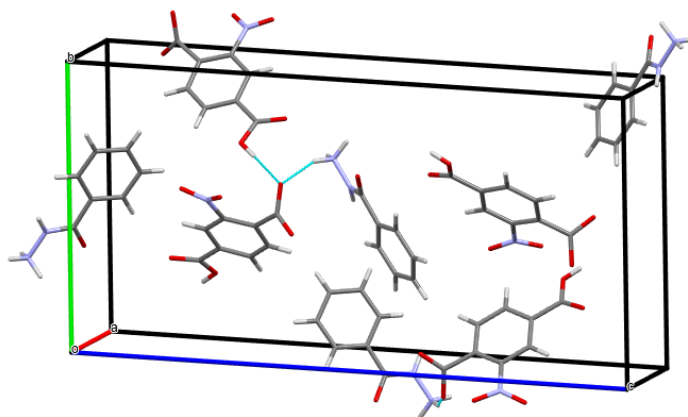


Figure 19: Crystal packing of benzhydrazide · NTPA co-crystal salt along the *b*-axis showing hydrogen bonding within the unit cell

The benzhydrazide molecule in the asymmetric unit adopts a nearly planar conformation. Torsion $\text{C}_{\text{ring}}\text{--C=O--N1--N2} = 179.15^\circ$, the terminal NH_3^+ group is extended $\text{C=O--N1--N2--H} = 177.84^\circ$, allowing it to participate in strong hydrogen bonding.

Table 9: Hydrogen Bonds for LM051

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
N1	H1	O7 ¹	0.88	1.98	2.709(3)	138.8
N2	H2A	O6 ¹	0.89	1.92	2.772(3)	161.6
N2	H2B	O1 ²	0.95(3)	1.81(3)	2.754(2)	172(3)
O3	H3A	O6 ³	0.84	1.79	2.626(2)	172.9

Symmetry transformations used to generate equivalent atom:

¹2-x,-1/2+y,1/2-z; ²1/2+x,3/2-y,1-z; ³1-x,-1/2+y,1/2-z

4.2.2 Carbamazepine (CBZ) · 2-Nitroterephthalic acid (NTPA) Co-crystal hydrate (LM052d)

The crystals obtained as shown in figure 20 appear as clusters rather than isolated single plate like crystals. Individual crystals within the cluster show well define edges and a uniform plate-like shape although the overall morphology is somewhat not clear.



Figure 20: Optical microscope image of carbamazepine · 2-Nitroterephthalic acid (LM052D) at 100×

The asymmetric unit of LM052D consists of one molecule of Carbamazepine, one molecule of 2-Nitroterephthalic acid and one molecule of water resulting in a co-crystal hydrate. This co-crystal crystallizes in the $P2_1/c$. A strong O–H $\cdots$ O hydrogen bond is observed between the hydroxyl group of the carboxylic acid moiety (O2–H2A) in NTPA and the carbonyl oxygen atom (O1) of carbamazepine, with an O2 $\cdots$ O1 donor–acceptor distance of 2.5772(13) Å and an O2–H2A $\cdots$ O1 bond angle of 165.4°. Additionally, the water molecule also forms a hydrogen bond with the hydroxyl hydrogen of the NTPA molecule.

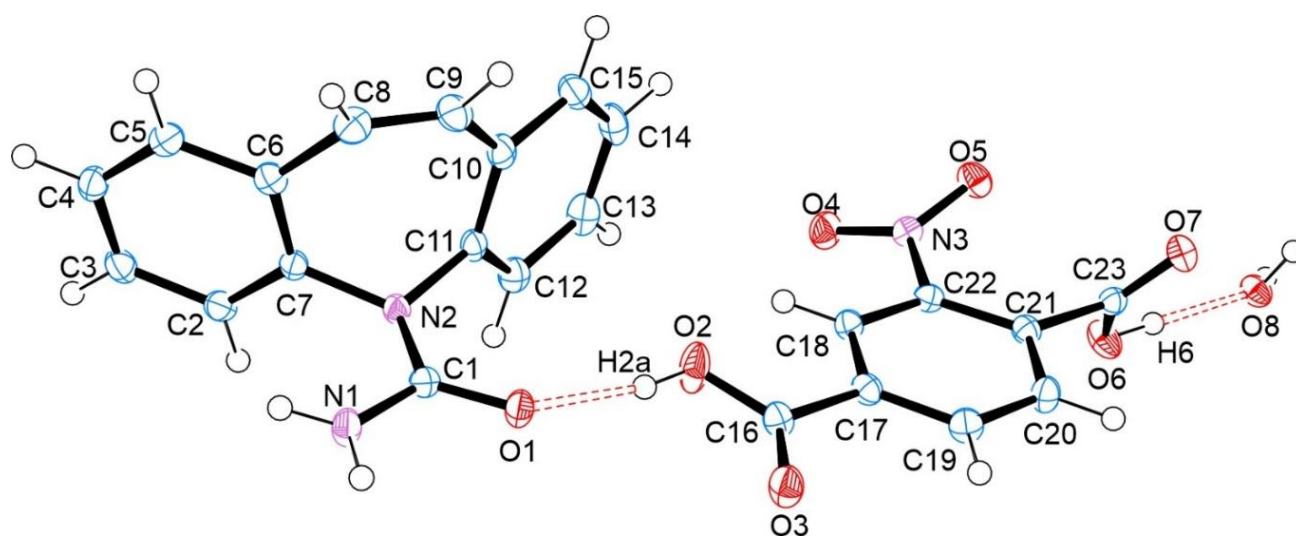


Figure 21: Asymmetric unit of Carbamazepine · nitroterephthalic co-crystal hydrate with ellipsoids at 50% probability

In terms on packing, the crystal structure shows a hydrogen-bonded (O–H $\cdots$ O) chain formed by NTPA molecules running parallel to the b -axis. The centroid–centroid distance between aromatic rings (7.437Å) indicates the absence of significant π – π stacking interactions, typical π – π is 3.3–3.8 Å.

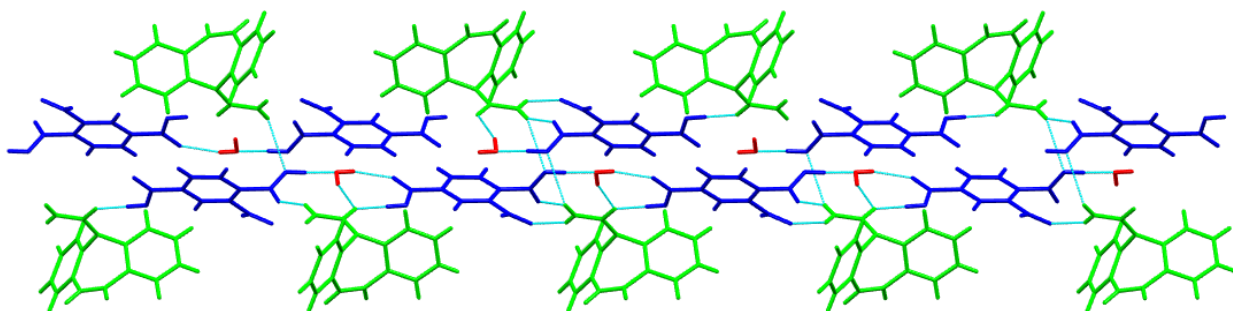


Figure 22: Crystal packing of CBZ· NTPA along the *b*-axis

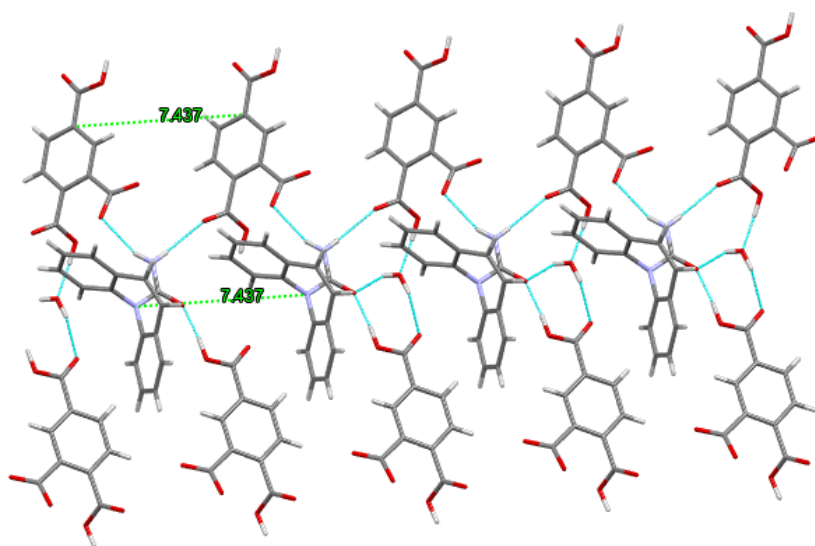


Figure 23: Crystal packing of CBZ· NTPA along the *c*-axis (interplanar distance = 7.437 Å)

Table 10: Hydrogen Bonds for LM052D

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O2	H2A	O1	0.84	1.76	2.5772(13)	165.4
O6	H6	O8	0.84	1.75	2.5906(12)	176.3
O8	H8A	O3 ¹	0.87	1.98	2.8216(13)	161.4
O8	H8B	O1 ¹	0.87	1.89	2.7247(13)	160.8

Symmetry transformation equivalent used to generate equivalent:

$$1+x, 3/2-y, 1/2+z$$

4.2.3 Niacinamide · 2-nitroterephthalic acid salt hydrate (LM053D)

Large, transparent block-like crystals were yielded after three days using slow evaporation method. The crystals, shown in figure 24 show well-defined edges and very little impurities on the crystals. A suitable single crystal was selected for SCXRD analysis. Characterization of these crystals confirmed the formation of a salt-hydrate instead of a pure co-crystal.



Figure 24: Optical image of niacinamide · 2-nitroterephthalic acid (LM053D) at 100×

The crystal crystallizes in the $P2_1/c$ space group. The asymmetric unit consists of one molecule of Niacinamide, one molecule of NTPA and one water molecule. The hydroxyl in the carboxylic acid group of NTPA donated a proton to the ring nitrogen of niacinamide, resulting in a protonated niacinamide cation and deprotonated NTPA anion. A strong N-H $\cdots$ O hydrogen bond is observed between hydrogen of the protonated nitrogen of niacinamide and NTPA oxygen as illustrated in figure 24. Another hydrogen bond is observed between the water molecule and NTPA molecule, the water molecule acts as a hydrogen bond acceptor to the hydroxyl group of NTPA.

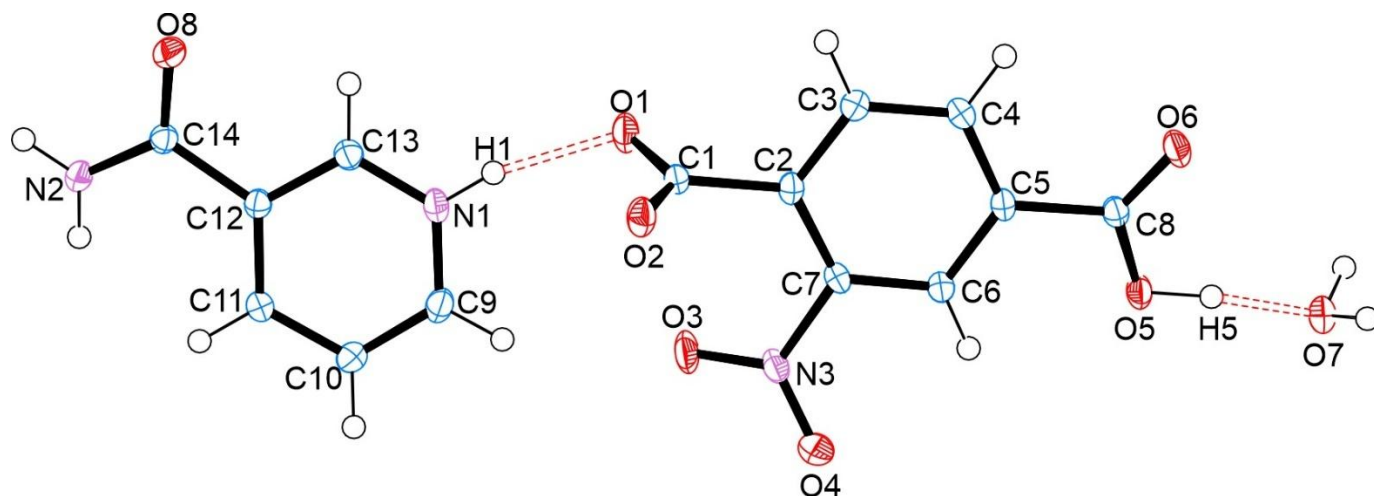


Figure 25: Asymmetric unit of Niacinamide. NTPA salt hydrate with ellipsoids at 50% probability

In terms of packing, the three different molecules form a hydrogen-bounded linear chain which propagates along the *c*-axis.

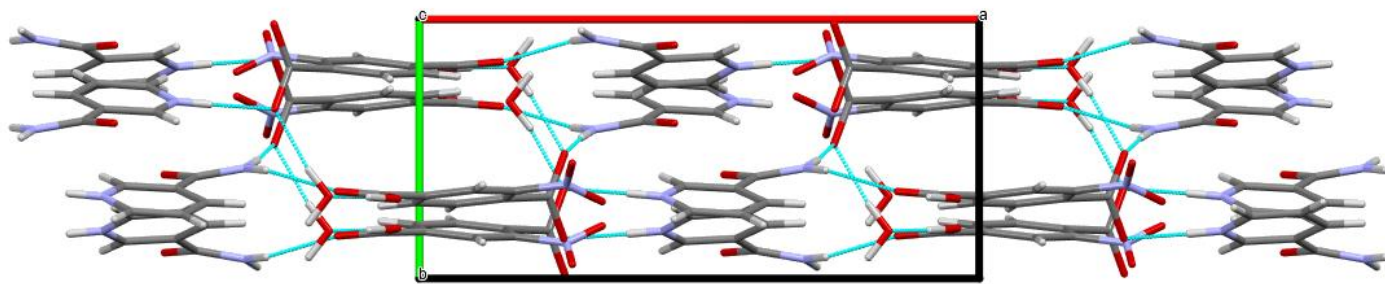


Figure 26: Crystal packing of the niacinamide–NTPA salt hydrate showing linear one-dimensional hydrogen-bonded chains along the *c*-axis

Table 11: Hydrogen Bonds for LM053D.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O5	H5	O7	0.973(17)	1.542(17)	2.5099(11)	172.8(16)
O7	H7A	O2 ¹	0.87	1.83	2.6895(12)	171.0
O7	H7B	O1 ²	0.87	1.83	2.6927(12)	170.6
N1	H1	O1	0.88	1.83	2.6900(12)	165.9

Symmetry transformations used to generate equivalent atoms:

¹2-x,-1/2+y,3/2-z; ²2-x,1/2+y,3/2-z

4.2.4 Nicotinic hydrazide · 2-Nitroterephthalic acid (LM054D)

The crystals were obtained by slow evaporation. When viewed under the microscope the crystal appears to be sliding over each other resulting in a cluster as show in the figure below. The single crystals within the clusters are transparent, plate-like with well-defined edges.



Figure 27: Optical microscope image of co-crystal Nicotinic hydrazide · NTPA (LM054D) at 100×

SCXRD showed that the sample of Nicotinic hydrazide with 2-nitroterephthalic acid formed a salt rather than co-crystal. The crystal crystallizes in the $P2_1/c$ space group.

The asymmetric unit of this salt contains one molecule of nicotinic hydrazide and one molecule of 2-Nitroterephthalic acid. There is proton transfer from the hydroxyl of the carboxylic acid group in the NTPA molecule to the terminal amino group ($-NH_2$) of nicotinic hydrazide resulting in a protonated hydrazide cation ($-NH_3^+$) and deprotonated NTPA anion. There is a hydrogen bond observed between the two molecules, the amide nitrogen acts as a hydrogen bond donor to deprotonated carboxylate group in the NTPA resulting in a strong $N-H\cdots O$ hydrogen bond.

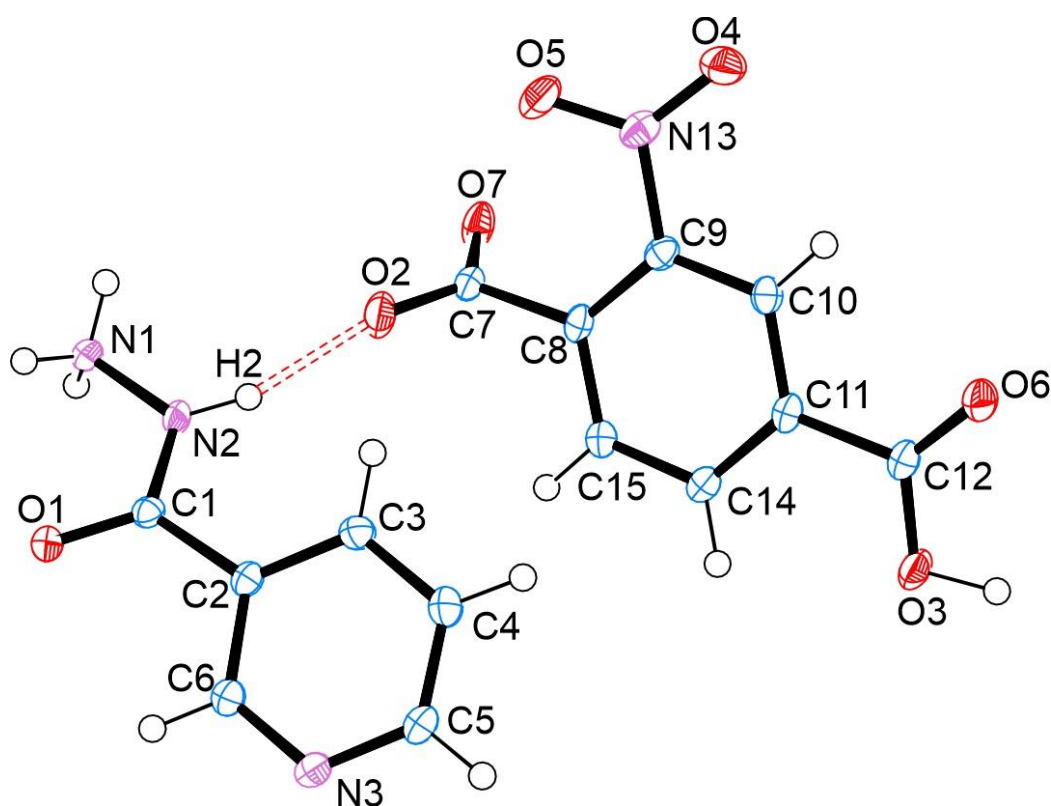


Figure 28: Asymmetric unit of nicotinic hydrazide · NTPA salt with ellipsoids at 50% probability

In terms of packing in the crystal lattice, each NTPA anion forms hydrogen bonds with two neighbouring nicotinic hydrazide cation, while each nicotinic hydrazide cation forms hydrogen bonds with two neighbouring NTPA molecule as shown in the figure below. The four molecules result in a cross like arrangement repeating down the a -axis.

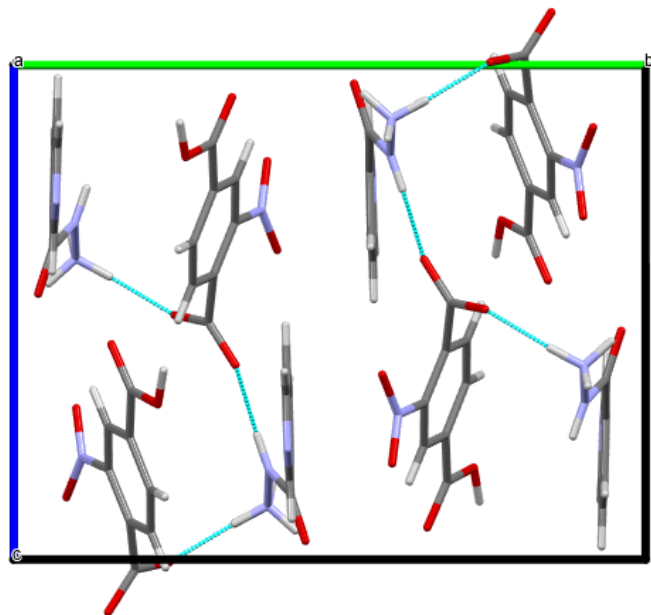


Figure 29: Crystal packing of the nicotinic hydrazone–NTPA salt down the *a*-axis

When Viewed down the *c*-axis the motif resembles w shaped like patterns as shown in the figure below.

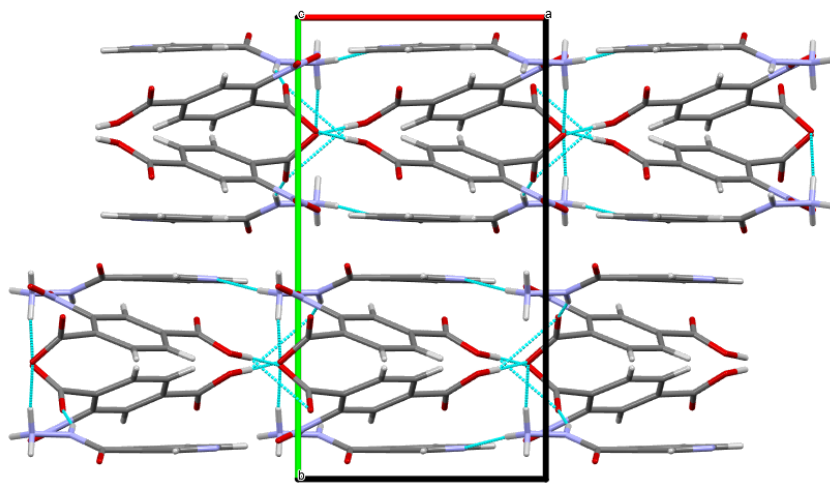


Figure 30: Crystal packing of nicotinic hydrazone-NTPA salt down the *c*-axis

Table 12: Hydrogen Bonds for LM054D

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O3	H3	O7 ¹	0.98(2)	1.58(2)	2.5544(14)	177.8(19)
N1	H1A	O1 ²	0.88	1.98	2.7997(15)	152.5
N1	H1B	N3 ³	0.89	1.86	2.7445(17)	173.0
N2	H2	O2	0.88	1.86	2.7153(16)	164.2
N1	H1C	O7 ⁴	0.905(19)	1.828(19)	2.7287(16)	173.0(17)

Symmetry transformation used to generate equivalent atoms

¹1+x,3/2-y,1/2+z; ²2-x,1-y,-z; ³3-1+x,+y,+z; ⁴4+x,3/2-y,-1/2+z

4.2.5 Methyl pyrazine-2-carboxylate · 2-nitroterephthalic acid (LM095C)

The co-crystallization reaction of pyrazinecarboxylic acid and 2-nitroterephthalic acid resulted in an unexpected esterification of the API, pyrazinecarboxylic acid. The starting material is believed to have undergone esterification where the OH group in the pyrazinecarboxylic acid was replaced by OCH₃ group from the methanol used as solvent. Slow evaporation method was used, the solution was heated at about 70 °C to facilitate complete dissolution of component.

The morphology of this co-crystal was not clear, despite that a suitable clear plate-like crystal was obtained and characterized using the SCXRD.

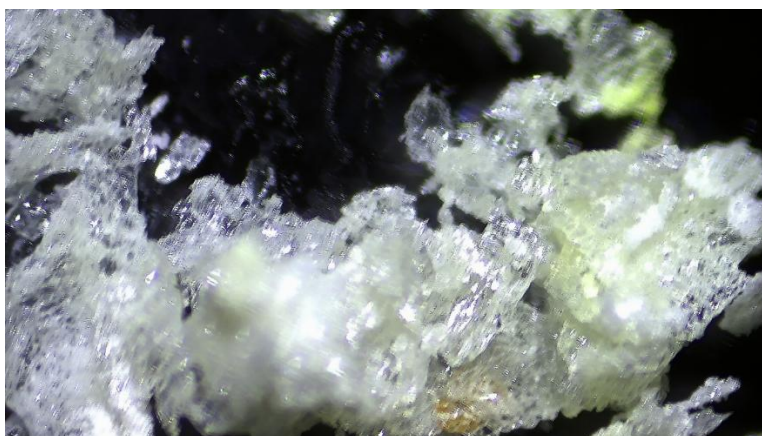


Figure 31: Optical microscope image of co-crystal methyl pyrazine-2-carboxylate · 2-nitroterephthalic acid (LM095C) at 100×

The asymmetric unit of methyl pyrazine-2-carboxylate · 2-nitroterephthalic acid co-crystals consists of one molecule of NTPA and two molecules of methyl pyrazine-2-carboxylate confirming complete conversion with no residual pyrazinecarboxylic acid detected ($R_1 = 0.0373$). The refinement, well below the 0.05 threshold for small molecules proves the pure ester phase crystallized, ruling out mixed pyrazinecarboxylic acid/ester crystals or impurity crystallization. All ester methoxy group atoms refined to full occupancy of 1 with no significant residual electron density requiring additional modeling.

NTPA forms a Bis (COOH $\cdots$ N_{pyrazine}) heterosynthon system with robust acid-pyrazine hydrogen bonds O1–H1 $\cdots$ N1 and O2–H2 $\cdots$ N2 consistent with the strong propensity of carboxylic acids and pyrazine derivatives to assemble through predictable O–H $\cdots$ N heterosynthon reported by Aakeröy et al. (2007).

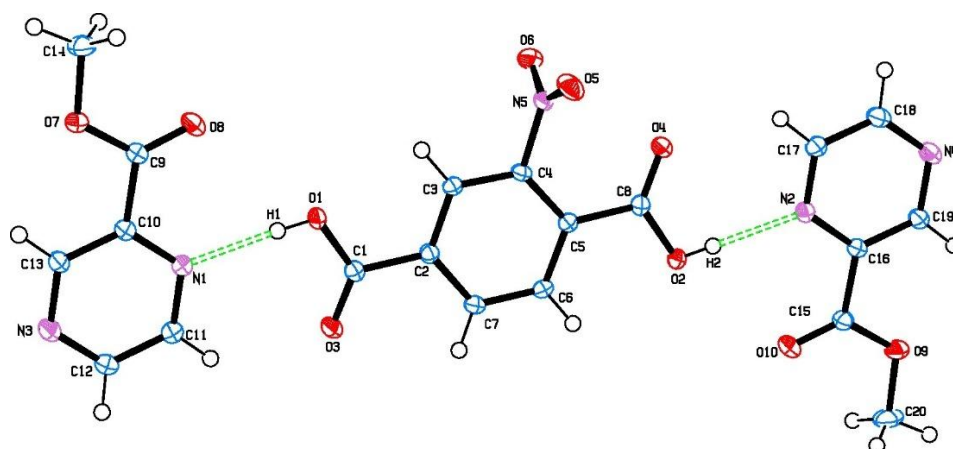


Figure 32: asymmetric unit of methyl pyrazine-2-carboxylate · 2-nitroterephthalic acid (LM095C) with ellipsoids at 50% probability

In terms of packing, the co-crystal exhibits a planar molecular arrangement parallel to the *a*-axis, with all three molecules in the asymmetric unit lying roughly in the same plane.

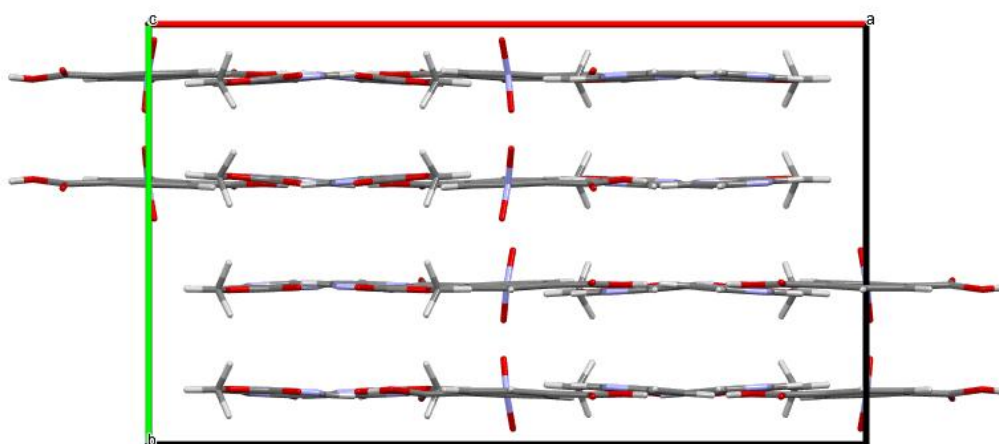


Figure 33: Packing of methyl pyrazine-2-carboxylate · 2-nitroterephthalic acid along the *a*-axis

Table 13: Hydrogen Bonds for LM95C

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O3	H3	N6	0.784(19)	1.99(2)	2.7750(13)	176.2(17)
O5	H5	N3	0.79(2)	1.95(2)	2.7452(13)	175.6(17)

Hirshfeld surface analysis was performed for the esterified product to further examine the intermolecular interactions and quantify the contributions of close contacts to crystal packing. The surfaces mapped over d_{norm} (Figure 34a–c) revealed distinct red spots corresponding to regions of significant close contact. For both methyl pyrazine-2-carboxylate molecules, intense red areas were observed near the pyridine nitrogen atoms, indicating strong N $\cdots$ H hydrogen bonding with the hydroxyl group of the co-former. Notably, although each API molecule contains two nitrogen atoms in the aromatic ring, the same nitrogen position is involved in hydrogen bonding in both cases, suggesting a preferred interaction geometry.

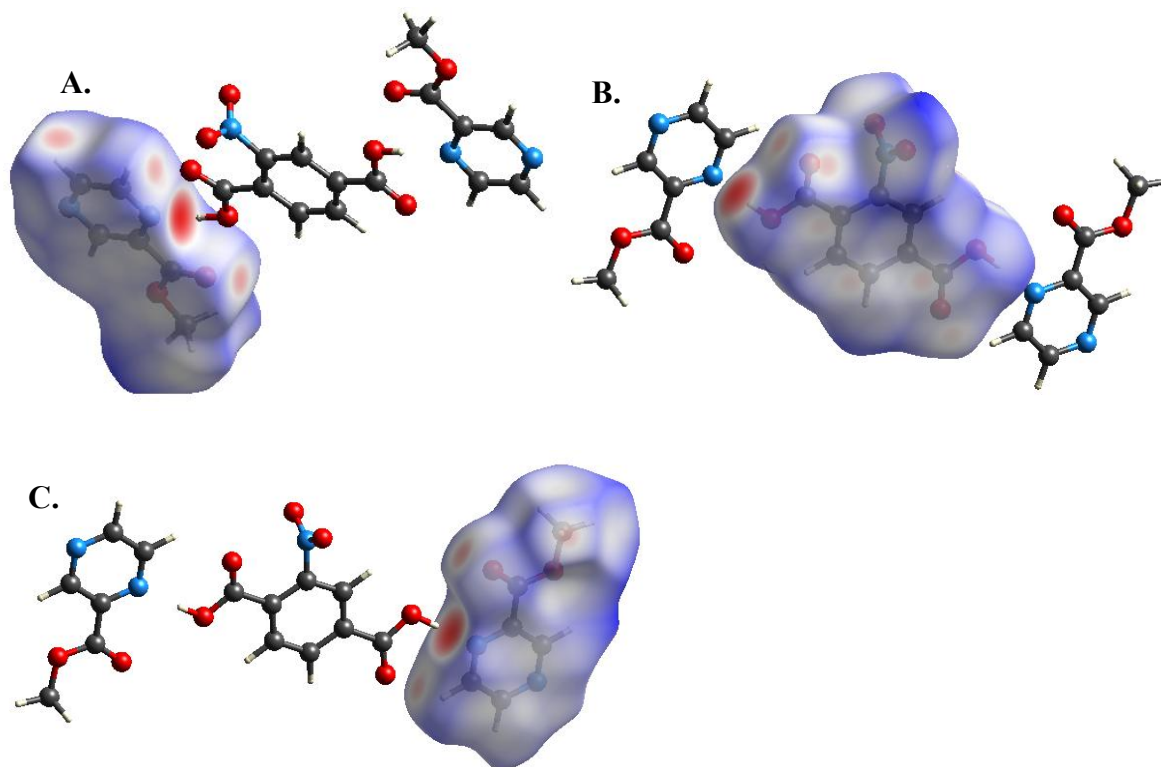


Figure 34: Hirshfeld surfaces mapped over d_{norm} for the co-crystal components: A. API 1, B. Co-former and C. API 2, indicating various intermolecular bonds in red, blue and white.

The fingerprint plot on figure 35 for API 1 showed major contributions from $\text{O}\cdots\text{H}$ (35%) figure 35C, figure 35D show $\text{H}\cdots\text{H}$ (24%), and figure 35 B show $\text{N}\cdots\text{H}$ (14%) contacts, while API 2 displayed similar values, Figure 37C show $\text{O}\cdots\text{H}$ (36.5%), figure 37D show $\text{H}\cdots\text{H}$ (23.9%), and 37B show $\text{N}\cdots\text{H}$ (12.5%). The co-former surface featured a pronounced red region at its hydroxyl group, confirming its role as a hydrogen bond donor to both APIs, along with weaker red spots around other oxygen atoms. Its fingerprint plot was dominated by $\text{H}\cdots\text{O}$ (45%) shown in figure 36C, followed by $\text{H}\cdots\text{H}$ (12%) in figure 36 D and $\text{H}\cdots\text{N}$ (11.3%) contacts shown in figure 36C. These findings confirm that $\text{N}\cdots\text{H}\cdots\text{O}$ hydrogen bonding is the principal interaction stabilizing the co-crystal, supported by van der Waals contacts involving hydrogen atoms and electron-rich oxygen regions.

The corresponding fingerprint plot of API 1 revealed a sharp spike at short contact distances, confirming the presence of strong, directional $\text{N}\cdots\text{H}$ hydrogen bonds. The broad region associated with $\text{O}\cdots\text{H}$ contacts reflects weaker, non-directional interactions such as $\text{C}-\text{H}\cdots\text{O}$, consistent with

the surface analysis. $\text{H}\cdots\text{H}$ contacts appeared as a broad central area, indicating typical van der Waals packing contributions.

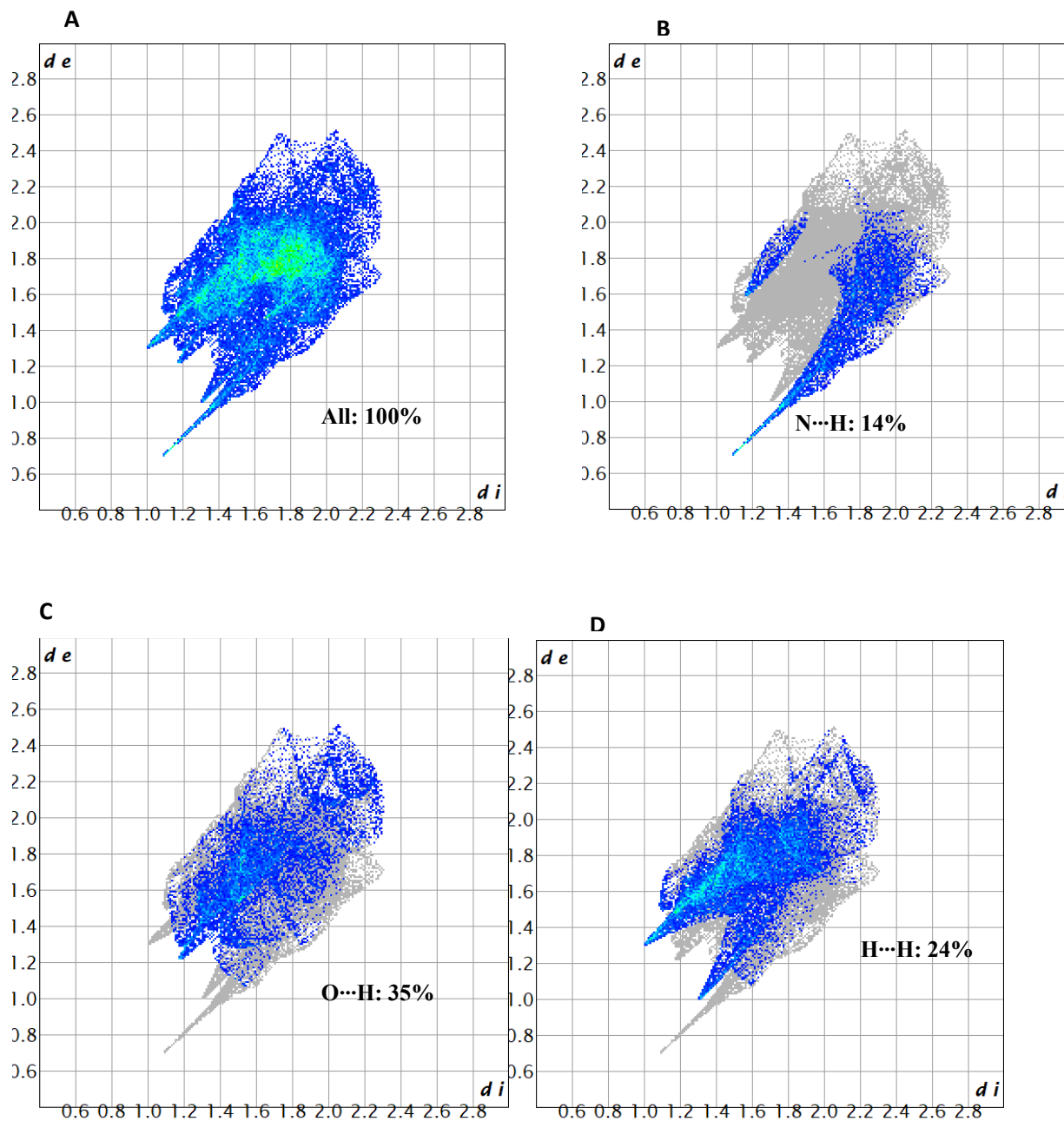


Figure 35: 2D fingerprint plot for API 1 in the co-crystal of methyl pyrazine-2-carboxylate · 2-nitroterephthalic acid, showing the percentage contribution of intermolecular contacts.

The fingerprint plot for the co-former (Figure 36) shows a sharp spike corresponding to $\text{H}\cdots\text{N}$ contacts (11.3%). This indicates a strong and well-defined hydrogen bond between the hydroxyl group of the co-former and the nitrogen atom in the API ring. The sharpness of the spike suggests that this interaction is geometrically consistent and contributes significantly to the crystal packing.

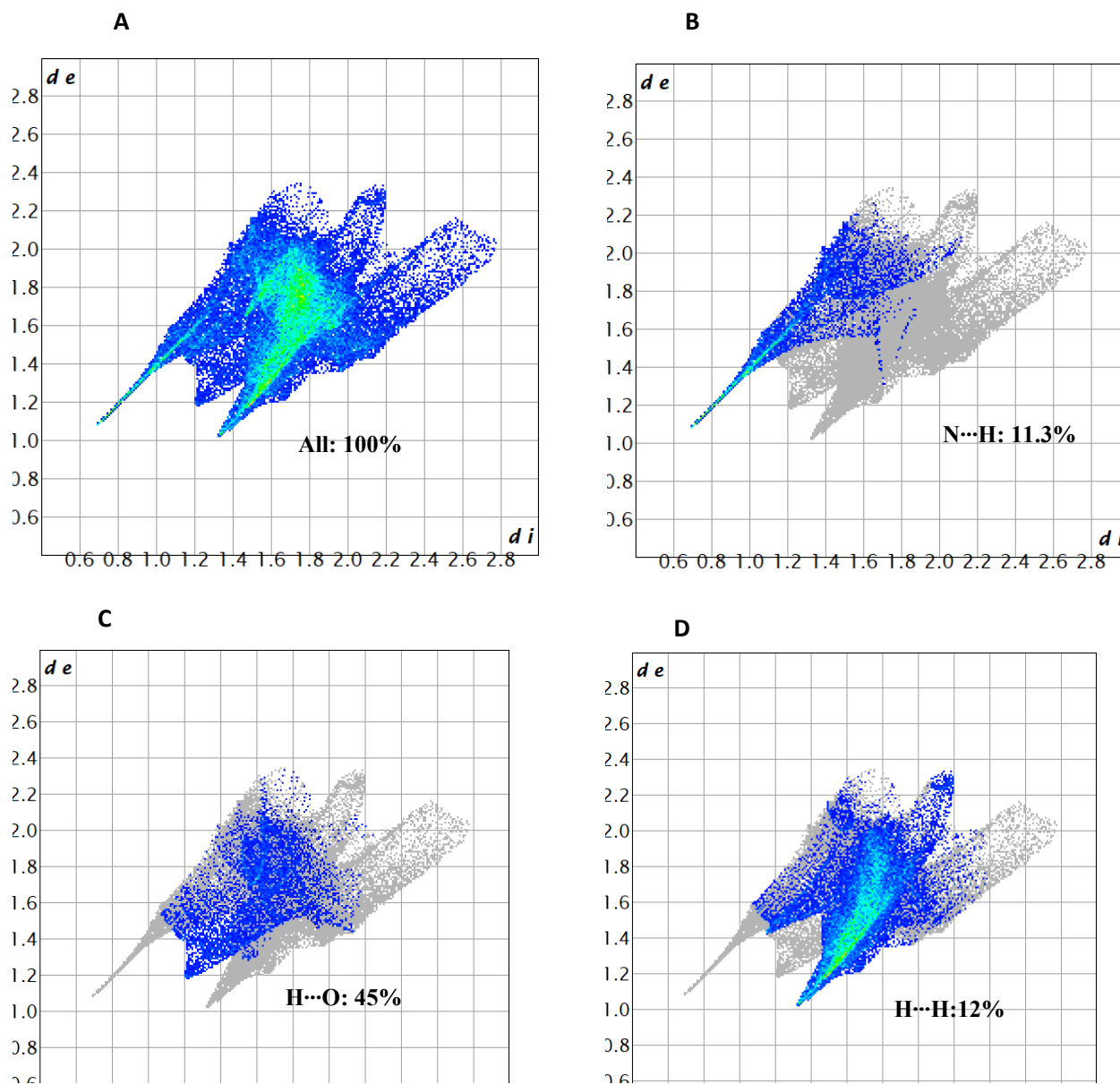


Figure 36: 2D fingerprint plot for co-former in the co-crystal of methyl pyrazine-2-carboxylate and 2-Nitroterephthalic acid, showing the percentage contribution of intermolecular contacts.

The fingerprint plot for API 2 shown in figure 37 below shows a sharp spike corresponding to $N\cdots H$ contacts contributing 12.5% of the intermolecular contact. This indicates a strong and well-defined hydrogen bond between the hydroxyl group of the co-former and the nitrogen atom in the API ring. The sharpness of the spike suggests that this interaction is geometrically consistent and contributes significantly to the crystal packing.

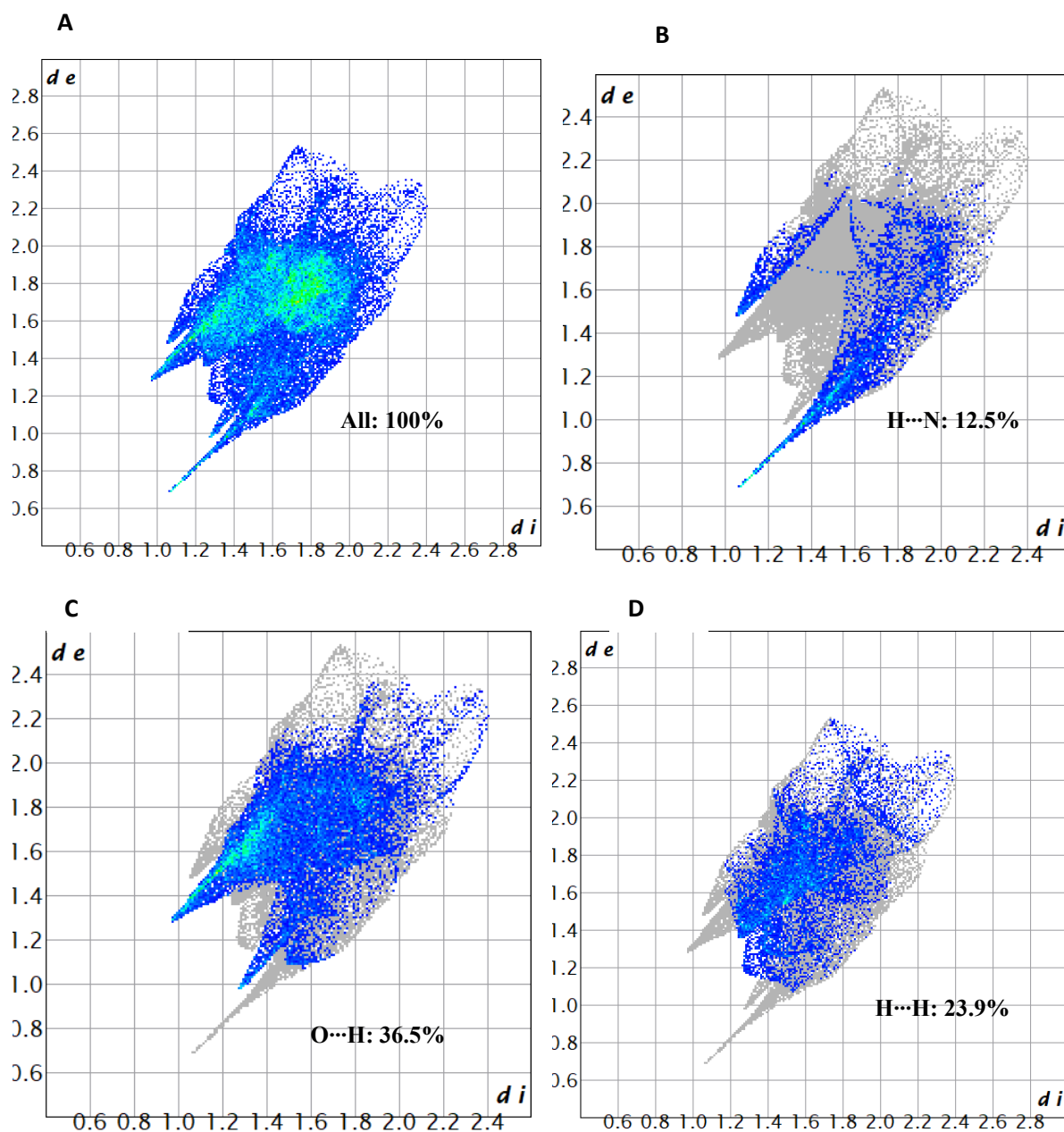


Figure 37: 2D fingerprint plot for methyl pyrazine-2-carboxylate

The FTIR spectrum of co-crystal LM095 provide evidence of both esterification and co-crystal formation. The spectrum exhibited a strong absorption band at 1705 cm^{-1} , assigned to C=O stretching of the ester group, confirming methylation of the pyrazinecarboxylic acid with characteristic C-O stretches at 1300 , 1248 , and 1184 cm^{-1} . The peak at 1539 cm^{-1} corresponds to the asymmetric stretch of the NO_2 moiety of nitroterephthalic acid.

as expected for the co-former. Complete loss of broad O-H stretch ($>3000\text{ cm}^{-1}$) from pyrazinecarboxylic acid confirms conversion to ester phase. The absorption at 767 cm^{-1} indicates out-of-plane bending of aromatic C-H bonds.

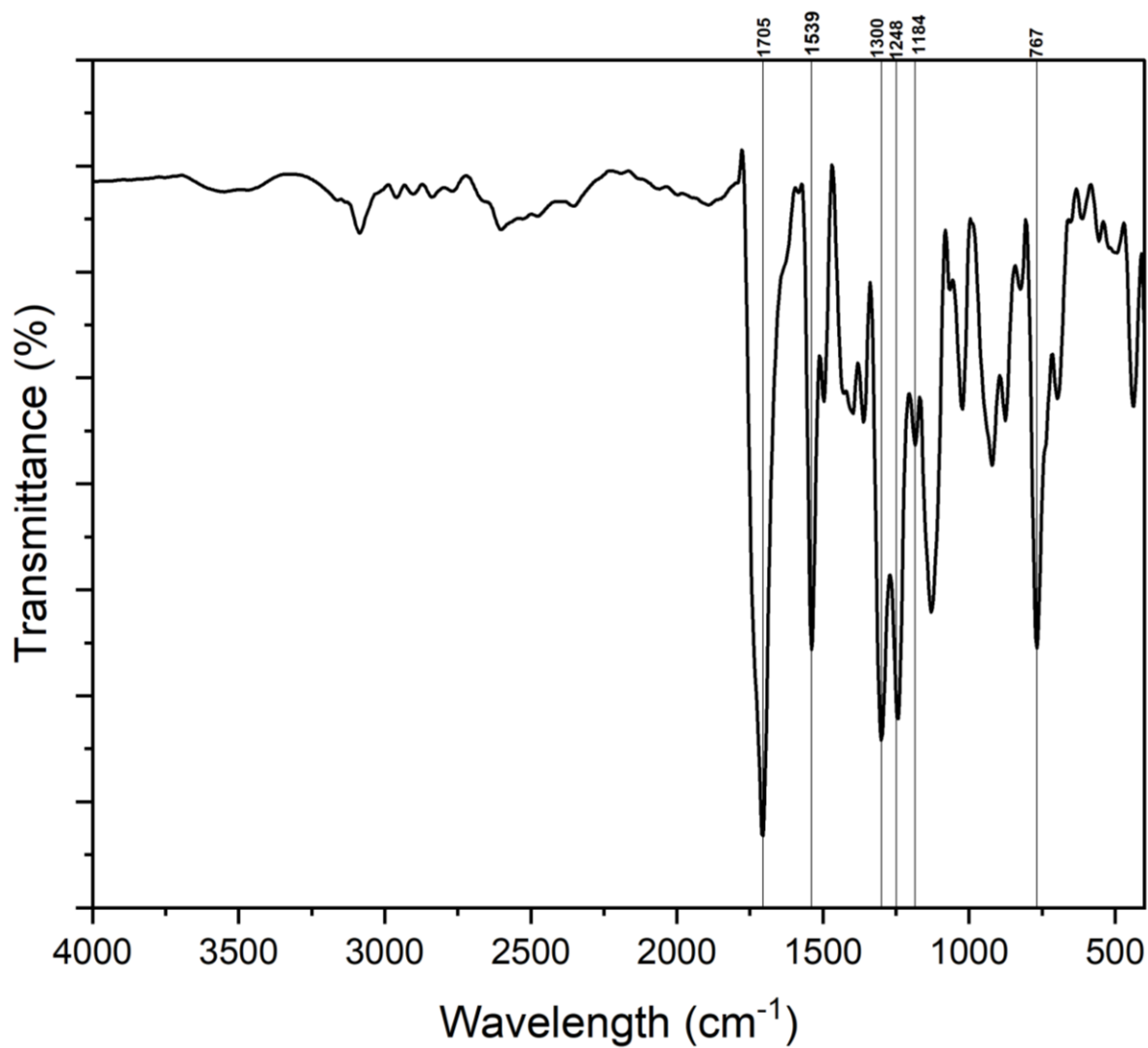


Figure 38: FTIR spectrum for LM095C esterification product

Table 14: FTIR spectral data analysis LM095 esterification product

Wavenumber (cm ⁻¹)	Assignment
1705	C=O stretching (ester group)
1539	Asymmetric NO ₂ stretch
1309	Symmetric NO ₂ stretch

1248	C-O stretching (ester)
1184	C-N stretch
767	Aromatic C-H out of plane bend

The esterification of the pyrazinecarboxylic acid was further confirmed by ^1H NMR and ^{13}C NMR. Crystals of the compound were dissolved in deuterated DMSO- d_6 containing 1% tetramethylsilane and transferred to an NMR tube for analysis. The samples were analysed using an Agilent 500 MHz spectrometer at 26°C (299.15 K), and the nuclear magnetic resonance (NMR) spectra were captured (^1H at 500 MHz and ^{13}C at 125 MHz). ^1H NMR showed a singlet at δ 4.2 ppm which is indicative of a methoxy group. Furthermore, the ^{13}C NMR showed a peak at δ 53.2 ppm which indicates the presence of a methoxy group.

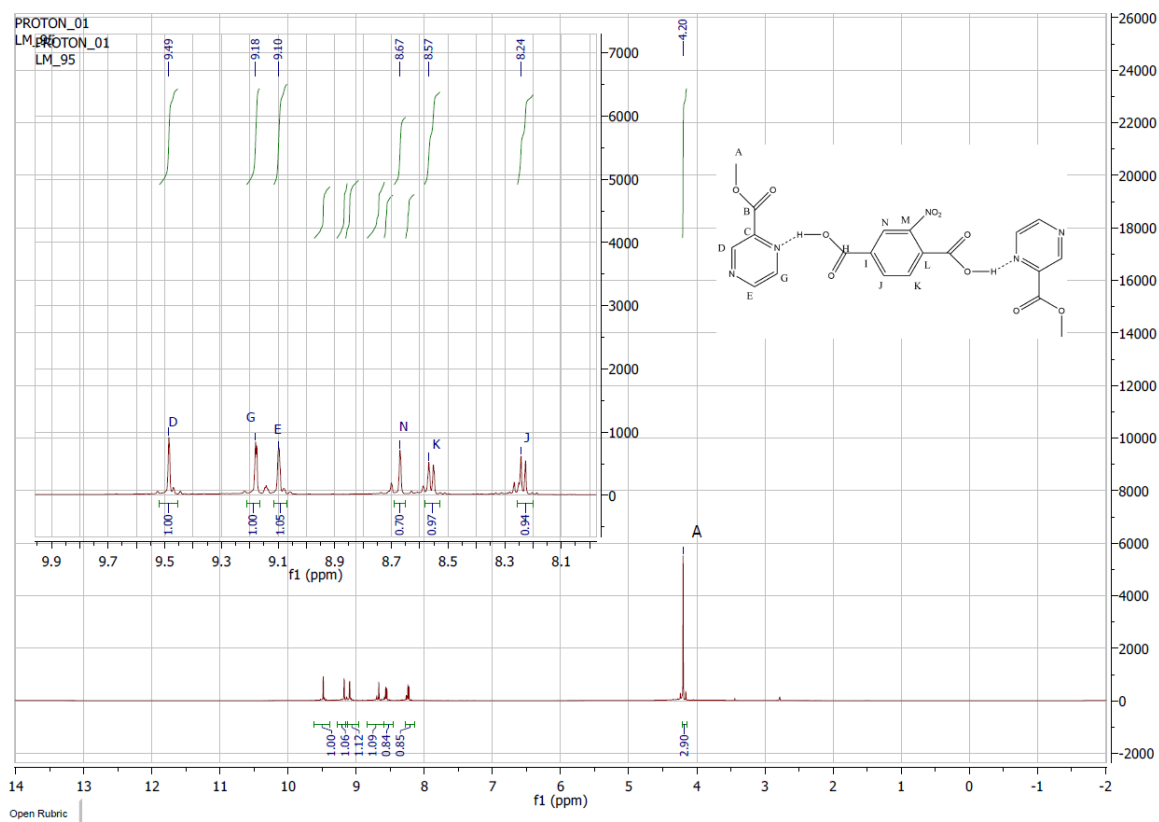


Figure 39: ¹H NMR spectrum of the esterified methyl pyrazine-2-carboxylate · NTPA product

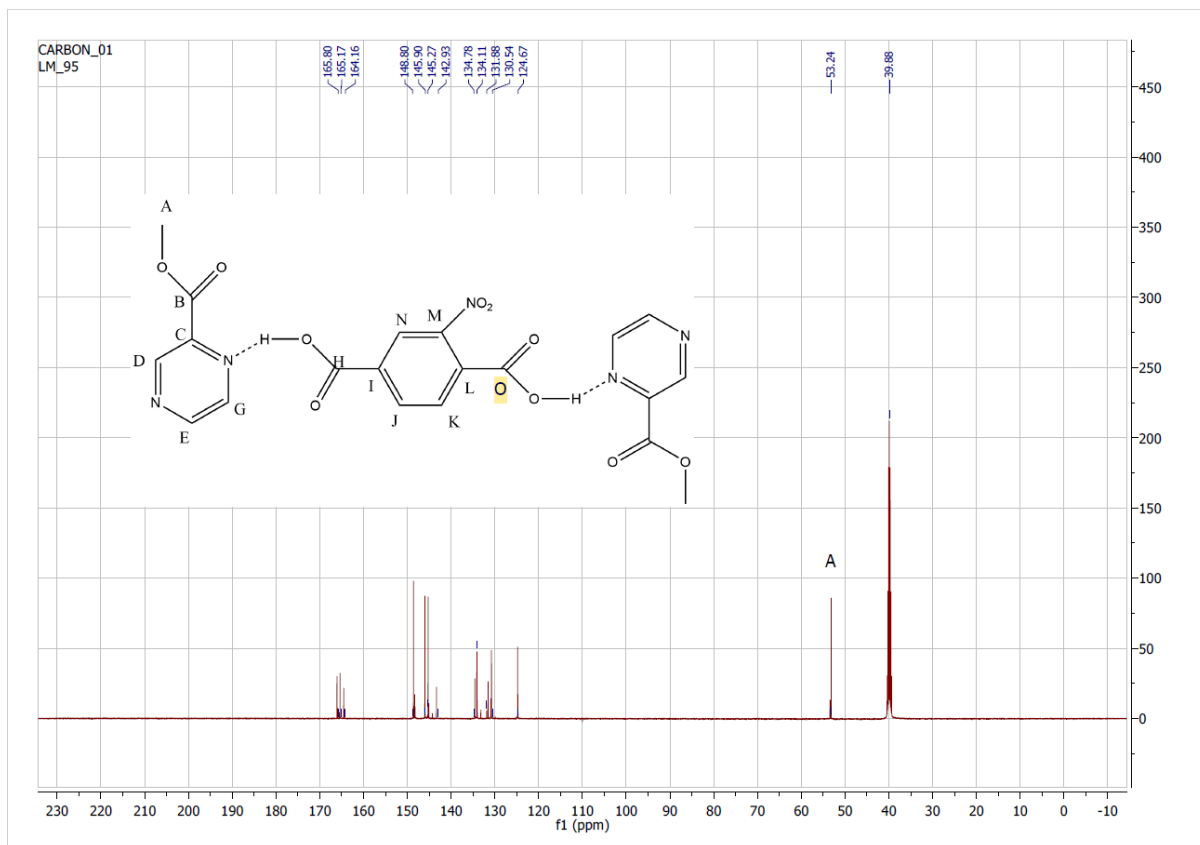


Figure 40: ^{13}C NMR spectrum of the esterified methyl pyrazine-2-carboxylate · NTPA product

4.2.6 Isonicotinamide · Nitroterephthalic acid (LM101)

Cluster-like crystals were obtained by ball milling. Although the overall morphology was not well-defined, as shown in figure 41, a single plate-like crystal was selected for SCXRD characterization.



Figure 41: Optical microscope image of isonicotinamide · NTPA (LM101) at 100×

The asymmetric unit comprises one molecule of INA and one molecule of NTPA, together with one DMSO solvent molecule. The API and co-former are linked via classical O–H···O and N–H···O hydrogen bonds involving the carboxylic acid group of NTPA and the amide functionality of isonicotinamide. These interactions generate a centrosymmetric $R_2^2(8)$ heterosynthon, characteristic of carboxylic acid–amide supramolecular motifs. The DMSO solvent molecule is present in the asymmetric unit and exhibits disorder at the sulfur atom, which is distributed over two crystallographically equivalent positions with refined occupancies of 0.5 each.

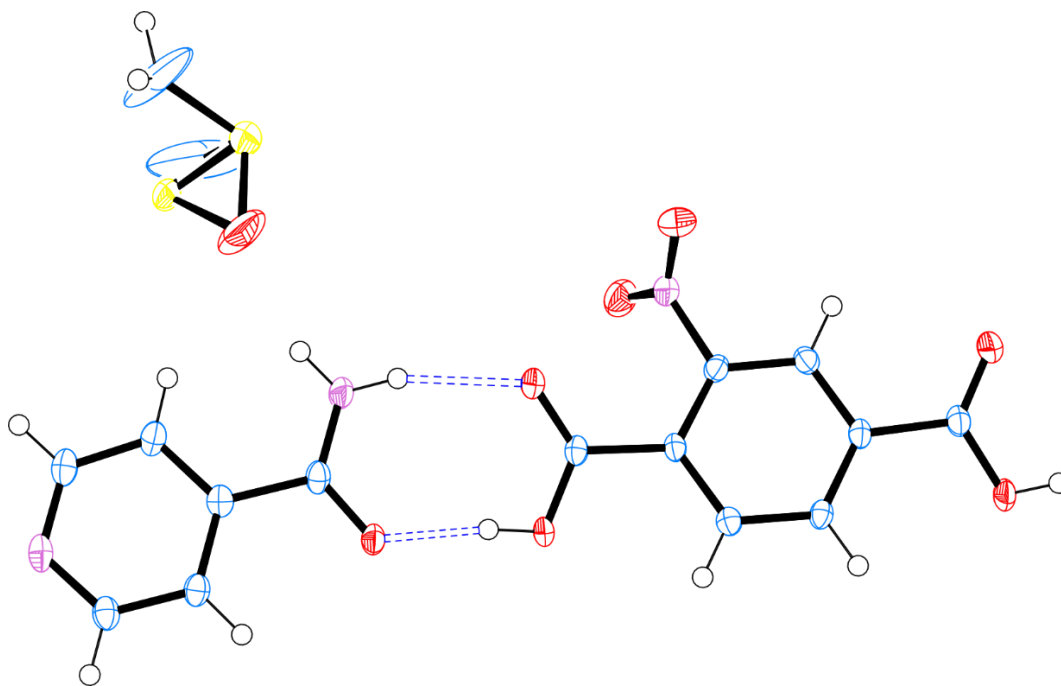


Figure 42: Asymmetric unit of Isonicotinamide · NTPA (LM101)

In terms of packing, the isonicotinamide and NTPA molecules propagate in a nearly planar fashion, generating one-dimensional chains along the *c*-axis. The disordered DMSO molecules also a parallel chain within the structure.

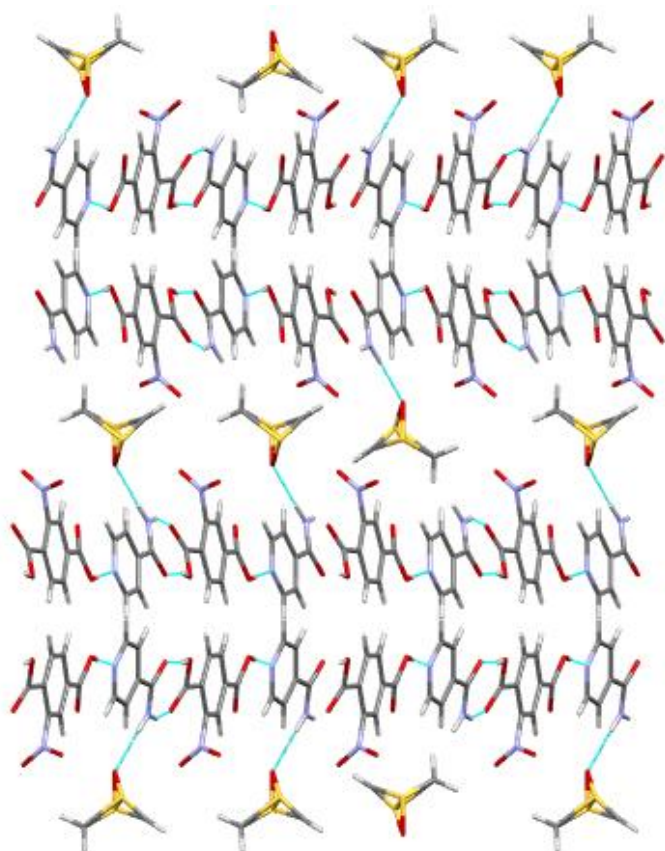


Figure 43: Crystal packing of isonicotinamide · NTPA along the *c*-axis

4.2.7 Key observations and discussion of nitroterephthalic acid structures

Co-crystallization of NTPA with APIs yielded structurally diverse solid forms comprising two salt co-crystals, two neutral co-crystals, one salt hydrate and one neutral solvate. Out of the six structures, four monoclinic systems crystallizing in the $P2_1/c$ space group were observed. This observation is consistent with literature studies involving NTPA and *N*-heterocyclic APIs where the monoclinic system, $P2_1/c$ space group is mostly observed (Severance et al., 2012) supported by CSD survey. The frequency of $P2_1/c$ can be attributed to its centrosymmetric nature, which favors hydrogen-bonding motifs such as $R_2^2(8)$ rings and charge-assisted $\text{NH}^+ \cdots \text{COO}^-$ interactions (Tomlin et al., 2000). The esterified product from methyl pyrazine-2-carboxylate, along with the benzhydrazide·NTPA co-crystal, represent exceptions to the dominant $P2_1/c$ monoclinic systems. These crystallized in orthorhombic space groups, $Pccn$ and $P2_12_12_1$, respectively.

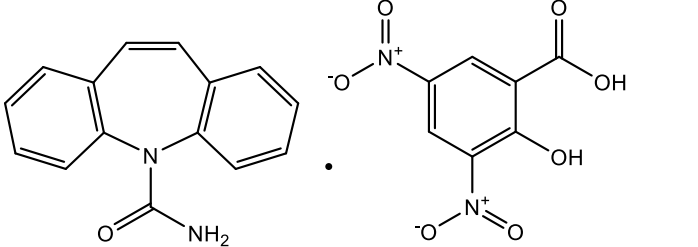
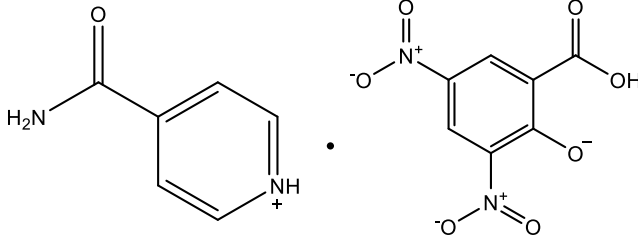
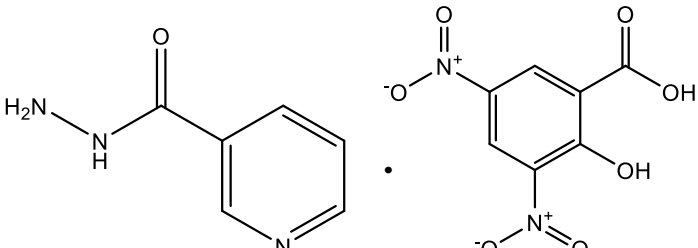
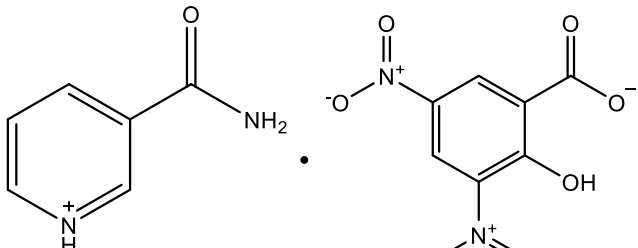
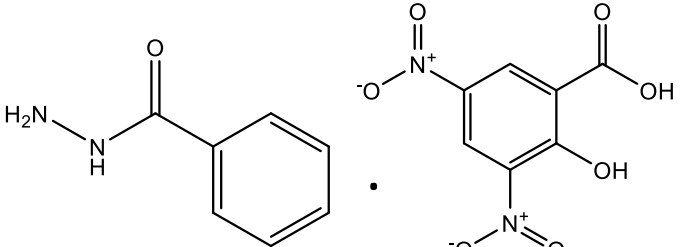
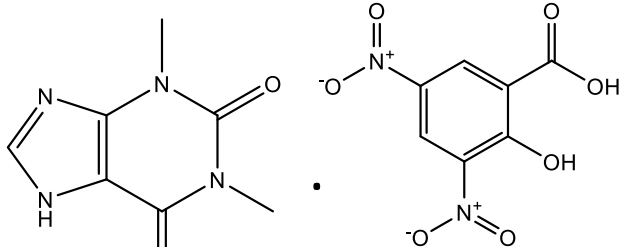
The NTPA series demonstrates significant solid-form diversity, with salts (niacinamide·NTPA hydrate, nicotinic hydrazide·NTPA), neutral co-crystals (benzhydrazide·NTPA, carbamazepine·NTPA, isonicotinamide·NTPA DMSO solvate), and an unexpected esterified product (methyl pyrazine-2-carboxylate ·NTPA). This diversity stems from NTPA two carboxylic acid groups, which enable proton transfer or neutral hydrogen bonding depending on API basicity and crystallization conditions. Stronger bases like niacinamide ($\Delta pK_a > 2$) favor charge-assisted salts, while moderate bases like carbamazepine promote robust R₂²(8) co-crystal synthons; solvent competition yields hydrates/solvates, and pyrazinecarboxylic acid's reactivity led to rare esterification.

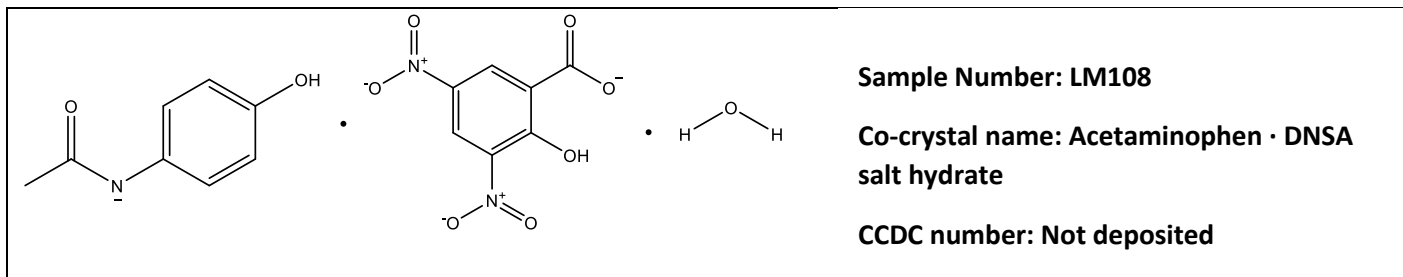
In this series of NTPA, hydrogen bonding dominates the supramolecular interactions, in the salt systems, protonated heterocyclic nitrogen formed charge-assisted N⁺–H···OOC interactions with NTPA carboxylates forming the strongest and most directional hydrogen-bond patterns expected when acids and bases interact in crystals. Carbamazepine and benzhydrazide, which are an exception in this study as they are not *N*-heterocyclic API, did not undergo proton transfer, yielding neutral crystals due to their basicity. Carbamazepine formed O–H···O bond where carbamazepine's amide carbonyl oxygen was the hydrogen acceptor. This outcome is consistent with literature reports on carbamazepine co-crystals, where acid–amide interactions commonly predominate (Ouyang et al., 2024). Similarly, the lack of proton transfer in the benzhydrazide system suggests that hydrogen bonding is governed more by donor–acceptor complementarity than by full acid–base ionization (Arya et al., 2019).

4.3 Series of Co-crystals of 3,5-dinitrosalicylic acid (DNSA)

This sub section present a series of co-crystals obtained from co-crystallizing DNSA with seven APIs, of which four are pure co-crystals, two are salts co-crystals and one is a co-crystal hydrate. It highlights resulting supermolecule synthons, how DNSA interacts with structurally diverse APIs, hydrogen bonding patterns and crystal packing. Six of the seven crystals were synthesized using the slow evaporation method and one using ball milling method. The co-crystals discussed in this chapter are listed in table 16 .

Table 15: Co-crystal structures of 3,5-dinitrosalicylic acid series

 Sample Number: LM089 Co-crystal name: Carbamazepine · DNSA co-crystal CCDC number: Not deposited	 Sample Number: LM90D Co-crystal name: Isonicotinamide · DNSA salt co-crystal CCDC number: 2449096
 Sample Number: LM91B Co-crystal name: Nicotinic Hydrazide · DNSA salt co-crystal CCDC number: 2470541	 Sample Number: LM92 Co-crystal name: Niacinamide · DNSA salt co-crystal CCDC number: 2570512
 Sample Number: LM094B Co-crystal name: Benzhydrazide · DNSA CCDC number: 2449006	 Sample Number: LM102 Co-crystal name: Theophylline · DNSA CCDC number: 2449011



Suitable crystals were selected under a polarizing microscope and mounted on a Bruker APEX-II CCD diffractometer. Data collection was performed at 100.00 K using Mo-K α radiation ($\lambda = 0.71073$ Å). The structures were solved using X-seed and Olex2, employing the charge flipping algorithm. Table 17 summarizes the crystallographic data of the series of co-crystals of 3,5 dinitrosalicylic acid obtained from SCXRD analysis which include unit cell dimensions, space group and other relevant crystallographic data.

Two structures within this series, LM089 and LM108, exhibited relatively elevated refinement parameters. LM089 displayed an R1 value of 0.0716 ($I \geq 2\sigma(I)$) together with a comparatively high internal agreement factor ($R_{int} = 0.3717$), while LM108 exhibited an R1 value of 0.1066 ($I \geq 2\sigma(I)$). These elevated values may be attributed to the intrinsic diffraction quality of the selected crystals. To exclude potential instrumental or data processing errors, both crystals were remeasured on the SCXRD instrument, yielding comparable refinement statistics.

Table 16: Crystallographic data and structure refinement for 3,5-Dinitrosalicylic acid co-crystals

Identification code	LM089	LM090D	LM091B
Empirical formula	C ₂₄ H ₁₄ N ₂ O ₈	C ₁₉ H ₁₆ N ₆ O ₉	C ₂₇ H ₂₆ N ₁₀ O ₁₇
Formula weight	458.387	472.38	762.58
Temperature/K	104.00	100.00	100.00
Crystal system	Monoclinic	Triclinic	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>c</i>	<i>P</i> -1	<i>P</i> 2 ₁ / <i>n</i>
<i>a</i> /Å	10.5300(4)	3.6730(3)	7.3691(3)
<i>b</i> /Å	6.5198(3)	14.7216(13)	30.5704(12)
<i>c</i> /Å	30.6231(12)	18.8817(17)	14.3320(6)
α /°	90	109.636(3)	90
β /°	98.881(2)	91.899(4)	102.068(2)
γ /°	90	91.752(4)	90
Volume/Å ³	2077.18(15)	960.13(15)	3157.3(2)
<i>Z</i>	4	2	4
ρ_{calc} /cm ³	1.466	1.634	1.604
μ /mm ⁻¹	0.112	0.133	0.136
<i>F</i> (000)	944.8	488.0	1576.0
Crystal size/mm ³	0.222 × 0.697 × 0.835	0.114 × 0.321 × 0.516 mm	0.82 × 0.529 × 0.106
Radiation	Mo K α (λ = 0.71073)	MoK α (λ = 0.71073)	MoK α (λ = 0.71073)
2 Θ range for data collection/°	3.92 to 54.98	4.296 to 55.318	3.942 to 56.656
Index ranges	-13 ≤ <i>h</i> ≤ 13, -8 ≤ <i>k</i> ≤ 8, -39 ≤ <i>l</i> ≤ 39	-4 ≤ <i>h</i> ≤ 4, -19 ≤ <i>k</i> ≤ 19, -24 ≤ <i>l</i> ≤ 24	-9 ≤ <i>h</i> ≤ 9, -40 ≤ <i>k</i> ≤ 40, -19 ≤ <i>l</i> ≤ 19
Reflections collected	80599	45391	88250
Independent reflections	4783 [<i>R</i> _{int} = 0.3717, <i>R</i> _{sigma} = 0.1339]	4446 [<i>R</i> _{int} = 0.0655, <i>R</i> _{sigma} = 0.0385]	7858 [<i>R</i> _{int} = 0.1048, <i>R</i> _{sigma} = 0.0555]
Data/restraints/parameters	4783/0/317	4446/0/308	7858/0/512
Goodness-of-fit on <i>F</i> ²	1.024	1.003	1.068
Final <i>R</i> indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0716, <i>wR</i> ₂ = 0.1630	<i>R</i> ₁ = 0.0483, <i>wR</i> ₂ = 0.1272	<i>R</i> ₁ = 0.0451, <i>wR</i> ₂ = 0.1081
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0872, <i>wR</i> ₂ = 0.1749	<i>R</i> ₁ = 0.0685, <i>wR</i> ₂ = 0.1413	<i>R</i> ₁ = 0.0682, <i>wR</i> ₂ = 0.1279
Largest diff. peak/hole / e Å ⁻³	0.70/-0.83	0.43/-0.31	0.47/-0.42

Identification code	LM092	LM094B	LM102
Empirical formula	C ₁₃ H ₁₀ N ₄ O ₈	C ₁₄ H ₁₁ N ₄ O ₈	C ₁₄ H ₁₂ N ₆ O ₉
Formula weight	350.246	363.266	408.286
Temperature/K	100.00	100.00	107.00
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i> /Å	4.7298(3)	9.3367(3)	15.7317(6)
<i>b</i> /Å	21.2608(13)	34.4225(9)	5.9421(2)
<i>c</i> /Å	13.8951(9)	9.6242(2)	18.6127(8)
α /°	90	90	90
β /°	94.602(2)	99.098(1)	112.622(1)
γ /°	90	90	90
Volume/Å ³	1392.78(15)	3054.23(14)	1606.04(11)
<i>Z</i>	4	8	4
ρ_{calc} /cm ³	1.670	1.580	1.689
μ /mm ⁻¹	0.142	0.133	0.144
<i>F</i> (000)	720.7	1497.3	840.8
Crystal size/mm ³	0.964 × 0.546 × 0.268	0.381 × 0.336 × 0.144	0.559 × 0.24 × 0.226
Radiation	Mo K α (λ = 0.71073)	Mo K α (λ = 0.71073)	Mo K α (λ = 0.71073)
2 Θ range for data collection/°	4.84 to 55.28	4.44 to 55.1	4.48 to 55.22
Index ranges	-6 ≤ <i>h</i> ≤ 6, -27 ≤ <i>k</i> ≤ 27, -18 ≤ <i>l</i> ≤ 18	-12 ≤ <i>h</i> ≤ 12, -44 ≤ <i>k</i> ≤ 44, -12 ≤ <i>l</i> ≤ 12	-20 ≤ <i>h</i> ≤ 20, -7 ≤ <i>k</i> ≤ 7, -24 ≤ <i>l</i> ≤ 24
Reflections collected	44403	99046	48782
Independent reflections	3230 [<i>R</i> _{int} = 0.0548, <i>R</i> _{sigma} = 0.0284]	7018 [<i>R</i> _{int} = 0.0414, <i>R</i> _{sigma} = 0.0198]	3722 [<i>R</i> _{int} = 0.0366, <i>R</i> _{sigma} = 0.0180]
Data/restraints/parameters	3230/0/249	7018/0/473	3722/0/270
Goodness-of-fit on <i>F</i> ²	1.054	1.070	1.067
Final <i>R</i> indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0424, <i>wR</i> ₂ = 0.1221	<i>R</i> ₁ = 0.0422, <i>wR</i> ₂ = 0.1295	<i>R</i> ₁ = 0.0370, <i>wR</i> ₂ = 0.1011
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0459, <i>wR</i> ₂ = 0.1267	<i>R</i> ₁ = 0.0525, <i>wR</i> ₂ = 0.1435	<i>R</i> ₁ = 0.0418, <i>wR</i> ₂ = 0.1067
Largest diff. peak/hole / e Å ⁻³	0.43/-0.39	0.99/-0.68	0.40/-0.32

Identification code	LM108B
Empirical formula	C ₁₅ H ₁₃ N ₃ O ₁₀
Formula weight	395.28
Temperature/K	100.00
Crystal system	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>n</i>
<i>a</i> /Å	8.2879(6)
<i>b</i> /Å	17.9304(12)
<i>c</i> /Å	11.6115(7)
α /°	90
β /°	97.410(2)
γ /°	90
Volume/Å ³	1711.1(2)
Z	4
$\rho_{\text{calc}}/\text{cm}^3$	1.534
μ/mm^{-1}	0.132
F(000)	816.0
Crystal size/mm ³	0.839 × 0.471 × 0.211
Radiation	MoK α (λ = 0.71073)
2 Θ range for data collection/°	4.204 to 54.98
Index ranges	-10 ≤ <i>h</i> ≤ 10, -23 ≤ <i>k</i> ≤ 23, -15 ≤ <i>l</i> ≤ 14
Reflections collected	46375
Independent reflections	3915 [<i>R</i> _{int} = 0.0740, <i>R</i> _{sigma} = 0.0386]
Data/restraints/parameters	3915/0/259
Goodness-of-fit on F ²	1.033
Final R indexes [<i>I</i> ≥ 2σ (<i>I</i>)]	<i>R</i> ₁ = 0.1066, <i>wR</i> ₂ = 0.3095
Final R indexes [all data]	<i>R</i> ₁ = 0.1221, <i>wR</i> ₂ = 0.3232
Largest diff. peak/hole / e Å ⁻³	1.80/-0.52

4.3.1 Carbamazepine·3,5-dinitrosalicylic acid (LM089)

The co-crystals formed through slow evaporation exhibited a long needle-like habit. The crystals are transparent and colourless, with smooth, well-defined faces. No inclusions or defects are visible, and the uniformity in size and shape suggests consistent crystallization conditions.



Figure 44: Optical microscope image of co-crystal Carbamazepine · DNSA (LM089) at 100×

The asymmetric unit of carbamazepine · 3,5-dinitrosalicylic acid as show in figure 45, consists of one molecule of carbamazepine and one molecule of 3,5-dinitrosalicylic acid. The co-crystal crystallizes in the monoclinic system with space group $P21/c$.

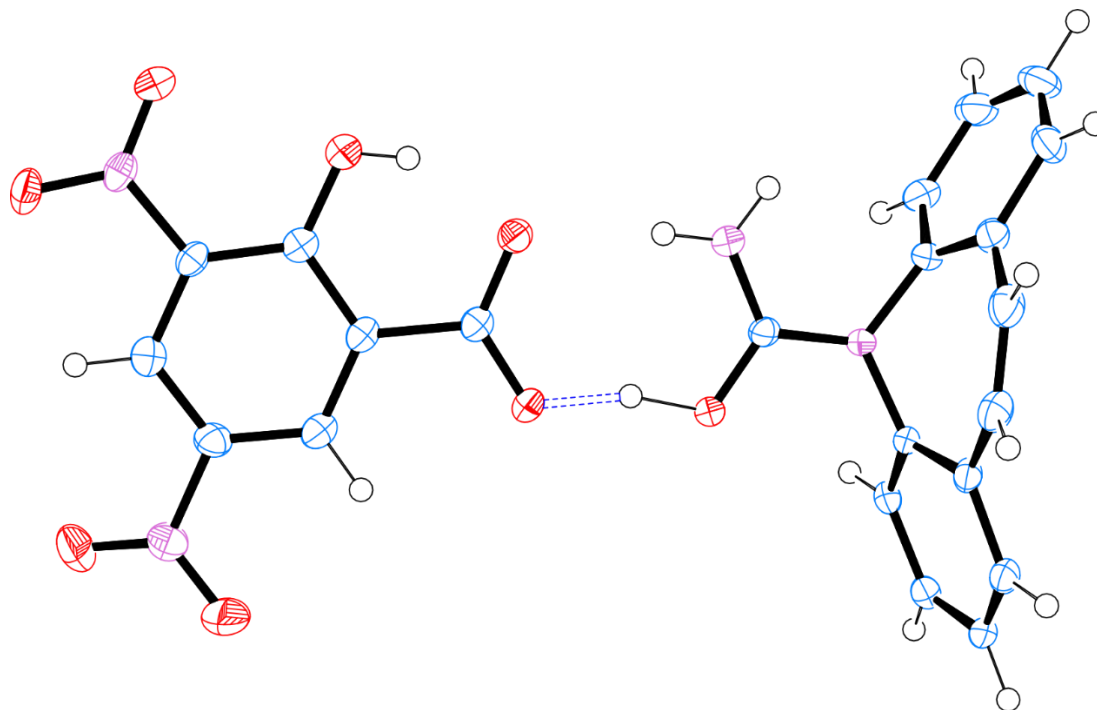


Figure 45: Asymmetric unit of carbamazepine · DNSA (LM089)

4.3.2 Isonicotinamide · 3,5-dinitrosalicylic acid (LM090D)

The co-crystal of isonicotinamide and 3,5-dinitrosalicylic acid was formed through slow evaporation which yielded plate-like crystals after two days. These crystals exhibited a cloudy appearance as shown in figure 46, suggesting the presence of impurities which may have resulted from unreacted starting material or other impurities during the crystallization process. Despite the visual imperfection, a suitable single crystal was selected for x-ray diffraction analysis.

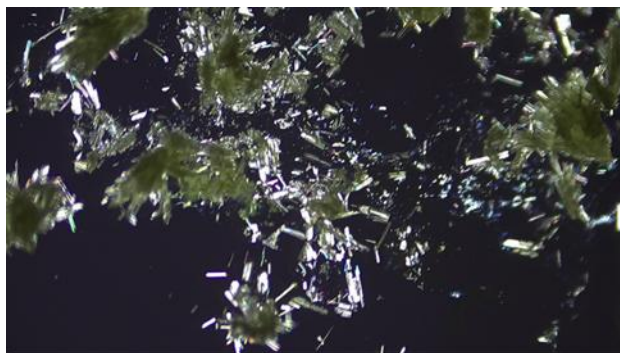


Figure 46: Optical microscope image of co-crystal Isonicotinamide · DNSA (LM090D) at 100×

The asymmetric unit of Isonicotinamide · 3,5-dinitrosalicylic acid comprises of two molecules of isonicotinamide and one 3,5-dinitrosalicylic acid (DNSA) molecule. Notably, each isonicotinamide molecule exhibits a protonated pyridine nitrogen with an occupancy of 0.5 at each site, indicates that the proton is disordered over the two sites. When refined with 0.5 site occupancy on each nitrogen atom, the R1 value decreased from 0.050 to 0.0483, indicating an improved fit to the observed data. In contrast, assigning full occupancy to one nitrogen and removing the hydrogen from the other resulted in an increase in R1 and the appearance of residual electron density near the unprotonated nitrogen atom in the difference Fourier map.

The DNSA molecule on the other hand has been deprotonated in the hydroxyl group resulting in the formation of a phenolate anion. The proton transfer from DNSA to isonicotinamide leads to the formation of a salt co-crystal, characterized by ionic interactions between the resulting cationic and anionic species. This proton transfer is further stabilized by a network of hydrogen bond interactions, which contribute to the overall stability and packing of the crystal structure.

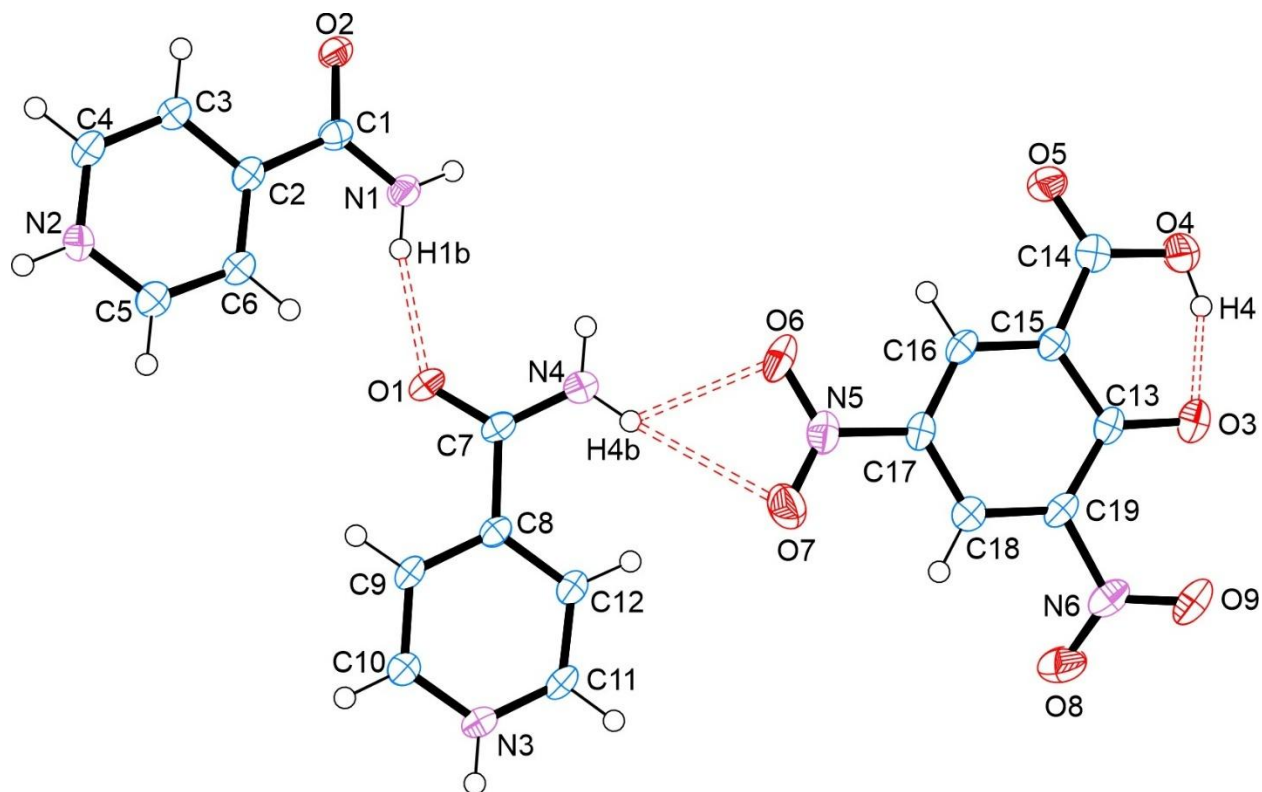


Figure 47: Asymmetric unit of Isonicotinamide · DNSA (LM090D)

Within the DNSA molecule, an intramolecular O–H···O hydrogen bond is observed between the deprotonated oxygen of the hydroxyl group and the adjacent carboxylic acid group. This interaction forms a six-membered ring, a common feature in salicylic acid derivatives, which stabilizes the molecular conformation

There is an observed bifurcated hydrogen bond between the nitro group of one INA molecule and the DNSA molecule. Specifically, there's a hydrogen bond between N4–H4b···O6 and N4–H4b···O7. This bifurcated interaction enhances the connectivity between the co-former and the API. The same INA molecule involved in bifurcated bonding with DNSA also forms a hydrogen bond with the second INA molecule. This interaction occurs between the amide N–H donor of one INA and the carbonyl oxygen acceptor of the other, facilitating the formation of INA dimers or chains within the crystal lattice.

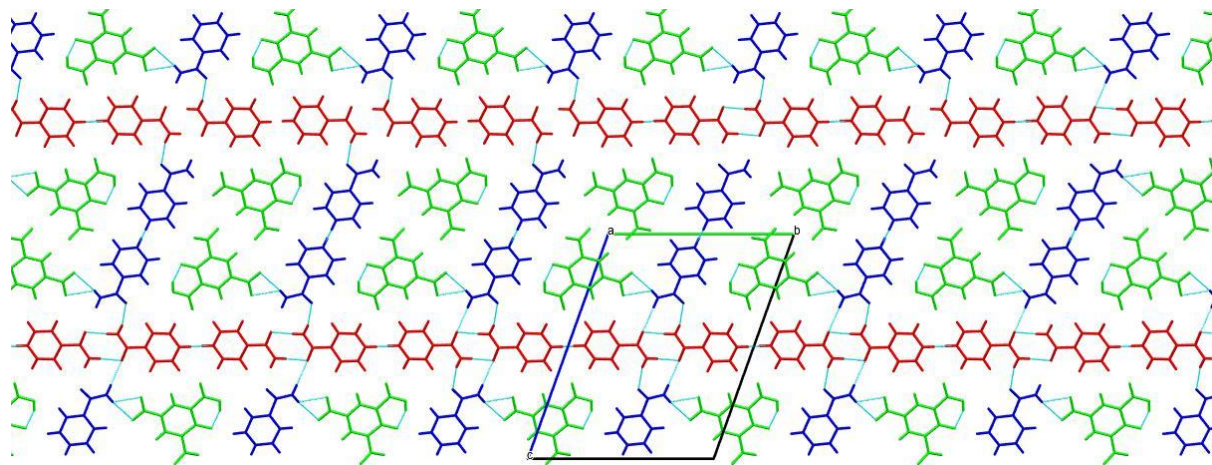


Figure 48: Packing of LM090D parallel to the *b*-axis

The packing of co-crystal LMO90D reveals distinct structural motifs, One INA molecule forms continuous ribbons parallel to the *b*-axis. These ribbons are stabilized by N–H···O hydrogen bonds between amide groups, resulting in the formation of $R_2^2(8)$ ring motifs—a common and robust supramolecular synthon in amide-containing compounds. The other INA molecule alternates with the DNSA molecule and form a continues chain parallel to the INA ribbon, this repeats twice before observing another INA ribbon.



Figure 49: INA ribbon showing the formation of $R_2^2(8)$ ring motifs between the amide groups of the INA molecule and hydrogen bonding between adjacent pyridine nitrogen atoms

Within the INA ribbon, there is evidence of a dynamic hydrogen bonding between adjacent pyridine nitrogen atoms. This interaction is due to the shared proton that is disordered over the two positions, each with an occupancy of 0.5. This means that, statistically, the proton resides on one nitrogen atom 50% of the time and on the adjacent nitrogen atom the remaining 50%.

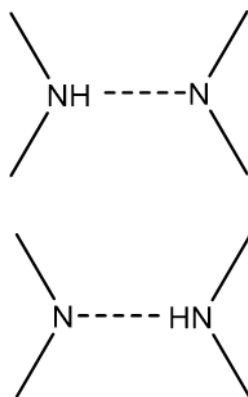


Figure 50: Dynamic hydrogen bonding between adjacent pyridine nitrogen atoms

Analysis along the *a* and *b* axis reveals significant π – π stacking interactions with centroid–centroid distance between aromatic rings (3.673 Å) between the aromatic rings of INA and DNSA molecules. Which contribute in stabilizing the co-crystal.

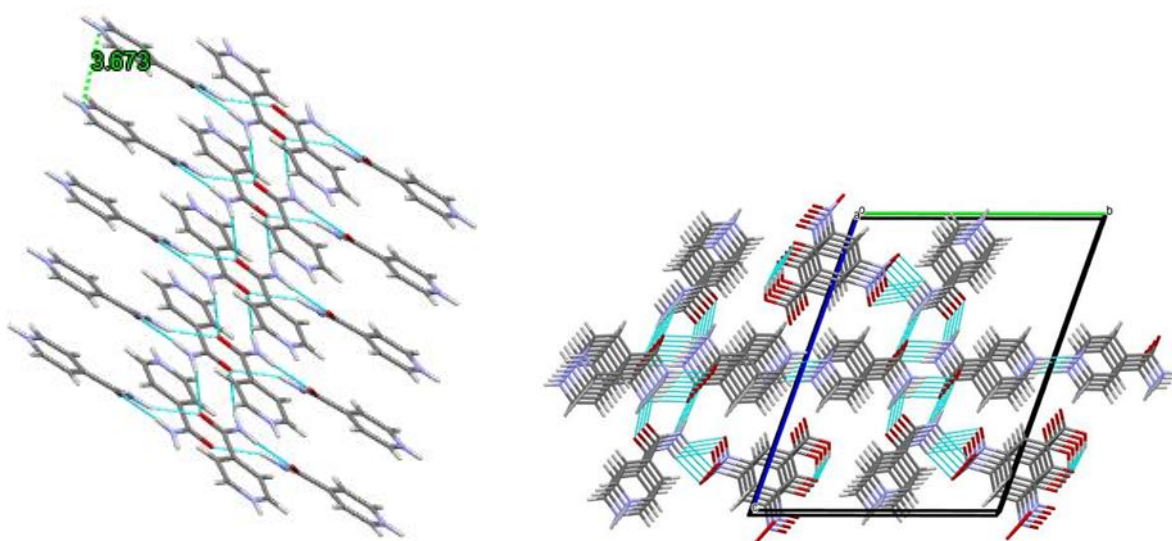


Figure 51: π – π stacking interactions between the aromatic rings of INA and DNSA molecules along the *b* and *c*-axis

Table 17: Hydrogen Bonds for LM090D

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
N3	H3	N3 ¹	0.88	1.77	2.648(3)	174.8
N1	H1B	O1	0.88	2.04	2.894(2)	165.1
N2	H2	N2 ²	0.88	1.82	2.700(3)	173.0
O4	H4	O3	0.84	1.70	2.482(2)	154.6

Symmetry transformations used to generate equivalent atoms:

1-x,1-y,-z; 22-x,2-y,1-z

4.3.3 Nicotinic hydrazide · 3,5-dinitrosalicylic acid (LM091)

The co-crystal of nicotinic hydrazide and 3,5-dinitrosalicylic acid was produced using slow evaporation method. Needle like crystals as shown in the figure below were formed after a day of slow evaporation. The crystals were clear and showed the presence of little or no impurities in the sample. A single suitable crystal was selected and analysed using SCXRD.

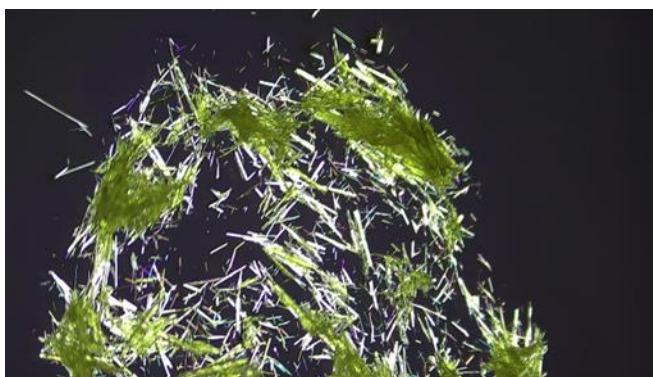


Figure 52: Optical microscope image of co-crystal Nicotinic hydrazide · DNSA (LM091) at 100×

The asymmetric unit of LM091 consist of two molecules of DNSA (co-former 1 and co-former 2), 2 molecules of nicotinic hydrazide (API) and one methanol molecule. The API molecules differ in protonation state and hydrogen bonding behaviour. One molecule (API 1) is protonated at the pyridine nitrogen resulting in pyridinium cation, whereas the other molecule (API 2) is protonated at the terminal hydrazide nitrogen forming -NH_3^+ . The terminal hydrazide -NH_2 group exhibits significantly higher basicity than the pyridine ring nitrogen, preferentially capturing the proton from the weaker DNSA phenolic OH to form the thermodynamically favored hydrazinium cation (-NH_3^+), while the pyridine nitrogen of API 1 protonates via competition with the stronger DNSA carboxylate base ($\text{pK}_a \approx 2\text{-}3$).

The different molecules in the asymmetric unit are held together by a network of hydrogen bonds. There are intramolecular hydrogen bonding observed within both molecules of DNSA happening between the carboxylic acid group forming a six membered ring S(6).

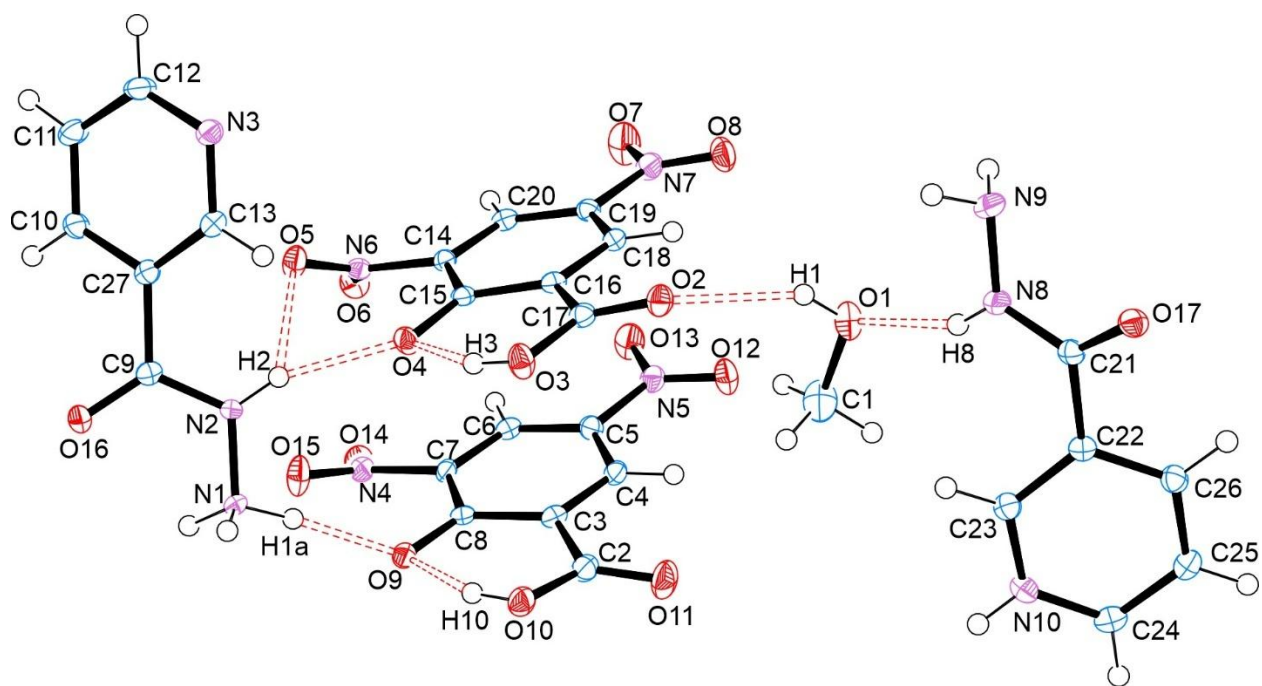


Figure 53: Asymmetric unit of co-crystal LM091 with ellipsoid at 50% probability

There are intermolecular hydrogen bonds between the molecules in the asymmetric unit holding the co-crystal together. Key hydrogen bonds include $\text{N-H}\cdots\text{O}$ between API 1 and the methanol

molecule, O-H $\cdots$ O between co-former 1 and the methanol molecule, a bifurcated hydrogen bond between API 2 and co-former 1; Specifically, N-H $\cdots$ O aryl interaction with the oxygen linked to the aromatic ring, and a N-H $\cdots$ O NO₂ interaction with a nitro oxygen, another N-H $\cdots$ O hydrogen bond was observed between API 2 and co-formers 1.

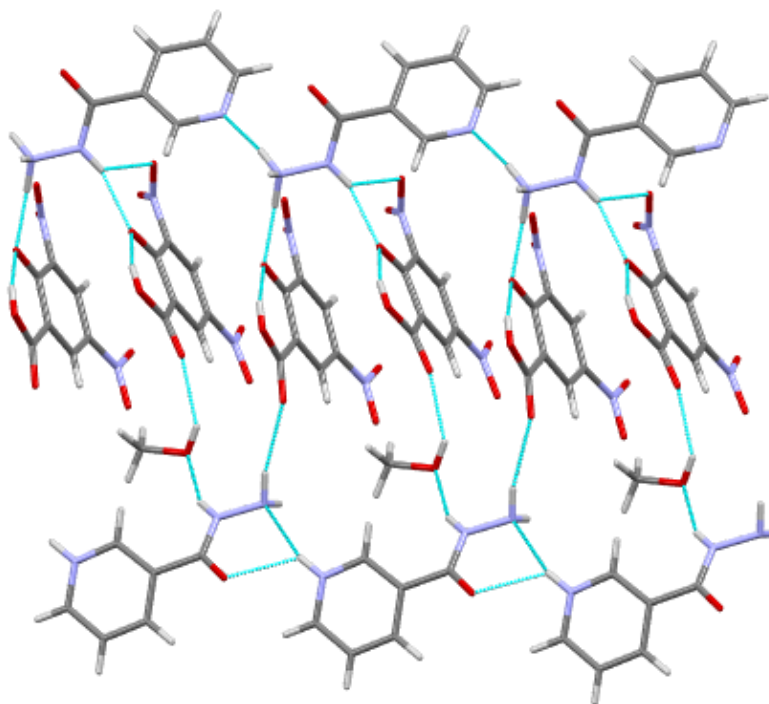


Figure 54: Crystal packing of Nicotinic hydrazide · DNSA along the *a*-axis

The crystal packing reveals a complex but ordered arrangement, when viewed along the *a*-axis, API 1 molecules extend into a one-dimensional chain below the co-former's molecules through bifurcated hydrogen bonds, forming a repeating hydrogen-bonded motif. Adjacent to this chain is the chain of methanol molecules which form hydrogen bonds with API 1 and the co-former molecules. The co-former molecules also form a chain, with co-former 1 slightly above co-former 2. The identical co-former molecules are aligned in a stacked fashion, appearing to form a pseudo-columnar arrangement perpendicular to the API chain. API 2 molecule forms a similar chain that propagates above the stacked co-formers, linked via intermolecular hydrogen bonds.

Table 18: Hydrogen Bonds for LM091B.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O1	H1	O2	0.82	2.13	2.8497(17)	146.9
O3	H3	O4	0.82	1.71	2.4760(16)	154.4
O10	H10	O9	0.82	1.76	2.5143(16)	153.1
N1	H1A	O9	0.96(2)	1.82(2)	2.7681(19)	168.3(18)
N1	H1B	O2 ¹	0.89(2)	1.98(2)	2.8570(19)	166(2)
N1	H1C	N3 ²	1.00(2)	1.77(2)	2.766(2)	174(2)
N2	H2	O4	0.86	1.99	2.8084(18)	158.6
N8	H8	O1	0.84(2)	2.06(2)	2.849(2)	156(2)
N10	H10B	O17 ²	0.86	2.25	2.8325(18)	125.1

Symmetry transformations used to generate atoms equivalents:

¹1-x,1-y,1-z; 21+x,+y,+Z

4.3.4 Niacinamide · 3,5-dinitrosalicylic acid (LM092)

Co-crystal LM092 was crystallize using slow evaporation method. Large, rectangular-prism shaped, transparent crystals with clear edges were yielded after 3 days. This sample showed no impurities, a single crystal was selected for characterization.



Figure 55: Optical microscope image of co-crystal Niacinamide · DNSA (LM092) at 100×

The asymmetric unit of co-crystal LM092 consist of one molecule of the API, niacinamide and one molecule of DNSA. The DNSA molecule exhibit positional disorder in the hydroxyl group position and the hydrogen position opposite to it. The hydroxyl group and the hydrogen atom are distributed between the two sites with occupancies of 0.75 and 0.25, respectively. In both disordered orientations, the hydroxyl group participates in an intermolecular O-H $\cdots$ O hydrogen bond with the adjacent carbonyl oxygen, forming a stable six-membered S (6) ring motif.

Hydrogen atoms involved in hydrogen bonding were located from difference Fourier maps and refined freely with isotropic displacement parameters, allowing accurate determination of protonation states and hydrogen-bond geometries. All other hydrogen atoms were placed in calculated positions and refined using a riding model with isotropic displacement parameters tied to their parent atoms

Another key observation in the structure deprotonation of the carboxylic acid group in the DNSA molecule resulting in a carboxylate, the proton is transferred to the ring nitrogen atom of niacinamide confirming salt formation rather than a neutral co-crystal.

The resulting protonated nitrogen atom of niacinamide participate in an intermolecular O $\cdots$ H-N with one of the nitrogen atoms in the DNSA molecule.

Together, the combination of positional disorder, intramolecular hydrogen bonding, proton transfer, and intermolecular charge-assisted hydrogen bonding leads to a robust and stabilized supramolecular assembly in the crystal lattice.

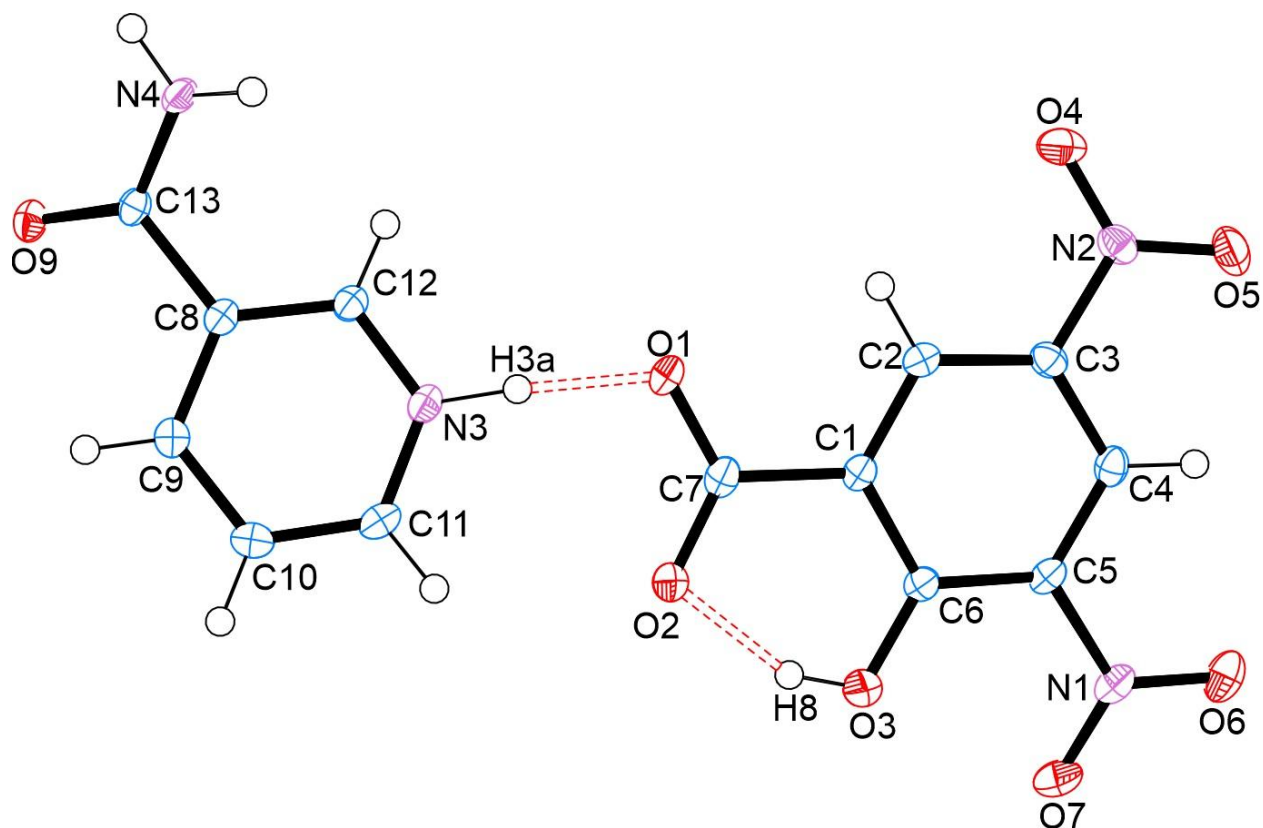


Figure 56: Asymmetric unit of Niacinamide · DNSA with ellipsoids at 50% probability

In terms packing, the molecules are linked through a network of hydrogen bonds forming a chain extending along *a*-axis

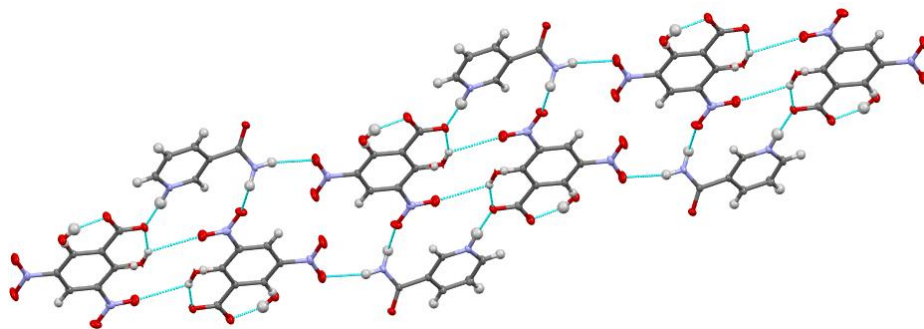


Figure 57: Crystal packing of niacinamide · DNSA along *a*-axis

Table 19: Hydrogen Bonds for LM092

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O8	H8	O2	0.8200	1.666(2)	2.4231(16)	152.4(4)
O3	H3	O1	0.8200	1.663(5)	2.420(3)	152.4(9)
N3	H3a	O1	1.00(2)	1.61(2)	2.6164(14)	176.2(19)

4.3.5 Benzhydrazide · 3,5-dinitrosalicylic acid (LM094)

Clear, needle like co-crystals of benzhydrazide and 3,5-dinitrosalicylic acid were yielded using slow evaporation. The shape and size of the crystals appear to be uniform suggestion minimum or no impurities in the sample. A single suitable crystal was selected under the microscope for characterization.



Figure 58: Optical microscope image of co-crystal benzhydrazide · DNSA (LM094) at 100×

The asymmetric unit of LM094 consists of two molecules of benzhydrazide and two molecules of 3,5-Dinitrosalicylic acid. Both DNSA molecules exhibit intramolecular O-H $\cdots$ O hydrogen bonds typical of salicylic acid derivatives, where the phenolic OH donates to the adjacent carboxylic carbonyl O, forming a six-membered S(6) ring motif. In terms of intermolecular hydrogen bond, each of the API molecules participate in bifurcated hydrogen bonding interaction with adjacent co-former molecules forming N-H $\cdots$ O bonds. Additionally, there is a N-H $\cdots$ O hydrogen bond between the two API molecules.

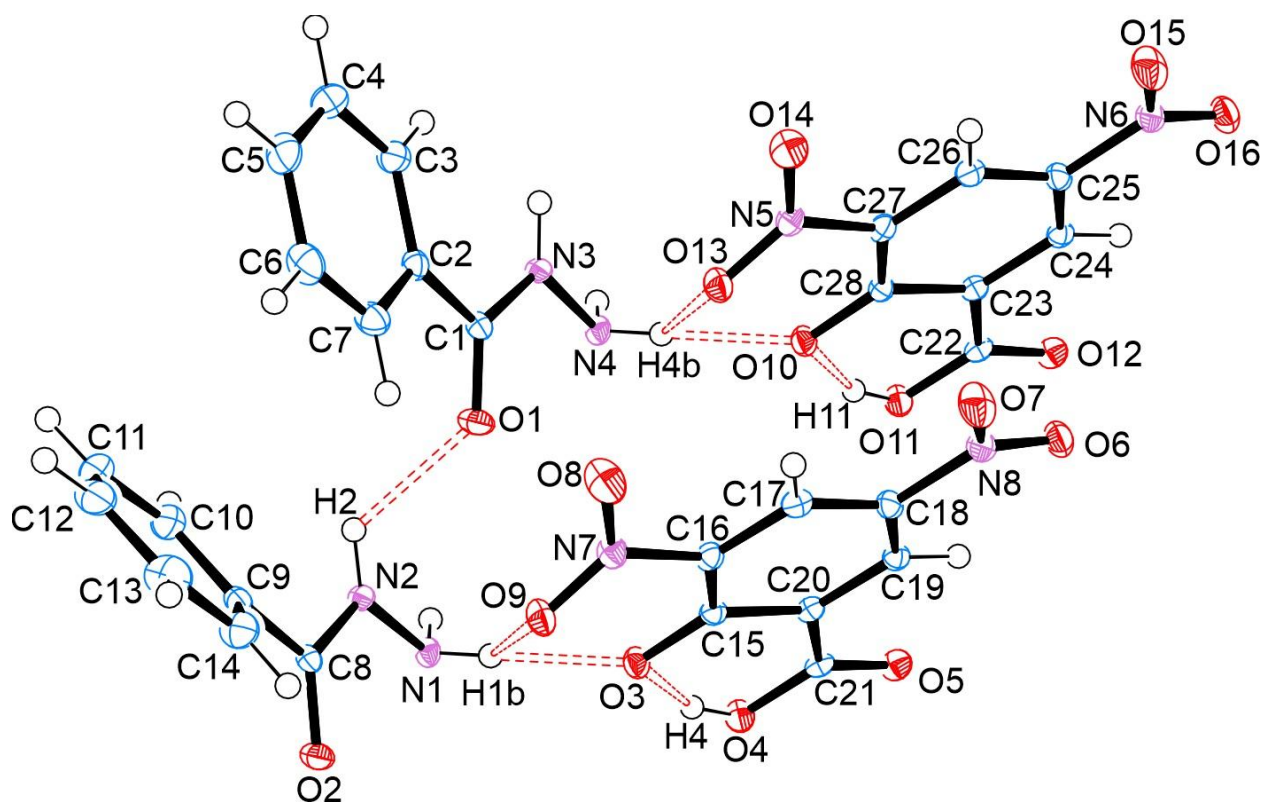


Figure 59: Asymmetric unit of LM094 with ellipsoids at 50% probability

No Significant π - π interactions are observed in the crystal lattice. The centroid-centroid distances between adjacent aromatic is 4.828 Å.

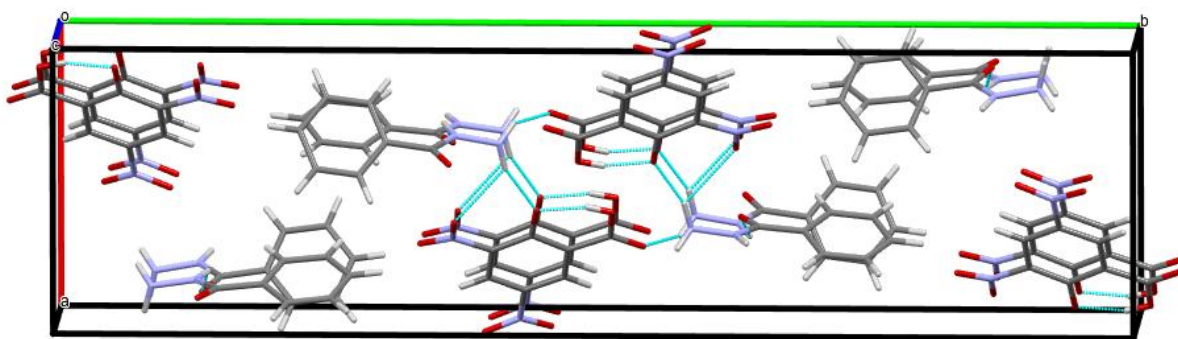


Figure 60: Packing diagram for benzhydrazone, 3,5-dinitrosalicylic acid co-crystal along the *c*-axis

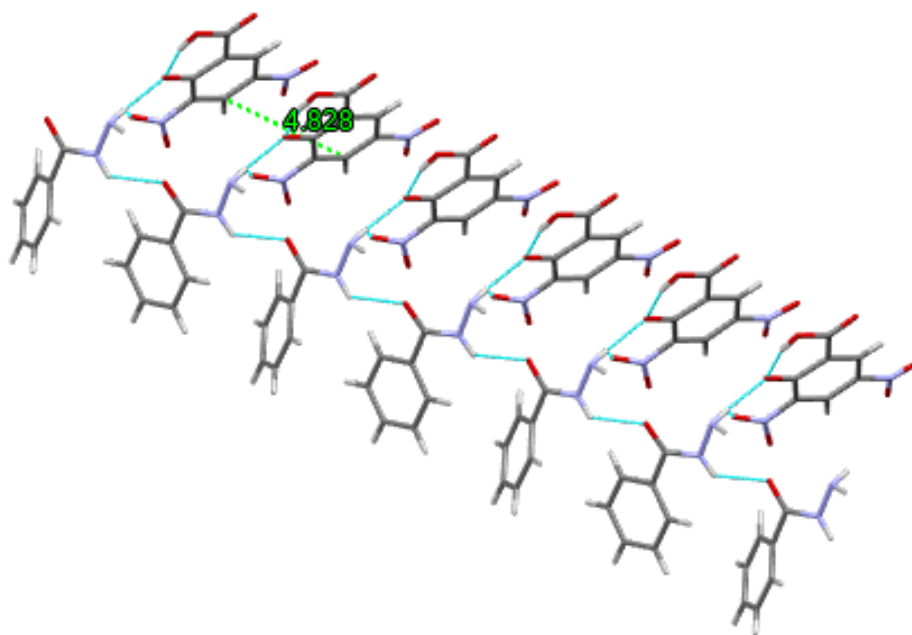


Figure 61: Packing diagram for benzhydrazide, 3,5-dinitrosalicylic acid co-crystal along the *a*-axis

Table 20: Hydrogen Bonds for LM094B

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
N1	H1a	O5 ¹	0.8800	2.093(6)	2.8447(14)	142.9(9)
N1	H1b	O3	0.8801	1.892(2)	2.7296(13)	158.5(5)
N2	H2	O1	0.8800	2.0920(14)	2.6930(14)	124.80(4)
N3	H3	O2 ²	0.8800	2.0611(14)	2.7186(14)	130.77(4)
N4	H4a	O12 ³	0.8800	2.114(7)	2.8548(14)	141.4(9)
N4	H4b	O10	0.8800	1.873(2)	2.7215(13)	161.3(6)
O4	H4	O3	0.8400	1.6293(13)	2.4200(12)	155.79(13)
O11	H11	O10	0.8400	1.6259(12)	2.4168(12)	155.82(5)

Symmetry operations used to generate equivalent atoms

$$^1 1-x, 1-y, 1-z; ^2 +x, +y, 1+z; ^3 1-x, 1-y, 2-z$$

4.3.6 Co-crystal of Theophylline · DNSA (LM102)

The co-crystal of Theophylline and DNSA was synthesized via slow evaporation method yielding needle-like and plate-like crystals. The morphology of this co-crystal was not clear, despite that a suitable clear plate-like crystal suitable for X-ray diffraction analysis were obtained and characterized using the SCXRD.

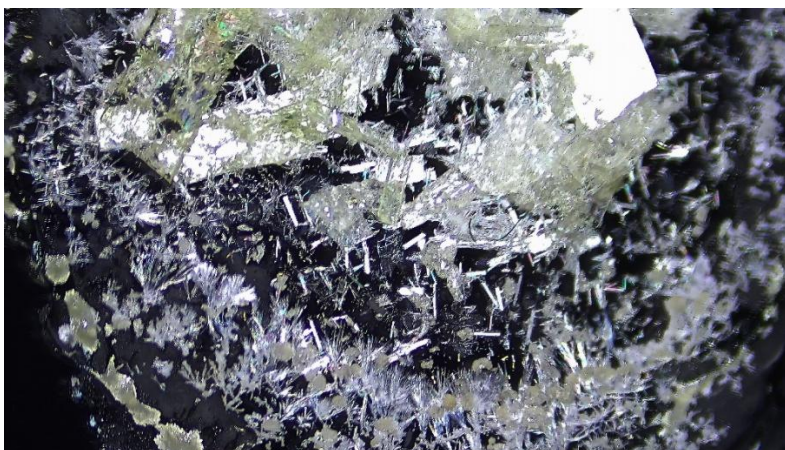


Figure 62: Optical microscope image of theophylline · DNSA crystals at 100×

SCXRD analysis revealed that the asymmetric unit comprises one molecule theophylline and one molecule of DNSA. Within the DNSA, an intramolecular hydrogen bond was observed, which contributes to the stabilization of its molecular conformation. In addition, intermolecular hydrogen bonding occurs between theophylline and DNSA molecules, confirming the successful formation of a co-crystal. The hydrogen bond is between the hydroxyl group of DNSA and the nitrogen atom in the imidazole of the API.

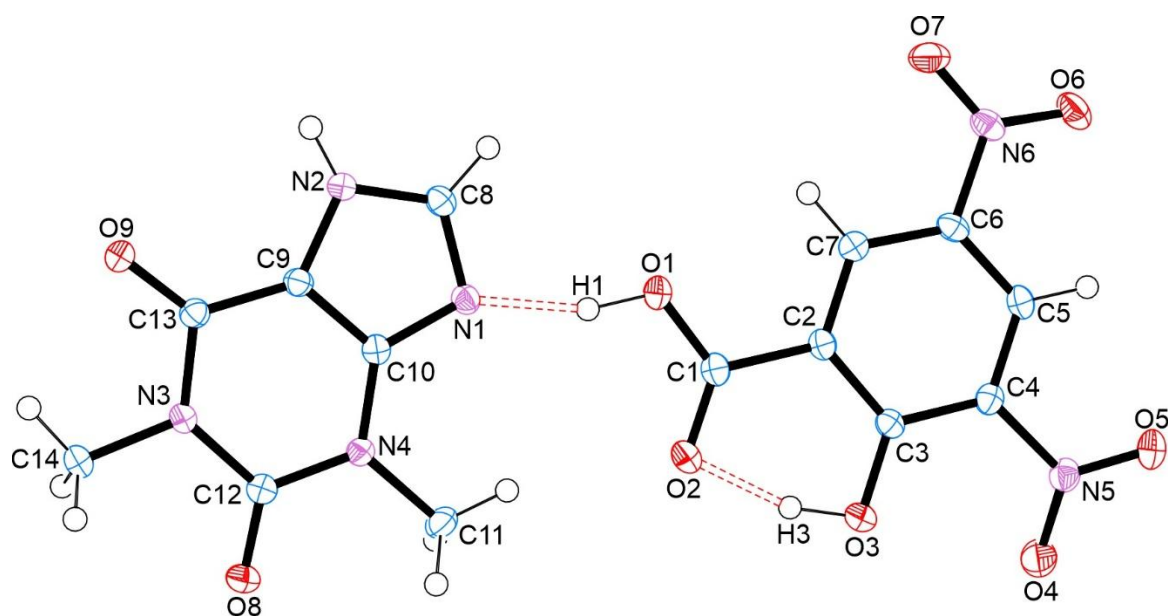


Figure 63: Asymmetric unit for Theophylline · 3,5-Dinitrosalicylic acid with ellipsoids at 50% probability

In terms of packing, both theophylline and 3,5-dinitrosalicylic acid molecules form distinct linear chains along the *a*-axis. Within this arrangement, the theophylline molecules align in alternating orientations—if one molecule is oriented with its purine ring pointing up, the next adjacent molecule is oriented down, creating a head-to-tail, anti-parallel alignment. A similar linear arrangement is observed for the co-former molecules.

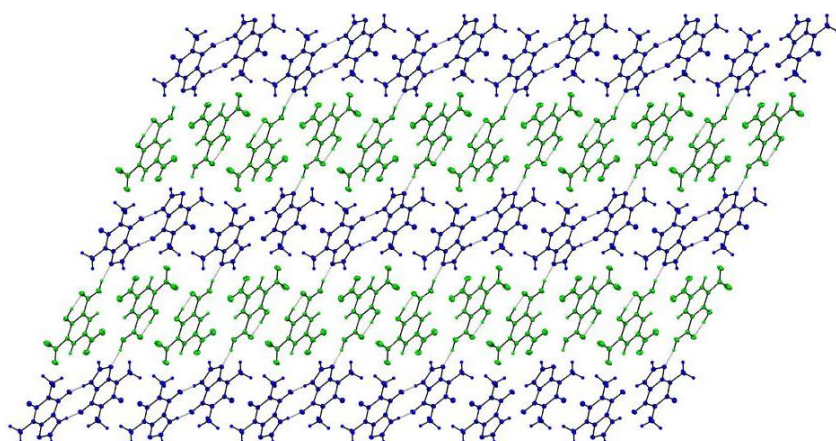


Figure 64: Crystal packing of LM102 co-crystal along the *a*-axis

Additionally, adjacent theophylline molecules from neighbouring asymmetric units are connected via hydrogen bonding in an R^2_2 (10) motif, which is a well-known graph-set descriptor indicating a pair of donors–acceptor hydrogen bonds forming a ten-membered ring. This interaction contributes to the robustness of the crystal packing.

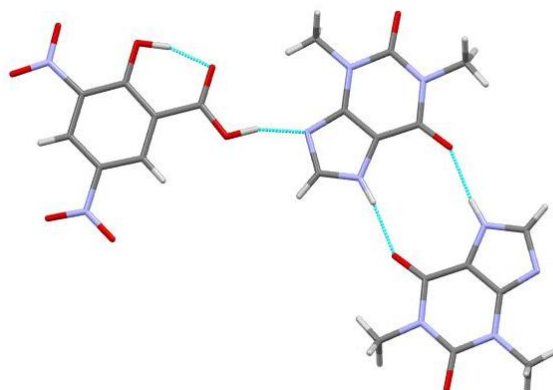


Figure 65: R^2_2 (10) ring motif observed within the crystal lattice

Table 21: Hydrogen Bonds for LM102

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O1	H1	N1	0.964(19)	1.650(19)	2.5833(14)	161.8(17)
O3	H3	O2	0.980(19)	1.555(19)	2.5038(13)	161.7(18)
N2	H2	O9 ¹	0.8800	1.8409(14)	2.7125(14)	170.34(4)

Symmetry transformation used to generate equivalents atoms:

¹2-x,1-y,1-z

4.3.7 Acetaminophen · DNSA (LM108)

Co-crystal LM108 was crystallized using the slow evaporation method and crystals were yielded after two days. The morphology of this crystal is not clear as they appear to be a mixture of powder and lumps. This might be because of poor crystallization of the API and co-former. Regardless of the morphology of these crystals, a single crystal was selected for characterization, and it appeared to be a clear plate-like crystal.

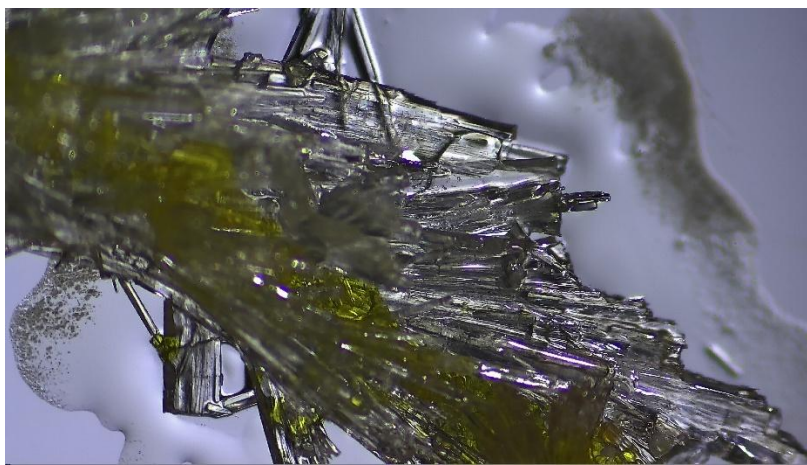


Figure 66: Optical microscope image of Acetaminophen · DNSA at 100×

The asymmetric unit of acetaminophen, DNSA co-crystal hydrate contains one acetaminophen molecule deprotonated at the amide nitrogen, one 3,5-dinitrosalicylic acid molecule deprotonated at the carboxyl group, and one water molecule. A hydrogen bond is formed where the hydroxyl oxygen atom of acetaminophen accepts a hydrogen atom from the water molecule. The second hydrogen atom of the water molecule donates a hydrogen bond to one of the carboxylate oxygen atoms of the 3,5-dinitrosalicylic acid, thus connecting all three components through a continuous hydrogen-bonding network.

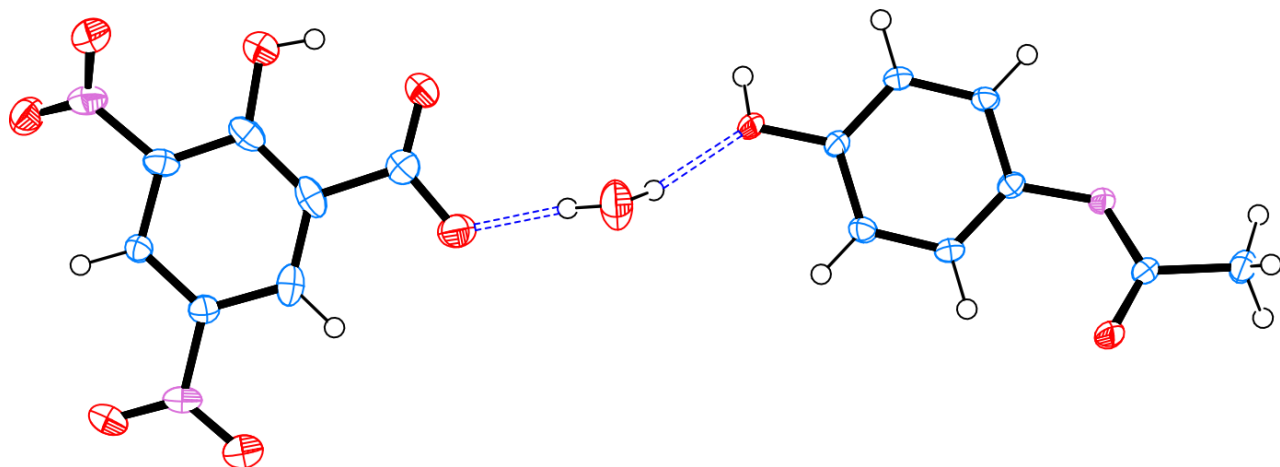


Figure 67: Asymmetric unit of Acetaminophen · 3,5-Dinitrosalicylic acid with ellipsoids at 50% probability

In terms of packing, the acetaminophen molecules align in an alternating fashion such that successive molecules are oriented in opposite directions, generating a one-dimensional chain. Parallel to this arrangement, the 3,5-dinitrosalicylic acid molecules also form chains. These two chains are interconnected by a network of hydrogen bonds, in which water molecules play a bridging role.

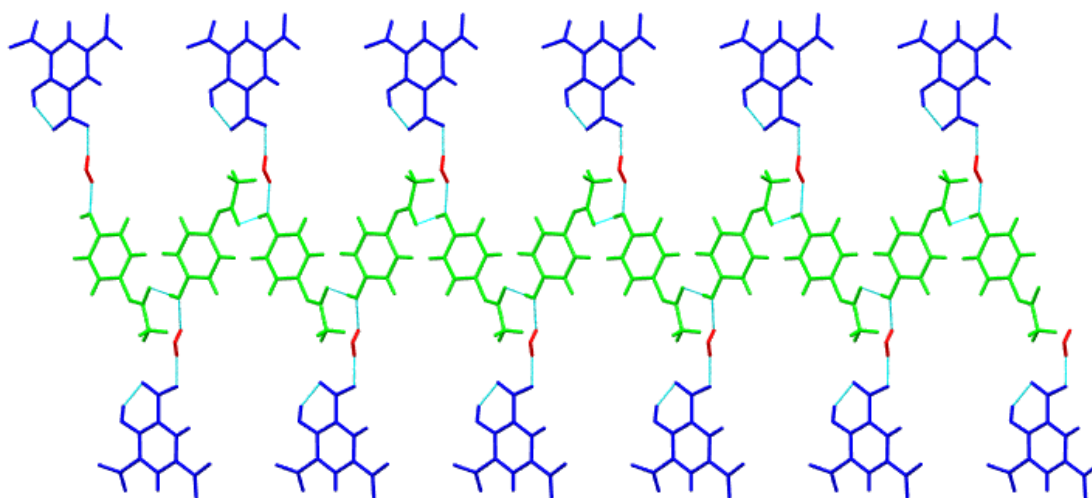


Figure 68: Crystal packing of LM108 co-crystal along the *a*-axis

4.3.8 Key observations and discussion of DNSA structures

In the series of DNSA, hydrogen bonding has dominated the interaction between chosen APIs and the DNSA co-former. Observed in almost all the structure is the intramolecular O–H···O hydrogen bond forming S(6) ring motif in the DNSA molecule. Carbamazepine·DNSA co-crystal was an exception. Such S(6) locking is widely reported for salicylic acid derivatives and is known to reduce conformational flexibility while directing intermolecular interactions outward through the carboxyl group (Amudha et al., 2015).

Consistent with prior crystal engineering studies, hydrogen bonding in this series preferentially involved the carboxylic acid group rather than the nitro substituents. Although nitro groups are good hydrogen-bond acceptors, literature shows they are typically secondary participants unless stronger donors or acceptors are unavailable (Robinson et., 2000). Here, the COOH group in the DNSA molecule acts either as a donor (hydrogen atom in COOH) or as a carboxylate acceptor (COO[−]) in salts. In the 1:1 asymmetric unit reported in this present study, deprotonation of DNSA led to charge-assisted N⁺–H···O[−] interactions, reflecting expected ΔpK_a -driven salt formation behaviour for nitro-substituted benzoic acids.

In contrast, structures containing two API and two DNSA molecules per asymmetric unit showed a network of hydrogen bonds where oxygen in the nitro group also participated in hydrogen bonding. This showed that once primary acid–base synthons are satisfied, secondary acceptors such as NO₂ groups participate in stabilizing the crystal lattice (Robinson et al., 2000). Isonicotinamide·DNSA structure particularly showed both nitro oxygen atoms engage in hydrogen bonding, forming a bifurcated hydrogen bond, indicating cooperative stabilization within a more densely connected hydrogen-bonding network.

Carbamazepine is a notable exception in this series as it does not contain basic heterocyclic nitrogen. As expected from literature on carbamazepine multicomponent systems, it formed a neutral co-crystal rather than a salt. The structure exhibits the robust R₂²(8) synthon, consistent with carbamazepine's well-known preference for amide–amide or amide–acid dimeric motifs (Ouyang et al., 2024). Similarly, Theophylline formed a co-crystal displaying an R₂²(10) motif, aligning with its bifunctional hydrogen-bond donor/acceptor character observed in previous solid forms (Guinet et al., 2023).

In the DNSA series, salt formation predominated for more basic APIs, including Isonicotinamide, Nicotinic hydrazide (salt hydrate), Niacinamide, Benzhydrazide, and Acetaminophen (salt hydrate). These outcomes follow established acid–base crystal engineering principles, where strongly electron-withdrawing nitro groups significantly enhance DNSA acidity, promoting proton transfer with suitable bases (Smith & Wermuth, 2014).

Additionally, significant π – π stacking were observed only in the isonicotinamide·DNSA structure. While DNSA possesses an aromatic core capable of stacking, hydrogen bonding clearly dominates the packing landscape in this present study.

4.4 Co-crystals of 2-(nitrophenyl)methanol

This sub-session presents two novel co-crystal structures of 2-(nitrophenyl)methanol with Isonicotinamide and carbamazepine. This chapter highlight the hydrogen bonding patterns between 2-(nitrophenyl)methanol as a co-former with the two different APIs, asymmetric unit and packing in the crystalline lattice. These co-crystals were synthesised using slow evaporation method. Structural characterization was done using SCXRD and SHELXT and SHELXL were used for solving and refining the crystal structures.

Part of this work has been published in *Zeitschrift für Kristallographie – Crystalline Materials* (October 2025) and is included in Appendix A.

Table 22: Crystallographic data and structure refinement for 2-(nitrophenyl)methanol

Identification code	LM049	LM050
Empirical formula	C ₁₃ H ₁₃ N ₃ O ₄	C ₂₂ H ₁₉ N ₃ O ₄
Formula weight	275.26	389.40
Temperature/K	173.15	100.00
Crystal system	Monoclinic	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>n</i>	<i>P</i> 2 ₁ / <i>n</i>
<i>a</i> /Å	3.7957(3)	5.0010(4)
<i>b</i> /Å	7.4395(5)	19.5759(17)
<i>c</i> /Å	43.878(3)	19.0909(17)
α /°	90	90
β /°	91.181(3)	91.343(3)
γ /°	90	90
Volume/Å ³	1238.77(16)	1868.5(3)
<i>Z</i>	4	4
ρ_{calc} /cm ³	1.476	1.384
μ /mm ⁻¹	0.112	0.097
<i>F</i> (000)	576.0	816.0
Crystal size/mm ³	0.116 × 0.151 × 0.763	0.104 × 0.284 × 0.104
Radiation	MoK α (λ = 0.71073)	MoK α (λ = 0.71073)
2 Θ range for data collection/°	3.714 to 55.13	4.162 to 55.188
Index ranges	-4 ≤ <i>h</i> ≤ 4, -9 ≤ <i>k</i> ≤ 9, -56 ≤ <i>l</i> ≤ 57	-6 ≤ <i>h</i> ≤ 6, -25 ≤ <i>k</i> ≤ 25, -24 ≤ <i>l</i> ≤ 24
Reflections collected	25907	4333
Independent reflections	2834 [<i>R</i> _{int} = 0.1010, <i>R</i> _{sigma} = 0.0612]	4333 [<i>R</i> _{int} = 0.0385, <i>R</i> _{sigma} = 0.0190]
Data/restraints/parameters	2834/0/193	4333/0/265
Goodness-of-fit on <i>F</i> ²	1.031	1.061
Final <i>R</i> indexes [<i>I</i> ≥ 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0513, <i>wR</i> ₂ = 0.1015	<i>R</i> ₁ = 0.0395, <i>wR</i> ₂ = 0.1084
Final <i>R</i> indexes [all data]	<i>R</i> ₁ = 0.0746, <i>wR</i> ₂ = 0.1131	<i>R</i> ₁ = 0.0450, <i>wR</i> ₂ = 0.1138
Largest diff. peak/hole / e Å ⁻³	0.26/-0.22	0.32/-0.25

4.4.1 Isonicotinamide · 2-(nitrophenyl)methanol (LM049)

CCDC number: 2451500

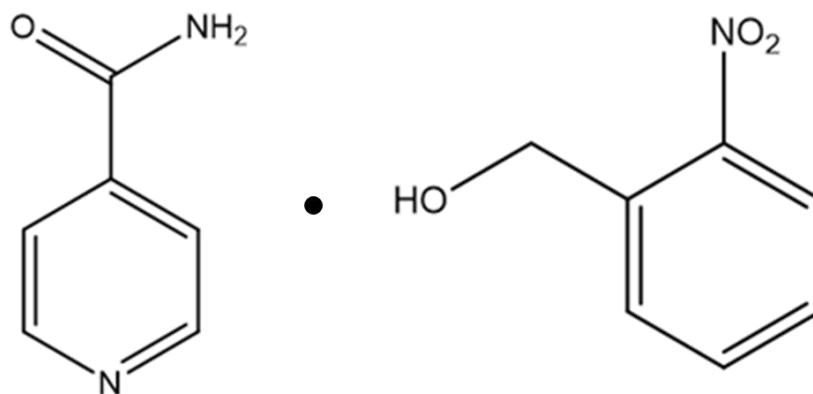


Figure 69: Co-crystal structure of Isonicotinamide and 2-(nitrophenyl)methanol

Long needle-like crystals were formed through slow evaporation. The crystals are colourless, with well-defined edges, a few yellowish crystalline fragments were also visible, which may correspond to unreacted starting material or an impurity phase.



Figure 70: Optical microscope image of isonicotinamide and 2-(nitrophenyl)methanol crystal (LM049) at 100×

The asymmetric unit comprises one molecule each of isonicotinamide and 2-(nitrophenyl)methanol and crystallizes in the monoclinic $P2_1/n$ space group. The two molecular components are associated through a discrete $O-H\cdots N$ hydrogen bond, in which the hydroxyl group of 2-(nitrophenyl)methanol acts as a hydrogen-bond donor to the pyridine nitrogen atom of isonicotinamide ($O2-H2A\cdots N2$), thereby linking the molecules within the asymmetric unit.

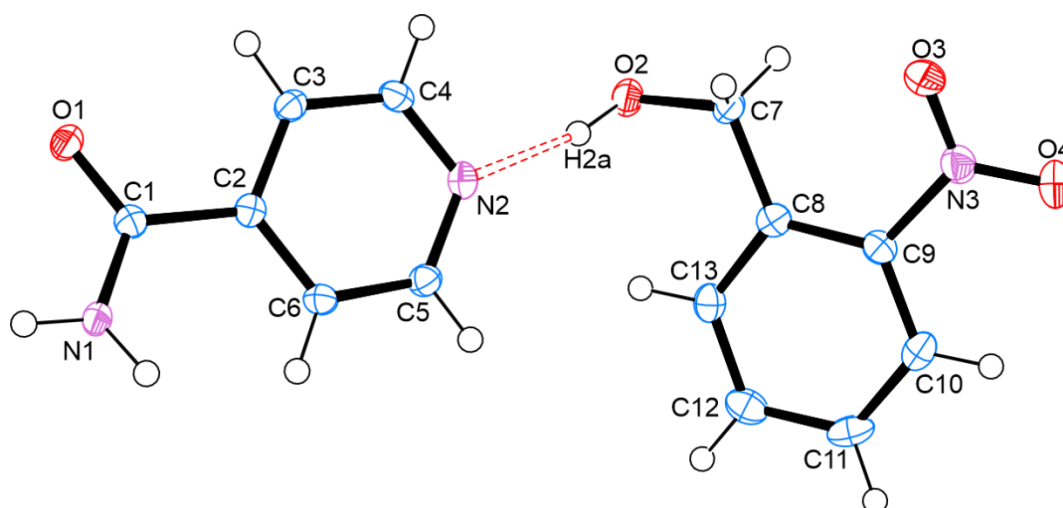


Figure 71: Asymmetric unit of Isonicotinamide · 2-(nitrophenyl)methanol showing 50% probability ellipsoids

In terms of packing, each isonicotinamide molecule acts as a hydrogen bond donor forming $O\cdots H-N$ hydrogen bond with adjacent 2-(nitrophenyl)methanol molecule outside the asymmetric unit. The isonicotinamide molecule also forms $R_2^2(8)$ ring motifs with neighbouring isonicotinamide molecule assigned using standard crystallographic graph set analyses.

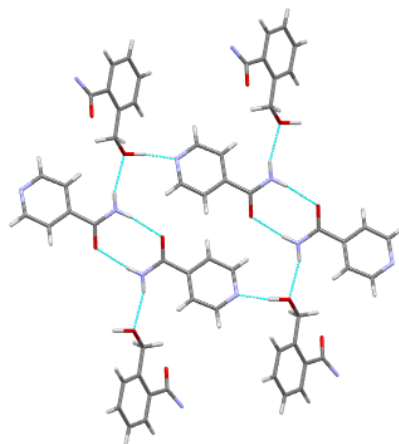


Figure 72: Crystal packing of Isonicotinamide · 2-(nitrophenyl)methanol co-crystal along the *a*-axis

Significant π – π stacking interactions were observed in this structure, centroid separation between the interacting rings is approximately 3.47 Å, which falls within the range commonly associated with significant $\pi \cdots \pi$ stacking interactions.

Table 23: Hydrogen Bonds for LM049

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
N3	H3B	O3 ¹	0.90(2)	2.13(2)	3.008(2)	164(2)
N3	H3C	O4 ²	0.89(2)	2.00(2)	2.889(2)	178(2)
O3	H3	N2	0.94(3)	1.84(3)	2.770(2)	168(3)
C3	H3A	O1 ³	0.95	2.65	3.219(2)	119.4

Symmetry operations used to equivalent atoms

¹1+x,-1+y,+z; ²2-x,-y,1-z; ³1/2-x,-1/2+y,1/2-z

4.4.2 Carbamazepine · 2-(Nitrophenyl)methanol (LM050)

CCDC NUMBER: 2486686

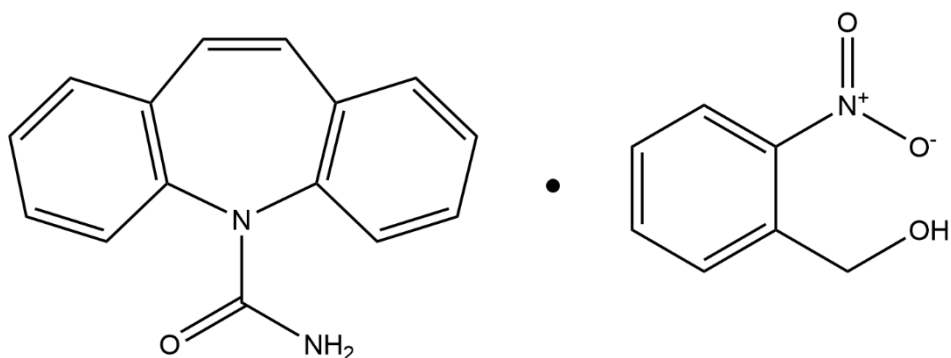


Figure 73: Co-crystal structure of Carbamazepine · 2-(nitrophenyl)methanol

Plate-like crystals were formed through slow evaporation. The crystals are colourless, with well-defined edges as shown in the figure below. A single crystal was selected for SCXRD analysis.



Figure 74: Optical microscope image carbamazepine·2-(nitrophenyl)methanol crystals at 100×

Asymmetric unit contains one molecule of carbamazepine and one molecule of 2-(nitrophenyl)methanol, which crystallizes in the monoclinic $P21/n$ space group. There is a single hydrogen bond observed between the two molecules formed between the oxygen acceptor of carbamazepine and the hydroxyl hydrogen donor of 2-(nitrophenyl)methanol, thus forming a $O1 \cdots H2a-O2$ hydrogen bond.

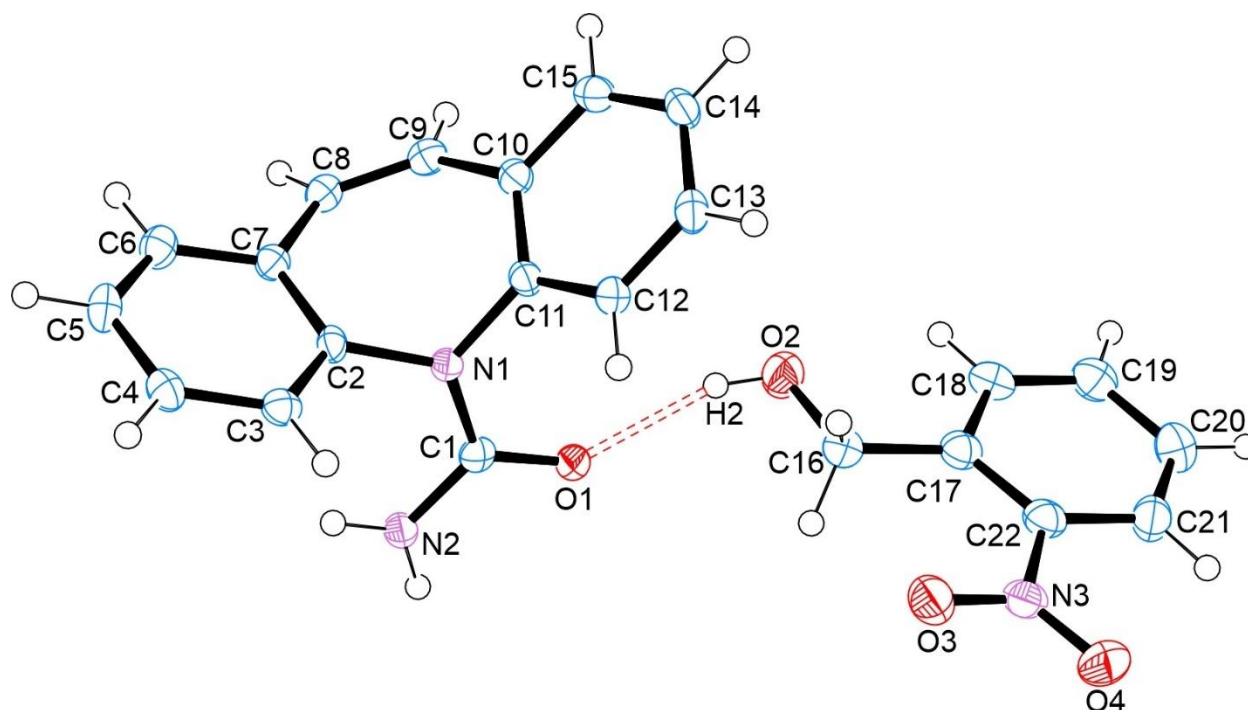


Figure 75: Asymmetric unit for carbamazepine · 2-(nitrophenyl)methanol co-crystal

In terms of torsion within the carbamazepine molecule, the amide group adopts an extended conformation with respect to the azepine ring, as indicated by the torsion angle $\tau(O1=C1-N1-C2) = 174.09^\circ$, which suggests that the amide moiety is nearly antiperiplanar to the adjacent azepine ring atom despite the non-planar nature of the seven-membered ring.

The orientation of the amide N–H bond relative to the carbonyl and ring confirms that the amide hydrogen is nearly in the plane of the molecule indicated by $\tau(C1-N1-H-C1) = -175.01^\circ$. The two aromatic rings connected to the azepine ring are nearly coplanar with the central ring, with torsion angles across the connecting bonds being close to $1-2^\circ$, reflecting a flat molecular conformation.

Regarding packing, each carbamazepine molecule is hydrogen bonded to two adjacent 2-(nitrophenyl)-methanol, O-H $\cdots$ O bond where carbamazepine acts as an acceptor and N-H $\cdots$ O where carbamazepine acts as a donor. Successive carbamazepine molecules are linked through R₂²(8) ring motif, a well-established hydrogen bond pattern in crystal engineering (Etter, 1990).

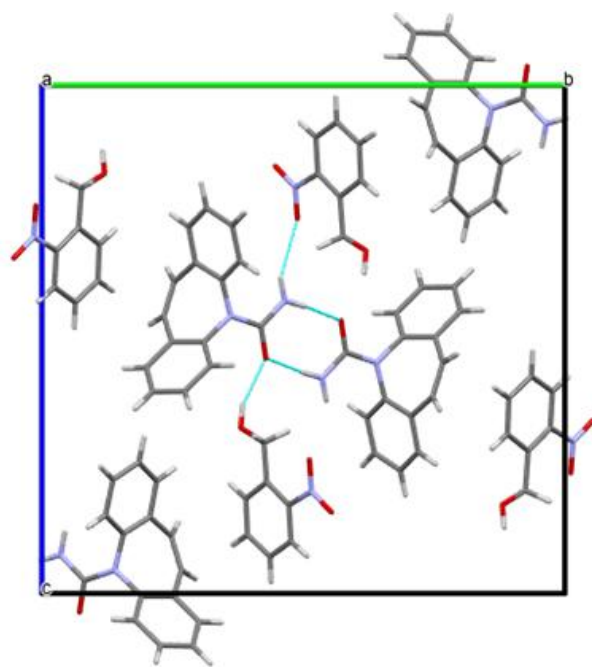


Figure 76: Crystal packing of Carbamazepine 2-(nitrophenyl)methanol co-crystal along the *a*-axis

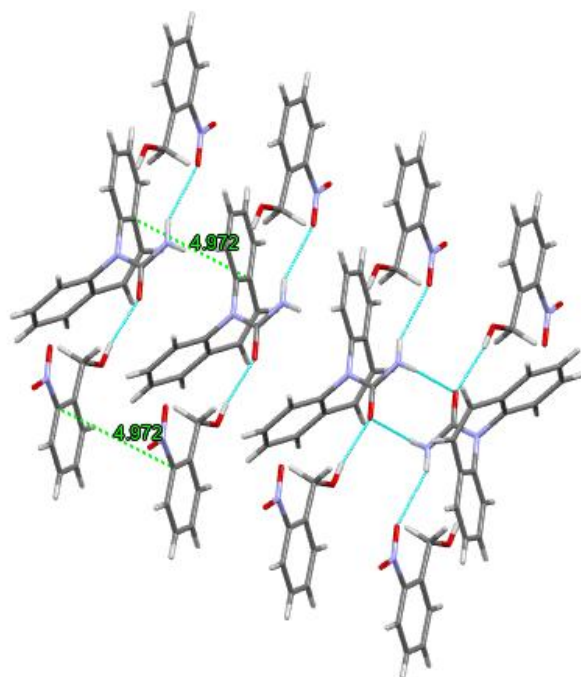


Figure 77: Crystal packing of carbamazepine 2-(nitrophenyl)methanol co-crystal along the *b*-axis

Table 24 Hydrogen Bonds for LM050

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
O2	H2	O1	0.8400	1.990(8)	2.796(2)	160(2)
N2	H2a	O1 ¹	0.8800	2.004(2)	2.865(2)	165.73(7)

Symmetry transformations used to generate equivalent atoms

¹-x,1-y,1-z

4.4.3 Key observation and discussion of 2-(nitrophenyl)methanol co-crystals

Co-crystallization of different APIs with 2-(nitrophenyl)methanol resulted in two novel co-crystal structures. Other samples were not successful, most of them did not co-crystallize, and some of them were confirmed using SCXRD to be starting materials. In both successful and unsuccessful co-crystallization of 2-(nitrophenyl)methanol, methanol was used as a solvent, and the slow evaporation method was employed.

In this series, hydrogen bonding interactions are observed through the hydroxyl group of 2-(nitrophenyl)methanol, the nitro group does not participate in the dominant supramolecular assembly, it contributes to crystal stabilization through a weak hydrogen-bonding interaction. This can be attributed to the comparatively weaker hydrogen-bond donor ability of the nitro group compared to hydroxyl group and their less favorable geometrical orientation. Both structures are further stabilized by amide-amide homosynthon which generates $R_2^2(8)$ ring motifs which is one robust and frequent synthon in the success of crystal engineering (Desiraju, 1995).

4.5 Limitations of the study

Although fifteen crystalline phases were successfully obtained and structurally characterized in this study, there are several limitations that should be acknowledged. The focus of this work was structural analysis using single-crystal X-ray diffraction. While this provided detailed information about hydrogen bonding, proton transfer and supramolecular interactions, additional bulk characterization techniques were only performed on some of the samples as this was not a core objective of the study.

Mechanochemistry yielded limited success under the conditions used. Only one crystalline phase suitable for single-crystal analysis was obtained from ball milling experiments. Further optimization of milling conditions, including frequency, time and the amount of liquid added, may improve results in future studies.

Although ΔpK_a considerations and crystallographic evidence were used to distinguish between salts and co-crystals, acid–base systems are not always straightforward. In some cases, classification depends strongly on structural interpretation, especially where ΔpK_a values fall within the intermediate range.

Solvent choice was shown to significantly influence crystallization outcomes. However, solvent screening was limited to solvents available during the period of the study, and exploring a wider range of solvents and solvent systems may lead to additional crystalline forms.

Lastly, the study was mainly limited to selected APIs and nitrobenzoic acid derivatives. Expanding the range of APIs and co-formers could provide a broader understanding of co-crystallization behavior in related systems.

Despite these limitations, the study provides important structural insight into multicomponent crystal formation and lays a foundation for further work in this area.

Chapter 5: Conclusions

This study investigated the co-crystallization of selected *N*-heterocyclic APIs with nitrobenzoic acid derivative co-formers using slow evaporation and mechanochemistry. Fifteen crystalline phases were successfully obtained, including neutral co-crystals, salts, salt hydrates and solvates. Single-crystal X-ray diffraction was used for full structural characterization and allowed the crystal forms to be classified using proton position, hydrogen bonding interactions, and C–O bond-length analysis.

Slow evaporation proved to be the most effective method, producing the majority of well-formed single crystals suitable for structural analysis. Mechanochemistry yielded mainly microcrystalline powders, with only one structure suitable for SCXRD. These results highlight the importance of controlled crystallization conditions in promoting ordered supramolecular assembly.

Hydrogen bonding played a major role in stabilizing the multicomponent crystals. Acid–pyridine and acid–amide interactions were the dominant supramolecular synthons observed. In several systems, proton transfer occurred, resulting in salt formation. This shows that the outcome of co-crystallization depends not only on the expected hydrogen-bonding patterns, but also on the relative acid–base strengths of the components and the crystallization conditions.

Solvent choice significantly influenced crystallization success. Methanol was the most effective solvent for most systems. Mixed solvent systems and the addition of DMSO improved solubility in certain cases and, in some instances, resulted in solvent molecules being incorporated into the crystal structure.

Overall, this study expands the structural understanding of multicomponent crystals formed between selected APIs and nitrobenzoic acid derivatives. The findings contribute to crystal engineering knowledge and provide a foundation for further investigation of the physicochemical properties of these systems.

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Appendix A: Scientific contributions

Title: The crystal structure of the co-crystal isonicotinamide – 2-(nitrophenyl)methanol (1/1)

Authors: Lindelwa N. Maziya, Hlobile Kgomo, Mark G. Smith

Journal: *Zeitschrift für Kristallographie – New Crystal Structures*

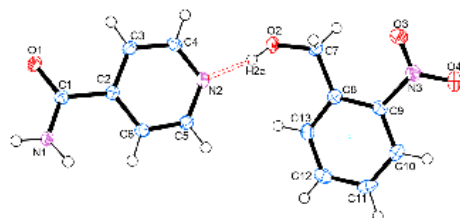
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Lindelwa N. Maziya, Hlobile Kgomo and Mark G. Smith*

The crystal structure of the co-crystal isonicotinamide – 2-(nitrophenyl)methanol (1/1), $C_6H_6N_2O \cdot C_7H_7NO_3$



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Abstract

$C_6H_6N_2O \cdot C_7H_7NO_3$, monoclinic, $P2_1/n$ (no. 14), $a = 3.7957(3)$ Å, $b = 7.4395(5)$ Å, $c = 43.878(3)$ Å, $\beta = 91.181(3)^\circ$, $V = 1238.77(16)$ Å³, $Z = 4$, $R_{int}(I) = 0.0514$, $wR_{ref}(F^2) = 0.1138$, $T = 173$ K.

CCDC no.: 2451500

Keywords: crystal structure; co-crystal; isonicotinamide; 2-(nitrophenyl)methanol

Table 1: Data collection and handling.

Crystal:	Colorless needle
Size:	0.76 × 0.15 × 0.12 mm
Wavelength:	Mo K α radiation (0.71073 Å)
μ :	0.11 mm ⁻¹
Diffractometer, scan mode:	Bruker APEX3, ϕ and ω scans
θ_{max} , completeness:	27.6°, 100 %
$N(hkl)_{measured}$, $N(hkl)_{unique}$, R_{int} :	25907, 2837, 0.101
Criterion for $I_{obs} > 2 \sigma(I_{obs})$:	$I_{obs} > 2 \sigma(I_{obs})$, 2107
$N(parameters)_{refined}$:	193
Programs:	Bruker, ¹ SHELX, ^{2,3} WinGX, ^{4,5} Platon ⁶

The molecular structure is shown in the figure. Table 1 contains the crystallographic data. The list of the atoms including atomic coordinates and displacement parameters can be found in the cif-file attached to this article.

1 Discussion

Isonicotinamide, a pyridine-based compound with an amide group at the gamma-position, holds pharmaceutical significance due to its anti-tubercular, antipyretic, and antibacterial activities.¹ It readily forms co-crystals because of its ability to act as both a hydrogen bond donor and acceptor, and the robust heterosynthon formed between the pyridine nitrogen heteroatom and carboxylic acids and alcohols is well known in crystal engineering.² It is therefore often used in the formation of hydrogen-bonded networks with a wide range of pharmaceutical and functional molecules.³ The 2-(nitrophenyl)methanol has the possibility to be a good co-former in co-crystallization since it is able to engage in both hydrogen bonding through the hydroxyl group and π - π stacking

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Appendix B

A manuscript of Carbamazepine and 2-(nitrophenyl)methanol have been submitted for publication and, following reviewer corrections, is ready for resubmission (ACTA E, 2026).