**COCRYSTAL FORMATION BETWEEN DIDANOSINE AND TWO AROMATIC ACIDS**

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**ABSTRACT**

Objective: Cocystal is becoming attractive as solid form to be developed in the pharmaceutical industry. The formation of intermolecular bonds between active pharmaceutical ingredient (API) and coformer can change the physicochemical properties of an API without altering its pharmacological activity.

Methods: In this research, cocystal formation between an anti-HIV drug didanosine (DDI) and two aromatic acids (benzoic acid and salicylic acid) coformer in equimolar ratio have been prepared by solvent-drop grinding with methanol and acetonitrile as solvents. Cocystal formation of didanosine-benzoic acid (DDI-BA) and didanosine-salicylic acid (DDI-SAA) was characterized by powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), fourier transform infrared (FTIR) spectroscopy, and polarized microscopy methods.

Results: The PXRD patterns of DDI-BA and DDI-SAA after solvent-drop grinding are different from pure components. As the solvent-drop grinding proceed, the characteristic peaks of DDI in 6.1° and 10.7° 2θ disappeared, while new peaks appear at 5.0° and 10.1° 2θ in DDI-BA cocystal and 4.7° and 10.0° 2θ in DDI-SAA cocystal. In addition, physical characterization showed that DDI-BA and DDI-SAA cocystals after solvent-drop grinding have unique thermal, spectroscopic, and microscopic.

Conclusion: The characterization results indicate that didanosine form cocystals with benzoic acid and salicylic acid by solvent-drop grinding method with methanol and acetonitrile as solvents.

**Keywords:** Didanosine, Benzoic acid, Salicylic acid, Cocystal, Solvent-drop grinding.

**INTRODUCTION**

Solid forms of active pharmaceutical ingredient (API) can be classified into amorphous, polymorphs, solvates, hydrates, salts, and cocystals. Solid form design depends on the nature of the drug molecules and the types of physicochemical properties that will be faced in its development. Generally cocystals are defined as crystalline materials that contain two or more components that are solid at room temperature held together by non covalent forces such as hydrogen bonding, n-stacking, van der Waals forces, etc. [1]. In recent years, the formation of pharmaceutical cocystals has gained greater attention as a way to optimize the physicochemical properties of solid dosage forms [2]. Pharmaceutical cocystals may offer the potential improvement in solubility, dissolution rate, bioavailability, physical stability, chemical stability, compressibility, and hygroscopicity of an API [3]. Currently cocystal approach is a method that is very attractive to the pharmaceutical industry. Pharmaceutical companies are interested in cocystals for two main reasons. First, the physicochemical properties of active pharmaceutical ingredients can be modified while the pharmacological activities of these drug molecules remain the same. Second, the shelf life of APIs can be extended by using cocystals in pharmaceutical product [4,5]. As well as the formation of solid dispersions and inclusion complexes with cyclodextrin [6,7], cocystal formation can also increase the solubility and dissolution rate of an API.

Didanosine (2',3'-dideoxyninosine) is a synthetic analogue of deoxyribose nucleoside inosine has been reported to inhibit the replication of human immunodeficiency virus (HIV). Didanosine act as competitive inhibitors of reverse transcriptase. The drug is approved by the U.S. Food and Drug Administration for the treatment of adults and children over age above 6 months [8]. It has a hypoxanthine moiety bonded to a sugar ring. Like other glycosidic compounds, didanosine is subject to hydrolytic degradation in acidic solutions. Hypoxanthine is didanosine’s major degradation product [9]. Some didanosine properties are unfavorable from a pharmacokinetics point of view. These include the tendency of didanosine to hydrolyze at the stomach pH, its low oral bioavailability and very short biological half-life [10,11].

There are several approaches or tools to predicting the formation of cocystal between API and cocystal former molecule (coformer), such as solubility-based approach [12], Hansen solubility parameter tool [13], binary phase diagram using thermal analysis [14], computational the lattice energy landscape calculation [15], and supramolecular synthons-based approach [16,17]. Supramolecular synthon-based approach is the simple method to predicting cocystal formation. Supramolecular synthons are defined as structural units within supermolecules which can be formed and/or assembled by known or conceivable intermolecular interactions. Supramolecular synthons are categorized further into 2 classes (a) supramolecular homosynthons: composed of identical self-complementary functionalities, for example the interaction between carboxylic acid---carboxylic acid or amide---amide and (b) supramolecular heterosynthons: composed of different but complementary functionalities, for example the interaction between carboxylic acid---amide or carboxylic acid---pyridine [18].

Pharmaceutical cocystals are solid API forms constructed using a synthons-based design, wherein the API and a cocystal former molecule (coformer) are connected via strong supramolecular synthons [19]. Cocystal formation depends on the functional groups between API and coformer, to allow for the occurrence of hydrogen bonds or other forms of solid interaction. Crystal engineering is often based on surveying existing structures stored in the Cambridge Structural Database (CSD) to identify robust supramolecular synthons. CSD is an important tool in the field of crystal engineering. This database software can help to understand supramolecular synthons that can be formed between functional groups. The structure of didanosine is purine analogue. Its structure almost similar to xanthine derivative, such as caffeine and theophylline. It consists of a pyrimidine ring fused to an imidazole ring. Didanosine molecule has some hydrogen bond acceptors including an aromatic nitrogen (N==N) in the imidazole ring and a carbonyl group. To my knowledge, none of didanosine cocystal was contained in the CSD 2012. Carboxylic acids have hydrogen bond donors that are easy to participate in hydrogen bonding. In the CSD, molecules that mentioned above contain a type of hydrogen bond donor to interact with purine or xanthine derivative, such as caffeine and theophylline. There are many cocystals between
xanthine derivative and some carboxylic acids. Caffeine form cocrystal with benzoic acid [20], salicylic acid [21], and glutaric acid [22]. As well as caffeine, theophylline also formed cocrystal with benzoic acid [23] and salicylic acid [24]. Supramolecular heterosynthon of \( \text{N}^2\text{H}^+ - \text{COOH} \) have the greatest chance occurrence in the cocrystal between xanthine derivative and carboxylic acid. Based on the chemical structure, didanosine (Fig 1a) has a great chance to form cocrystal with benzoic acid (Fig 1b) and salicylic acid (Fig 1c). Benzoic acid and salicylic acid are safe chemical for human consumption selected from the GRAS (generally recognized as safe) list of the US FDA.

**Fig. 1:** Chemical structure of (a) didanosine, (b) benzoic acid, (c) salicylic acid

Based on the above, it is very important to prepare cocrystal between didanosine and two aromatic acids, such as benzoic acid and salicylic acid, so it can improve physicochemical properties of didanosine. The aim of this study was to prepare and characterize of didanosine-benzoic acid (DDI-BA) and didanosine-salicylic acid (DDI-SAA) cocrystals.

**MATERIAL AND METHODS**

**Materials**

Didanosine commercial material with purity of >99% was obtained from PT. Kimia Farma, tbk, Indonesia. Benzoic acid and salicylic acid were obtained from Wako Pure Chemical Industry Japan. Methanol, acetonitrile, and other reagents were purchased from Merck Chemicals without any purification.

**Preparation of cocrystal didanosine-benzoic acid and didanosine-salicylic acid by solvent-drop grinding**

Solvent drop grinding experiment was performed by combining equimolar ratios of didanosin and each coformer (benzoic acid and salicylic acid). Didanosine (118 mg, 0.5 mmol) and benzoic acid (61 mg, 0.5 mmol) or salicylic acid (69 mg, 0.5 mmol) was mixed in mortar, and 50 µL of solvent was added. Methanol and acetonitrile were used as solvents. The mixture was ground by variation of grinding time until completed cocrystallization. After grinding, the products were dried and stored at ambient temperature.

**Characterization by PXRD**

PXRD data were collected with Bruker D8 advance X-ray powder diffractometer with a LynxEye detector \((\text{Cu-K}α = 1.5406 \text{\AA})\). The X-ray tube was operated at 40 kV, 40 mA. Data collected at room temperature in the scan range of 20° to 45°, step size, 0.02° and scan speed, 1°/min. Sample powder was carefully placed into a glass holder, and the sample surface was flattened.

**Thermal Analysis by DSC**

Differential scanning calorimetry (DSC) was performed using Rigaku Thermo Plus Evo DSC 8230 Instrument (Japan). Five to ten mg of each sample was placed in crimped sample pan. The sample was heated from 30° to 300°C at a heating rate of 5°C/min under nitrogen purged.

**Characterization by FTIR**

Infrared spectra were recorded by using an FT IR-Affinity-1 spectrophotometer (DRS-8000) Shimadzu, Japan. The dried pure didanosine, benzoic acid, salicylic acid, and cocrystals samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 ratio of sample and KBr. The KBr powder was used as blank for background correction in FT-IR (DRS) studies. Forty five scans were obtained from 4000 to 400 cm\(^{-1}\).

**Characterization by polarized microscope**

One to two mg of physical mixture between didanosine and each coformer (benzoic acid and salicylic acid) was placed on object glass. A drop of methanol was added to each physical mixture until dissolved and allowed to recrystallize. Recrystallization process was observed under a polarizing microscope. The microscopic images were recorded with an Olympus SC-30 digital color camera attached to the Olympus BX-50 polarized microscope.

**RESULTS AND DISCUSSION**

**Preparation of DDI-BA and DDI-SAA cocrystals**

Many ways of producing cocrystals have been reported. In this research, to produce DDI-BA and DDI-SAA cocrystals was used solvent-drop grinding (also referred to liquid-assisted grinding, wet cocrinding) method. Solvent-drop grinding involves the grinding of two materials together and a small quantity of solvent [17]. This method only uses less solvent, so the method is more cost-effective and environmentally friendly than solution method [25,26]. This method is also reliable for the discovery of a new cocrystal. The presence of a small amount of the liquid phase can improve the rate of cocrystal formation [27]. The selection of solvent used in the solvent-drop grinding method is very important, where the solvent should be able to dissolve at least small portion of the parent’s components. Methanol and acetonitrile were used as liquid phase in preparation of DDI-BA and DDI-SAA cocrystals by solvent-drop grinding, because two coformers are soluble in these solvents, while the solubility of didanosine in methanol slightly better than in acetonitrile. In addition, methanol and acetonitrile are also volatile. Variation of grinding time is performed to determine the time required for the completed cocrystallization. During solvent-drop grinding process, the mixtures changed to amorphous phase and recrystallized rapidly. X-ray powder diffraction was used to monitor cocrystallization progress. Formation of intermediate phases such as amorphous phase during grinding process should exhibit enhanced mobility and/or higher energy of reactant molecules with respect to their starting crystalline forms [12,28]. The presence of methanol or acetonitrile as a liquid phase can increase the rate of cocrystal formation due to enhancement molecular diffusion.

**PXRD Pattern**

The formation of co-crystals is primarily characterized by powder X-ray diffractometer (PXRD). If the resulting PXRD pattern of the solid product after grinding pure solid compounds (API and coformer) is different from the reactants, it can be concluded that the new solid phase was formed [29]. The PXRD patterns for didanosine, benzoic acid, physical mixture of DDI-BA, and DDI-BA cocrystal are shown in Fig. 2a, whilst PXRD patterns for didanosine, salicylic acid, physical mixture of DDI-BA, and DDI-BA cocrystal are shown in Fig.2b. Didanosine has no polymorph form. PXRD pattern of didanosine matches with the pattern previously reported [30]. The diffractograms of the products after solvent-drop grinding are different from its physical mixture and starting components. The characteristic peaks of didanosine and benzoic acid were disappeared, whilst new peaks appear after solvent-drop grinding process. The changes in the position of the peaks after the solvent-drop grinding process indicate the formation of DDI-BA cocrystal in a 1:1 ratio. There was no difference in the PXRD pattern of cocrystal generated using methanol and acetonitrile solvents. As well as DDI-BA cocrystal, the position of peaks didanosine and salicylic acid changed after solvent-drop grinding process. This also indicates the formation of DDI-SAA cocrystal in a 1:1 molar ratio. Methanol and acetonitrile as solvents also showed the same PXRD pattern of cocrystal DDI-SAA. The peaks position of DDI-BA and DDI-SAA cocrystals are shown on the Table 1.
As previously mentioned, the selection of solvent used in the solvent-drop grinding method is very important, where the solvent should be able to dissolve at least small portion of the parent's components. The solvent here is used as a catalytic role, to enable the formation of cocrystals not obtained by neat grinding. The PXRD diffractograms of solvent-drop grinding results of didanosine and benzoic acid mixture after various grinding time using methanol and acetonitrile as solvents are show in the Fig. 3a and 3b. Solvent-drop grinding process caused intensity of characteristic peak of didanosine at 6.1° decreased, while intensity of the new peaks at 5.0° increased. Grinding time affect the completeness of cocrystal formation. All of the mixture of didanosine and benzoic acid formed cocrystal after grinding for 3 and 5 min with methanol and acetonitrile solvents, respectively. The perfection of DDI-BA cocrystal formation was characterized by the loss of didanosine peak at 6.1°. DDI-BA cocrystal formation by solvent-drop grinding method using methanol as solvent is faster than acetonitrile, because benzoic acid and didanosine more soluble in methanol than acetonitrile.

### Tabel 1: Main peaks of DDI-BA (1:1) and DDI-SAA (1:1) cocrystals compare to pure components

<table>
<thead>
<tr>
<th>2 Theta (°) position</th>
<th>DDI</th>
<th>BA</th>
<th>SAA</th>
<th>Cocystal DDI-BA</th>
<th>Cocystal DDI-SAA</th>
</tr>
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<tr>
<td>6.1</td>
<td>8.1</td>
<td>11.0</td>
<td>5.0</td>
<td></td>
<td>4.7</td>
</tr>
<tr>
<td>10.7</td>
<td>16.3</td>
<td>15.5</td>
<td>10.0</td>
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<td>10.0</td>
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<td>17.4</td>
<td></td>
<td>11.1</td>
</tr>
<tr>
<td>15.1</td>
<td>24.0</td>
<td>25.6</td>
<td>20.0</td>
<td></td>
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<tr>
<td>25.6</td>
<td>26.0</td>
<td>29.0</td>
<td>26.3</td>
<td></td>
<td>25.4</td>
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</table>

Fig. 3: Change in intensity of the peaks at 5.0° and 6.1° as solvent-drop grinding of equimolar of DDI-BA physical mixture with (a) methanol and (b) acetonitrile solvents
Fig. 4: Change in intensity of the peaks at 4.7° and 6.1° as solvent-drop grinding of equimolar of DDI-SAA physical mixture with (a) methanol and (b) acetonitrile solvents

The PXRD diffractograms of solvent-drop grinding results of didanosine and salicylic acid acid mixture after various grinding time using methanol and acetonitrile as solvents are show in the Fig. 4a and 4b. The characteristic peak of didanosine at 6.1° decreased, while intensity of the new peak at 4.7° increased. DDI-SAA cocrystal was obtained after solvent-drop grinding process for 5 and 7 min with methanol and acetonitrile solvents, respectively. The use of methanol as solvent in DDI-SAA cocrystal formation as well as in DDI-BA cocrystal formation was faster than acetonitrile, because the solubility of didanosine and salicylic acid in methanol is higher than acetonitrile.

**DSC Thermogram**

The melting point is a fundamental physical property, which is determined by the temperature at which the solid phase is at equilibrium with the liquid phase [31]. DSC is the preferred technique for obtaining comprehensive melting point data. As shown in Fig 5a, the melting point (T_peak) of pure didanosine was 186.6°C, allowed recrystallization become hypoxanthine at 191.9°C, whilst T_peak of benzoic acid was 134.4°C. T_peak of DDI-BA cocrystal was 118.0°C, which had lower than pure didanosine and benzoic acid. The difference of melting point of DDI-BA cocrystal from starting components is indicated that cocrystal was formed between didanosine and benzoic acid during the solvent-drop grinding. One endothermic peak at 127.8°C was caused by the fusion of benzoic acid sublimation result after the fusion of DDI-BA cocrystal. DSC analyses of DDI-SAA result after solvent-drop grinding are shown in Fig. 5b. DDI-SAA cocrystal exhibited a broad-single endothermic peak at 101.6°C. This endothermic peak was different from the melting points of either didanosine (186.6°C) and salicylic acid (168.3°C). This also indicates that cocrystal was formed between didanosine and salicylic acid during the solvent-drop grinding.

Fig. 5: DSC thermograms of (a) DDI-BA cocrystal, (b) DDI-SAA cocrystal after solvent-drop grinding compare to pure components
FTIR Spectra

Infrared spectroscopy can be a very powerful tool in detecting cocrystal formation, especially when a carboxylic acid is used as a coformer and/or when a neutral O-H···N hydrogen bond is formed between an acid and a base [31]. As shown in the Fig. 6a, didanosine have intense peak at 1717 cm⁻¹ due to C=O stretching. The hydrogen stretching region exhibits a broad band due to the –NH and –OH stretching band at 3400-3100 cm⁻¹. An O-H group of benzoic acid is present in the region 3100-2500 cm⁻¹, whilst intense peak at 1709 cm⁻¹ due to the C=O stretching. The C=O group stretching of didanosine and benzoic acid shift to 1713 cm⁻¹. The presence of shifting in the vibrational frequencies of didanosine and benzoic acid indicate the formation of supramolecular heterosynthon of the cocrystal. In Fig. 6b, salicylic acid have hydrogen-bonded hydroxyl group in the region 3500-2500 cm⁻¹ with strong peak at 3238 cm⁻¹. Similar to DDI-BA cocrystal, the shifting of carbonyl group also occurred in the DDI-SAA cocrystal. The intense peaks of carbonyl group of salicylic acid shift from 1676 cm⁻¹ to 1667 cm⁻¹, while intense peaks of carbonyl group of didanosine shift from 1717 cm⁻¹ to 1713 cm⁻¹. This also indicates the formation of supramolecular heterosynthon of the DDI-SAA cocrystal.

![FTIR spectra of (a) DDI-BA cocrystal, (b) DDI-SAA cocrystal after solvent-drop grinding compare to pure components](image)

Polarized microscope photo

The cocrystallization process of DDI-BA and DDI-SAA was observed under polarized microscope after addition a drop of methanol. As shown in Fig. 7a and 7b, both of DDI-BA and DDI-SAA cocrystals have different crystal habit from its physical mixtures. This indicates the formation of DDI-BA and DDI-SAA cocrystals after the addition of methanol solvent.

![Polarized microscopy photo of (a) DDI-BA physical mixture and cocrystal, (b) DDI-SAA physical mixture and cocrystal](image)
CONCLUSION
Cocrystals formation between didanosine and two aromatic acids (benzoic acid and salicylic acid) have been characterized by PXRD, DSC, and FTIR. The PXRD patterns shown DDI-BA (1:1) and DDI-SAA (1:1) cocrystal are different from its physical mixtures and starting components. DSC thermograms of DDI-BA (1:1) and DDI-SAA (1:1) cocrystals have unique thermal behavior, which had lower than each pure component. FTIR spectra show a shift of the carboxyl and hydroxyl groups of coformers due to supramolecular heterosynth with N\textsubscript{2} of imidazole ring of didanosine. The crystal habit of DDI-BA and DDI-SAA are different from its physical mixtures. This study establishes that didanosine form cocrystals with benzoic acid and salicylic acid by solvent-drop grinding method with methanol and acetonitrile as solvents.

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