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In Silico Coformer Screening for Mefenamic Acid Cocrystallization

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Abstract
Cocrystallization is a widely used approach to enhance the solubility and dissolution characteristics of poorly soluble drugs. A pharmaceutical cocrystal is a multicomponent system composed of a solid active pharmaceutical ingredient (API) and a coformer, governed by non-covalent interactions. Screening for suitable coformers is essential to obtain an optimal cocrystal for specific drugs. This study aims to determine the drug-coformer interactions to select the most suitable coformer for cocrystal formation using the molecular docking method. Mefenamic acid, classified as a class II drug in the biopharmaceutical classification system (BCS), was used as the model drug. Two-dimensional structures of mefenamic acid (PubChem CID: 4044) and potential coformers were sourced from PubChem. Geometric optimization of all compounds was performed using GaussView 5.0.8 and Gaussian09 with the 3-21G basis set and Density Functional Theory (DFT) B3LYP method. The optimized compounds were prepared by adding hydrogen atoms and calculating Kollman partial charges using AutoDock 4.2. A grid box of size 40 Å × 40 Å × 40 Å was generated, with a maximum radius of 0.375 Å set as the surface distance in each simulation. A hundred conformations were run using the Lamarckian Genetic Algorithm. Interaction types and binding energies were analyzed using VMD 1.9.2 and BIOVIA Discovery Studio 2020 to compare interactions between mefenamic acid and each coformer. The results revealed that most coformer compounds formed interactions with mefenamic acid via hydrogen bonding and π–interactions. Saccharin demonstrated the most optimal interaction with mefenamic acid, with a binding free energy of –3.1 kcal/mol. Saccharin was identified as the most suitable coformer for mefenamic acid cocrystal formation based on the molecular docking study. Further experimental validation of saccharin is recommended to confirm its effectiveness in cocrystallization with mefenamic acid.

Keywords: Cocrystal, Coformer, Screening, Molecular Docking, Mefenamic Acid.

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1. INTRODUCTION

To achieve optimal efficacy, solid active pharmaceutical ingredients (APIs) need to have certain aqueous solubility to ensure absorption through gastrointestinal fluid. However, despite their abundance and various pharmacological activities, solid crystalline APIs are still suffering from limited solubility (Bhandaru et al., 2015). Several drug modifications, such as by formation of different polymorphs, inclusion complexes, and cocrystals, have been extensively studied to overcome the physicochemical limitations of the drug in providing therapeutic effects (Censi & Di Martino, 2015; Putra et al., 2018).

A pharmaceutical cocrystal is a stoichiometrically defined multicomponent crystalline structure consisted of an API and a cocrystal former (coformer) (Karimi-Jafari et al., 2018). Several aspects, i.e., physical form, molecule size, functional group types, and "generally recognized as safe (GRAS)" status must be considered when using a compound as a coformer (Singh et al., 2023). From previous works, it is shown that several types of acids such as ascorbic acid, benzoic acid, citric acid, fumaric acid, stearic acid, and succinic acid, as well as nicotinamide and saccharin, were universally acknowledged as potential coformers in cocrystal formation (Wouters et al., 2011).

Several types of non-covalent intermolecular interaction, such as hydrogen bonding, halogen bonding, van der Waals force, or π-interaction may govern the binding between drug and coformer molecules (Berry & Steed, 2017). Among those interactions, hydrogen bonding is viewed as primary interaction in the cocrystal formation. Thus, to perform cocrystallization, both drug and coformer molecules must contain hydrogen bond donor or hydrogen bond acceptor sites (Li et al., 2018).

In the development of pharmaceutical cocrystal, selection of suitable coformer(s) is an important preliminary step to predict cocrystallization ability of drug-coformer, and subsequently obtaining cocrystal with better properties and performance compared to the parent drug (Mangesh et al., 2019). Both experimental approaches and computational approaches have been employed in coformer screening for designing cocrystals (Musumeci et al., 2011). Computational-based screenings are considered more favorable in terms of efficiency and cost-perspective. Several computational methods, such as crystal structure prediction using Cambridge Structural Database (CSD), conductor-like screening model for real solvents using COSMO-RS, molecular electrostatic potential surfaces (MEPS), lattice energy calculations, Hansen solubility parameters, and molecular docking-based screenings have been able to effectively predict the formation of cocrystal (Khalaji et al., 2021; Kumar & Nanda, 2021; Mohammad et al., 2011; Siswandi et al., 2015).

In this work, we use mefenamic acid (2-[(2,3-dimethylphenyl)amino]benzoic acid) as model drug for cocrystal formation. Mefenamic acid is a nonsteroidal anti-inflammatory drug (NSAID) with various pharmacological activities, including analgesic and antipyretic (Cimolai, 2013). According to the biopharmaceutical classification system (BCS), mefenamic acid is included in class II due to low solubility–high permeability characteristic (Nurhikmah et al., 2016). Mefenamic acid modification toward cocrystal form is a viable option to enhance its solubility and dissolution rate (Utami et al., 2017). The purpose of this study is to explore the interaction between mefenamic acid and several compounds as coformer candidates using molecular docking approach. Binding affinities of drug and coformer molecules were determined based on calculated interaction energy.

2. RESEARCH METHOD

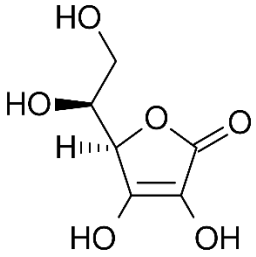
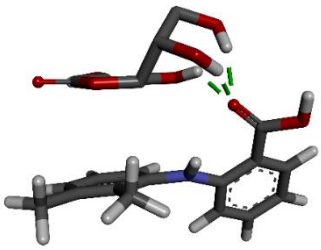
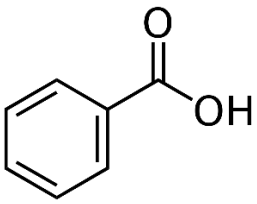
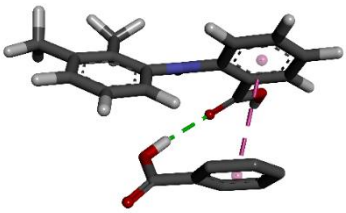
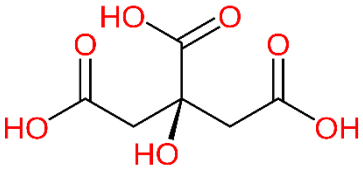
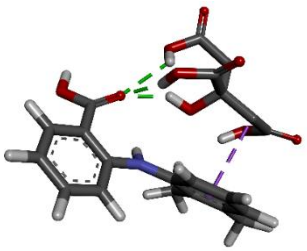
The simulation in this study was performed using 6-cores (@4.30 GHz) computing unit runs on dual operating systems (Windows 10 and Linux Ubuntu 18.10). Two-dimensional structures of mefenamic acid (PubChem CID: 4044) and the coformers were acquired from

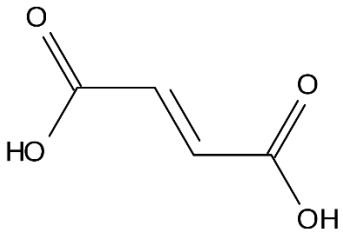
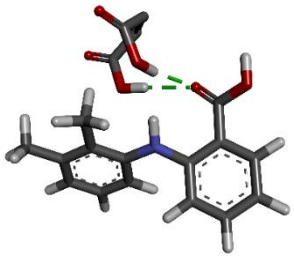
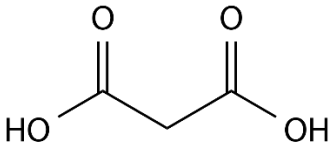
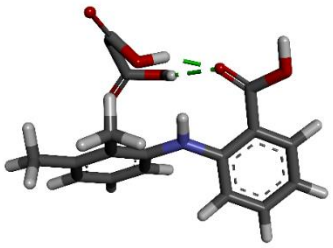
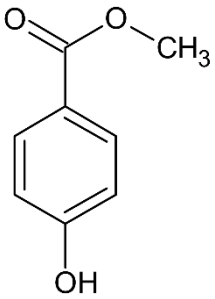
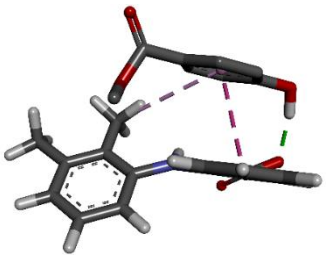
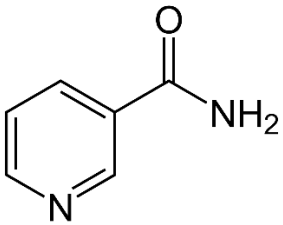
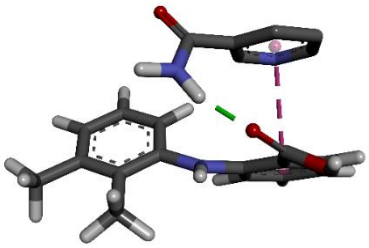
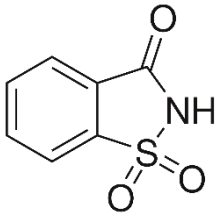
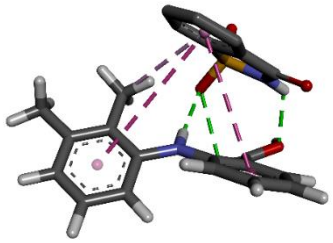
pubchem.ncbi.nlm.nih.gov. All compounds were geometrically optimized using GaussView 5.0.8 and Gaussian09 with the 3-21G base set Density Functional Theory (DFT) B3LYP method. Optimized compound files were then prepared by inserting hydrogen atoms and calculating Kollman partial charges using AutoDock 4.2. Grid box with the size of 40 Å × 40 Å × 40 Å was generated, and maximum radius of 0.375 Å was set as surface distance in each simulation. A hundred conformations were run using Lamarckian Genetic Algorithm throughout the process. Interaction type and binding energy of interaction were observed using VMD 1.9.2 and BIOVIA Discovery Studio 2020 to compare the interaction between mefenamic acid and each coformers.

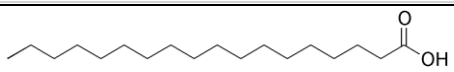
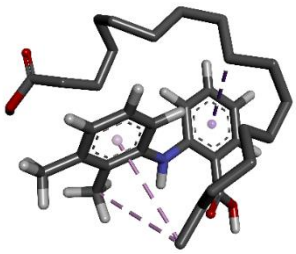
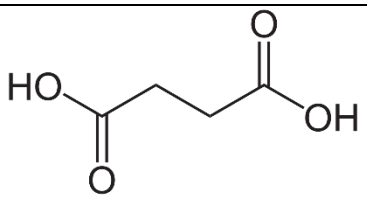
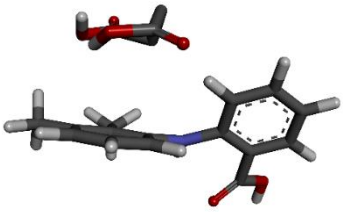
3. RESULTS AND DISCUSSION

The two-dimensional structure of coformers and visualized interaction types of mefenamic acid-coformer interaction from molecular docking simulation are presented in Table 1.

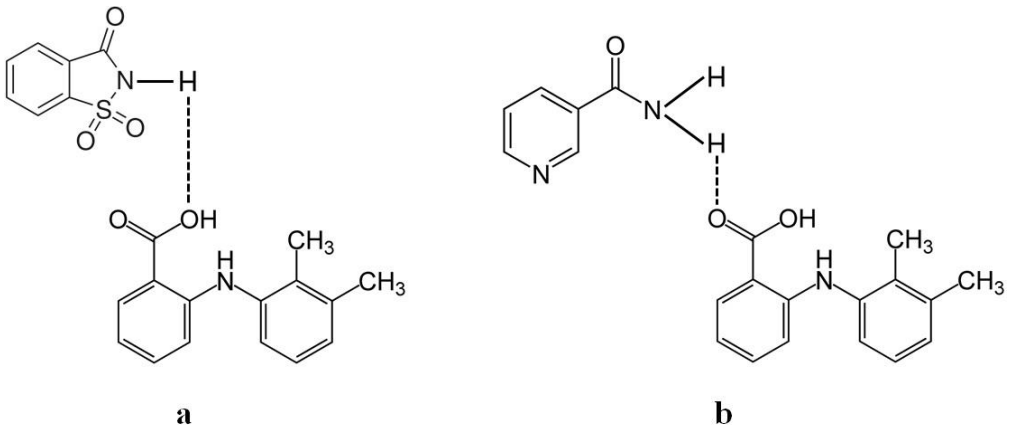
Table 1. Interaction types of mefenamic acid-coformer based on molecular docking result

Coformer	2D Structure	Interaction
Ascorbic Acid		 Hydrogen bond
Benzoic Acid		 Hydrogen bond π– π interaction
Citric Acid		 Hydrogen bond π– σ interaction

Fumaric Acid		 Hydrogen bond
Malonic Acid		 Hydrogen bond
Methylparaben		 Hydrogen bond π– π interaction
Nicotinamide		 Hydrogen bond π– π interaction
Saccharin		 Hydrogen bond π– π interaction

Stearic Acid		 π– σ interaction
Succinic Acid		 no interactions

Mefenamic acid contains two hydrogen bond donors (hydrogen atoms in –COOH and –NH), and three hydrogen bond acceptors (oxygen atoms in –COOH and nitrogen atom in –NH) (Gajjar et al., 2013). Intermolecular hydrogen bonding between mefenamic acid molecules and coformer molecules may be identified in these sites. According to the molecular docking result, it is observed that the majority of the compounds, with the exception of stearic acid and succinic acid, were able to generate hydrogen bonding interaction with mefenamic acid. In conventional synthesis, the presence of hydrogen bond reflects the stability of the cocrystal due to the strong complementary pairing of hydrogen bond donor and acceptor moieties (Taylor & Day, 2018). Thus, it is more favorable for the cocrystal system to have such interaction.



a **b**

Figure 2. Predicted hydrogen bonding of mefenamic acid-saccharin (a) and mefenamic acid-nicotinamide (b) based on molecular docking result.

Figure 2 illustrates a closer inspection of hydrogen bonding in mefenamic acid-saccharin and mefenamic acid-nicotinamide based on molecular docking prediction. It is shown that the hydrogen bond in both systems have resulted from amide-carboxylic acid (N–H···O) interaction. The acid-amide heterosynthon is considered the most commonly recognized hydrogen bonding interaction in cocrystal formation (Saha & Desiraju, 2018). Furthermore, it has been argued that the strength and high-directiveness of N–H···O may lead to a robust synthon, hence increasing the probability of stable cocrystal structure (Vener et al., 2014).

The presence of two aromatic rings in mefenamic acid structure is also worth pointing out, since these rings are responsible for intermolecular π-interactions between drug and the coformer. From geometrical perspective, aromatic rings can form interaction through edge-to-face (T-shaped), face-to-face stacked (sandwich), or parallel-displaced stacked motifs (Bora et al., 2018). The latter motif is considered the most energetically favorable, since its orientation enables attractive interaction between positively charged σ-bond with negatively charged π-electron density. While face-to-face motif considered unfavorable due to π–π repulsive interaction (Grimme, 2008). From docking results, it is indicated that π–σ interactions were present between mefenamic acid with citric acid and stearic acid. While π–π interactions were occurred between mefenamic acid with benzoic acid, methylparaben, nicotinamide, and saccharin. Both T-shaped and parallel-displaced π–π motifs were depicted solely on the interaction between mefenamic acid with saccharin, implying the more energetically favorable conformation. The energetic favorability of a system directly represents the spontaneity of a process (Sarcevica et al., 2013).

Table 2. Interaction energy of mefenamic acid-coformer based on molecular docking result

Coformer	ΔG (kcal/mol)	$E_{inter-mol}$ (kcal/mol)	E_{VHD} (kcal/mol)	E_{elec} (kcal/mol)	E_{total} (kcal/mol)	$E_{torsional}$ (kcal/mol)
Ascorbic Acid	–1.98	–3.77	–3.18	–0.60	–2.50	+1.79
Benzoic Acid	–1.38	–1.97	–2.36	+0.39	–0.02	+0.60
Citric Acid	+1.16	–1.53	–2.69	+1.17	–2.72	+2.68
Fumaric Acid	+0.07	–1.12	–1.96	+0.84	–0.33	+1.19
Malonic Acid	+0.21	–0.98	–1.98	+1.00	+0.05	+1.19
Methylparaben	–2.27	–3.16	–2.90	–0.26	–0.12	+0.89
Nicotinamide	–2.38	–2.68	–2.41	–0.26	+0.01	+0.30
Saccharin	–3.10	–3.10	–3.05	–0.05	–0.00	–0.00
Stearic Acid	+1.15	–3.92	–4.00	+0.08	–0.80	+5.07
Succinic Acid	+0.55	–0.95	–1.20	+0.25	–0.68	+1.49

Table 2 depicts the energies of involved interaction in mefenamic acid-coformer systems. ΔG estimated the free energy of binding, $E_{inter-mol}$ is the final intermolecular energy (E_{VHD} + E_{elec}), E_{VHD} (van der Waals + hydrogen bonding + desolvation energy), E_{elec} is the electrostatic energy, E_{total} is the final total internal energy, and $E_{torsional}$ is the torsional free energy. It is observed that the intermolecular interaction between mefenamic acid and coformer compound is dominated by E_{VHD}, signifying the contribution of non-covalent van der Waals and hydrogen bonding to the formation of cocrystal (Liu et al., 2018). Additionally, a more negative ΔG value indicates a more energetically favorable system, which subsequently demonstrates a more spontaneous process due to lower energy of formation (Zhang et al., 2017). Analysis of binding free energy also offers a quantitative approximation toward the stability of a thermodynamic system. This implies a direct relationship between the spontaneity of the process and the stability of the structure (Tahir et al., 2019). It can be seen that mefenamic acid interaction with saccharin generates the lowest binding free energy (–3.10 kcal/mol), thus offering the possibility of a stable cocrystal formation.

4. CONCLUSION

Coformer screening for mefenamic acid cocrystallization has been investigated using molecular docking method. From docking result, it is revealed that the majority of coformer

candidates were able to form interaction with mefenamic acid via hydrogen bonding and π–interaction. Based on calculated binding energy, saccharin (–3.1 kcal/mol) had the most optimal binding affinity with mefenamic acid, indicating the possibility of stable cocrystal formation. Further experimental studies may be performed to confirm the result and reveal the ability of coformer candidates in the formation of cocrystal with mefenamic acid.

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