



Preparation and Study on the Single-Crystal Structure of Flurbiprofen–Salicylamide Co-crystal

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Abstract

Keywords

- ▶ flurbiprofen
- ▶ salicylamide
- ▶ co-crystals
- ▶ density functional theory

Co-crystals can improve the physicochemical properties of pharmaceutical active ingredients, and thus have great potential in improving drug performance. Some studies have obtained the powder spectrum of the co-crystal of flurbiprofen (FBP) and salicylamide (2-OHBZA) but have not obtained a single-crystal structure. In this study, co-crystals of FBP-2-OHBZA with a molar ratio of 1:1 were obtained by slow evaporation using ethyl acetate and tetrahydrofuran for verification. Density functional theory was used to optimize the structure of the hydrogen bond and π – π stacking formed by the co-crystal of FBP and 2-OHBZA. The assay results of the co-crystals of FBP-2-OHBZA are in good agreement with the calculated values reported.

Introduction

The co-crystal strategy remains an appealing means to modify the physical and chemical properties of an active pharmaceutical ingredient (API). API combined with an appropriate conformer results in the formation of an API co-crystal that may demonstrate significant potential in improving solubility, stability, and bioavailability over the API itself. Flurbiprofen (FBP) is widely used as one of the nonsteroidal anti-inflammatory drugs for the treatment of mild to moderate pain, rheumatoid arthritis, and osteoarthritis.¹ Unfortunately, the therapeutic application and efficacy of the drug are limited due to its low solubility. Although much research including solid dispersion,² nanosuspension,³

etc., has been performed to solve this problem, the co-crystal strategy of the drug remained largely unknown.

Density functional theory (DFT) is a method for studying the electronic structure of multi-electron systems. It is widely used in physics and chemistry, especially to study the properties of molecules, and is one of the most commonly used methods in the fields of materials science and computational chemistry.⁴ Reduced density gradient (RDG) calculation results show noncovalent interactions, which are a way of visually understanding noncovalent interactions and a powerful tool to reveal noncovalent interactions, such as hydrogen bonding and electrostatic and van der Waals (vdW) interactions.⁵ Hirshfeld surface (HS) analysis and fingerprinting are powerful tools for analyzing molecular environments from different perspectives, interpreting and comparing molecular interactions. The sign of the electrostatic potentials (ESPs) in a particular region around a

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molecule is key to assessing its reaction properties. Regions which are positive are those to which electrophiles are initially attracted, in particular to the negative of the most negative potentials. There are molecular features: (1) long pairs of atoms such as O, N, F, S, P, Cl, Se, and Br; (2) pi regions, such as those found in the above-mentioned atoms; and (3) strained carbon–carbon bonds.^{6,7}

Surov et al attempted FBP co-crystallizations and reported FBP co-crystals with three amide compounds (including salicylic amide) through thermodynamic analysis of binary phase diagram.⁸ The co-crystals of FBP and salicylamide (2-OHBZA) were prepared through drop milling experiments assisted by ethanol solvents, but no single crystal was obtained. Based on high-resolution synchrotron radiation PXRD (powder X-ray diffraction), the eutectic structure prepared by the grinding method is determined and the TOPAS software is used to refine and solve the structure. This method is effective to obtain a rough structure when a single-crystal structure cannot be obtained. However, the workload is huge, with high professional requirements and equipment requirements. Surov et al also determined the crystal structure of the co-crystal by calculation model and data refinement. The relationship between the change of Gibbs free energy and the molecular volume of the co-crystal was further observed and discussed. Surov et al mainly studied lattice energy, solubility product, and binary phase diagram. However, single crystals have not been cultivated through experiments, and the solid-state characterization is incomplete. Thus, this experiment mainly focuses on the co-crystal of FBP-2-OHBZA obtained by evaporation crystallization. The solid-state characterization data of the FBP-2-OHBZA co-crystal were supplemented, and the single-crystal data obtained by the experiment were used for verification.

Materials and Methods

Materials

FBP and 2-OHBZA were provided by manufacturer Adamas Reagent Co., Ltd. Organic reagents were purchased from Sinopharm Group Co., Ltd (Shanghai, China). All chemicals were of reagent grade and used without further purification.

Preparation of Samples

The solubilities of FBP and 2-OHBZA were approximately obtained in solvent systems, such as ethanol, methanol, acetone, tetrahydrofuran, acetonitrile, ethyl acetate, dichloromethane, diethyl ether, and *n*-butanol. Although both molecules have a similar solubility in the above solvent systems, their 1:1 co-crystal was obtained only in ethyl acetate and tetrahydrofuran. Moreover, the co-crystal with the appropriate size for single-crystal X-ray diffraction (SXR) was only obtained from ethyl acetate. The single-crystal cultivation process is described as follows. Equimolar amounts of FBP and 2-OHBZA were weighed on an evaporating dish. Tetrahydrofuran/ethyl acetate was added to dissolve the solid components. The mixture was stirred on a magnetic stirrer at room temperature and evaporated to dryness. The solid powder obtained was then dried under

reduced pressure at 30°C for 4 hours. 10 to 20 mg of the above eutectic powder was reconstituted in 0.6 mL ethyl acetate. After stirring, the mixture was filtered through a filter head with a pore size of 0.45 μm into a 2 mL glass vial, which had been scrapped a few times on the vial wall with a steel spoon. The glass vial was sealed with parafilm and left standing still at room temperature for single-crystal cultivation. In the process, the sealing film was pierced with a few holes for a more controlled solvent volatilization rate. Finally, the FBP-2-OHBZA co-crystal of sufficient size was harvested. Several small blocks of single crystals were ground to powder.

Single-Crystal X-Ray Diffraction

SXR on a Bruker smart CCD diffractometer was measured at room temperature with Cu Kα radiation ($\lambda = 1.54178 \text{ \AA}$), in ϕ/ω scan mode, in the angular range of 3.13 to 69.99° for data collection. They were refined by full-matrix least-squares procedures by using SHELXL⁹ on F^2 with anisotropic displacement parameters for nonhydrogen atoms on the Olex2 crystallography software platform.¹⁰ The hydrogen atoms of the hydroxyl and amino groups were localized by differential Fourier transform and corrected using the isotropic temperature factor. All other hydrogen atoms are placed in geometrically idealized positions and constrained to their parent atoms. The simulated PXRD patterns were calculated using Mercury software (version 2020.1, Cambridge Crystallographic Data Center, United Kingdom)¹¹ with a starting angle of 3° and a final angle of 45° with a step size of 0.02°.

Powder X-Ray Diffraction

Part of the single-crystal sample was ground into powder and passed through a 100-mesh sieve to meet the analysis requirements. Appropriate amounts were subjected to PXRD test under the following conditions: Cu Kα radiation ($\lambda = 1.54178 \text{ \AA}$; Bruker D8 advance), voltage 40 kV, current 40 mA, test angle range 3° to 45°, step size 0.02°, scan speed 16°/min.

Computational Studies

HS analysis was performed using the CrystalExplorer 17 program¹² to display HS and intermolecular interactions in molecular crystals. The program accepts a structure input file in the .cif format.

ESP analysis was explored with DFT calculations. The structure was drawn on Gaussian, and then selected the function “Optimization and Frequency” for optimization. The most powerful conformation is found to be consistent with the single-crystal structure. The B3LYP/6–311G* functional was employed for molecular ESP energy analysis.

The B3LYP/6–311G* level was employed for RDG analysis. The Gaussian 16 package was utilized for all calculations.¹³ The Multiwfn 3.6 program was employed for all wave function analyses.¹⁴

Results and Discussion

SXR Analysis

The appearance of FBP-2-OHBZA crystal is colorless and transparent oblique prism crystal. The crystal structure

was analyzed using Olex2 software. The analytical results show that the crystal belongs to the triclinic crystal system, the space group is P-1, and with the unit cell parameters: $a = 6.0370(1) \text{ \AA}$, $b = 10.9021(2) \text{ \AA}$, $c = 14.8953(3) \text{ \AA}$, $\alpha = 72.305(1)^\circ$, $\beta = 84.163(1)^\circ$, $\gamma = 89.023(1)^\circ$; unit cell volume $V = 929.02(3) \text{ \AA}^3$, number of molecules in unit cell $Z = 2$. The least squares method was used to modify the structural parameters and identify the atomic species, and the geometric calculation method and the difference Fourier method were used to obtain the positions of all hydrogen atoms. The final reliability factors were $R_1 = 0.0388$, $wR_2 = 0.1112$, and $S = 1.037$.

The crystallographic data and hydrogen bond list of the FBP-2-OHBZA co-crystal are shown in ►Tables 1 and 2, the ellipsoid diagram of the asymmetric unit is shown in ►Fig. 1A, and the unit cell packing diagram is shown in ►Fig. 1B. The results show that the FBP-2-OHBZA co-crystal is composed of one molecule of FBP and one molecule of 2-OHBZA as an asymmetric unit. The molecular arrangement in the crystalline state belongs to the second space group, and the crystal has no optical activity. In the asymmetric unit, two pairs of intermolecular hydrogen bonds are formed between the carboxyl group of FBP and the amide of 2-OHBZA. At the same time, the hydroxyl group in the 2-OHBZA molecule and the carbonyl group of the amide bond form a pair of intramolecular hydrogen bonds, see ►Fig. 1A for details.

There are three benzene ring structures in the smallest asymmetric unit of FBP-2-OHBZA (►Fig. 1C). May be there are also π – π stacking interactions, as shown in ►Fig. 1C. Interestingly, the benzene ring stacking of 2-OHBZA are π – π stacking between two molecules a and b, the centroid distance is 3.827 \AA , and the slip angle is 48° . There is no obvious π – π stacking interaction between b and c, the centroid distance is 8.958 \AA , and the slip angle is 0.99° , which is close to a plane. Discontinuous accumulation of π – π stacking is uncommon. The center-of-mass distance between the two molecules of the FBP benzene ring is 6.037 \AA , and the slip angle is 64° , so there is no obvious π – π stacking interaction between the molecules.

Surov et al obtained many possible results using the synchrotron radiation technique, used differential scanning calorimetry to establish a two-dimensional phase diagram, and compared the differences in melting points to determine that the eutectic stoichiometric ratio is 1:1. To validate the crystal structures of the FBP co-crystals determined from the PXRD data, Surov et al performed the relaxation of the positions of all the atoms with fixed unit cell parameters and calculated the crystal lattice energy. The final Rietveld

Table 1 Crystallographic data of FBP-2-OHBZA co-crystal

	Co-crystal FBP-2-OHBZA
Empirical formula	$C_{22}H_{20}FNO_4$
Formula weight	381.39
Temperature	296(2) K
Wavelength	1.54178 \AA
Crystal system, space group	Triclinic, P-1
Unit cell dimensions	$a = 6.0370(1) \text{ \AA}$; $\alpha = 72.305(1) \text{ deg.}$ $b = 10.9021(2) \text{ \AA}$; $\beta = 84.163(1) \text{ deg.}$ $c = 14.8953(3) \text{ \AA}$; $\gamma = 89.023(1) \text{ deg.}$
Volume	$929.02(3) \text{ \AA}^3$
Z, calculated density	2, 1.363 Mg/m ³
Absorption coefficient	0.835 mm^{-1}
F(000)	400
Crystal size	0.40 mm × 0.10 mm × 0.02 mm
Theta range for data collection	3.13 to 69.90 deg.
Limiting indices	$-6 \leq h \leq 7$, $-12 \leq k \leq 13$, $-17 \leq l \leq 17$
Reflections collected/unique	9648/3190 [$R(\text{int}) = 0.0230$]
Completeness to theta = 68.90	92.7%
Max. and min. transmission	0.9835 and 0.7312
Refinement method	Full-matrix least-square on F^2
Data/restraints/parameters	3190/0/270
Goodness-of-fit on F^2	1.037
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0388$, $wR_2 = 0.1079$
R indices (all data)	$R_1 = 0.0419$, $wR_2 = 0.1112$
Extinction coefficient	0.0055(8)
Largest diff., peak, and hole	0.280 and $-0.167 \text{ e. \AA}^{-3}$

refinement showed good agreement with the simulated and measured PXRD patterns. In this experiment, the stoichiometric ratio of FBP-2-OHBZA was directly obtained by SXRD and was 1:1. The unit cell parameters were compared in ►Table 3. SXRD is the most accurate and convenient method to obtain the absolute configuration. The acquisition

Table 2 Hydrogen bond data of FBP-2-OHBZA co-crystal

D–H...A	$d(D\cdots H)/\text{\AA}$	$d(H\cdots A)/\text{\AA}$	$d(D\cdots A)/\text{\AA}$	$\angle(D-H\cdots A)^\circ$
O(2)–H(2A) ...O(3)	0.90(3)	1.75(3)	2.6346(16)	167(2)
O(4)–H(4A) ...O(3)	0.91(3)	1.75(3)	2.5651(17)	148(2)
N(1)–H(1A) ...O(1)	0.88(2)	2.19(3)	3.062(2)	171.1(19)

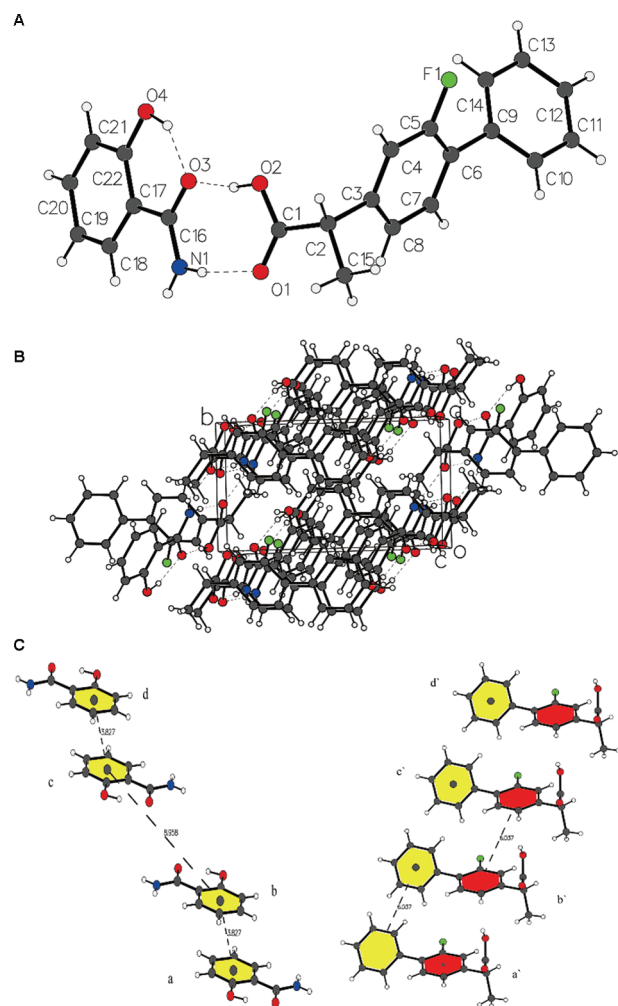


Fig. 1 (A) Ellipsoidal diagram of FBP-2-OHBZA co-crystal molecules; (B) FBP-2-OHBZA packing of co-crystal structures; (C) the stacking interactions of 2-OHBZA and FBP molecules.

of single-crystal data helped make us understand the bonding mode of eutectics more clearly. Comparing the unit cell parameters and hydrogen bonding modes obtained by the two methods, we can see that the unit cell parameter and hydrogen bonding results obtained by the high-resolution radiation technique are consistent with the experimental results. However, the workload of this study is much smaller than Surov et al's.

PXRD Analysis

The simulated standard spectrum of FBP-2-OHBZA and the measured spectrum of the eutectic sample PXRD are displayed in ►Fig. 2. The results show that the co-crystal sample prepared by the solvent evaporation method has high purity,

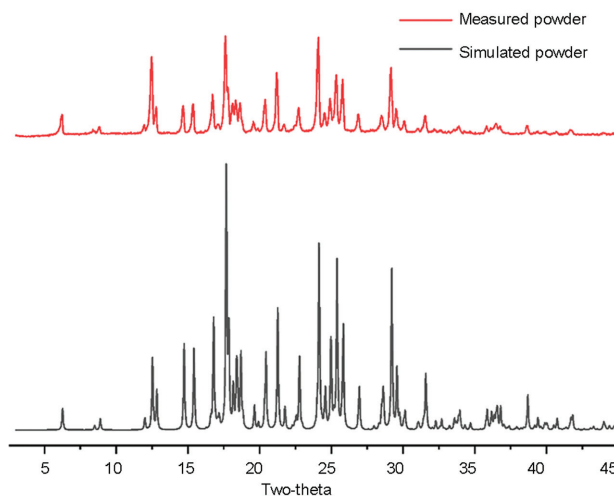


Fig. 2 PXRD patterns of single crystal simulated and measured. PXRD, powder X-ray diffraction.

and its PXRD pattern is consistent with the single-crystal simulation pattern.

Theoretical Calculation

HS Analysis

HS (mapped with dnorm) provides a three-dimensional image of close contacts in crystals in ►Fig. 3. Fingerprints summarize information about intermolecular interactions in ►Fig. 4, including the plot of d_i versus d_e , where d_i is the distance to the nearest atomic center inside the surface, d_e is the distance to the nearest atomic center outside the surface, and $dnorm$ is the normalized contact distance. It is based on d_e and d_i and the vdW radius of the atom, d_e and d_i reflect the distance from the surface to the nearest outer and inner atoms, respectively. The percentage contribution of the close contacts of molecules to the HS area is given using pie charts in ►Fig. 5. The HS analysis uses FBP as the main molecule and 2-OHBZA as the main molecule, respectively.

Surface mapping for d_e used the ranges 0.6 Å (red) and 2.6 Å (blue). Large circular depressions (dark red) visible on the molecular surfaces indicate hydrogen bonding contacts. ►Fig. 3 shows the different HS in the co-crystal FBP-2-OHBZA. Each molecule of FBP and 2-OHBZA contains three red sunken circles, indicating that they both have three hydrogen bonding sites. Typically, in a two-dimensional fingerprint in ►Fig. 4, the upper peaks ($d_e > d_i$) correspond to hydrogen bond donors, and the lower peaks ($d_e < d_i$) correspond to hydrogen bond acceptors. In general, the spikes flanking each plot are due to dimeric H...O hydrogen bonds. All interactions in ►Fig. 3 show two sharp peaks,

Table 3 Obtained unit cell parameters by two methods

Method	<i>a</i> (Å)	<i>b</i> (Å)	<i>c</i> (Å)	α (deg)	β (deg)	γ (deg)	<i>V</i> (Å ³)
1	6.0370(1)	10.9021(2)	14.8953(3)	72.305(1)	84.163(1)	89.023(1)	929.02(3)
2	6.0366(2)	10.9003(4)	14.8907(5)	72.305(2)	84.124(3)	88.989(2)	928.46(6)

Note: Method 1 represents single-crystal data obtained in this work; method 2 represents single-crystal data obtained by synchrotron radiation.

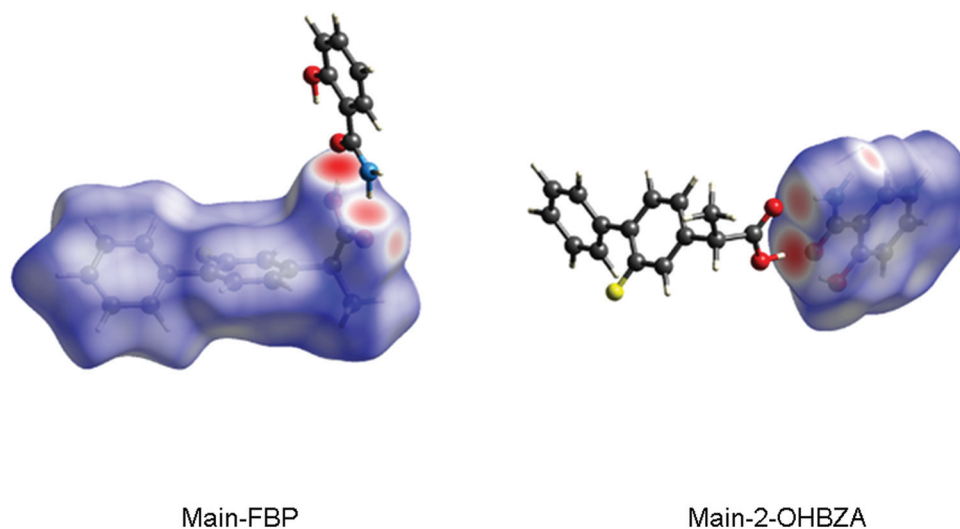


Fig. 3 Hirshfeld surface (mapped with dnorm).

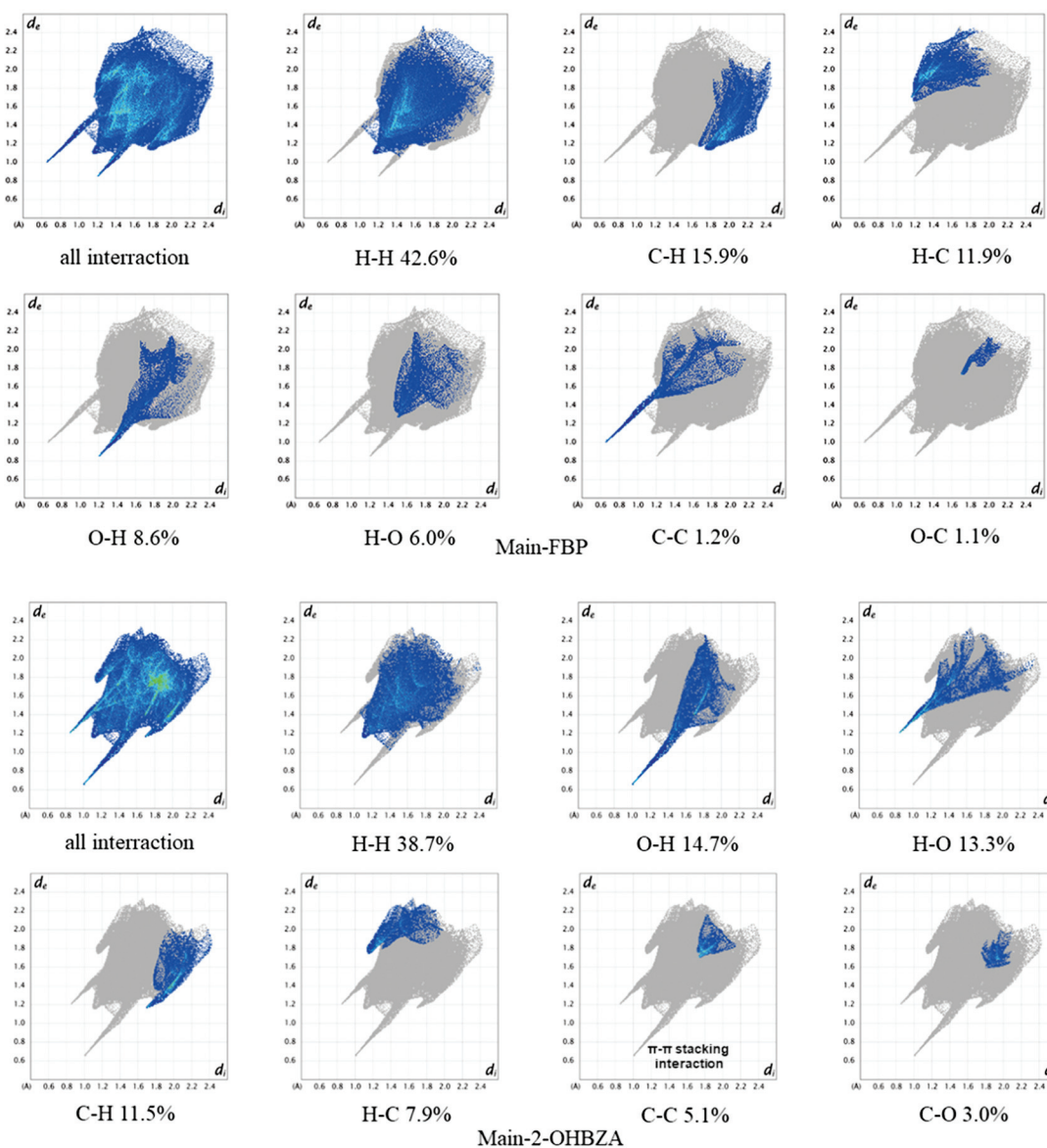


Fig. 4 Fingerprint plots for FBP and 2-OHBZA.

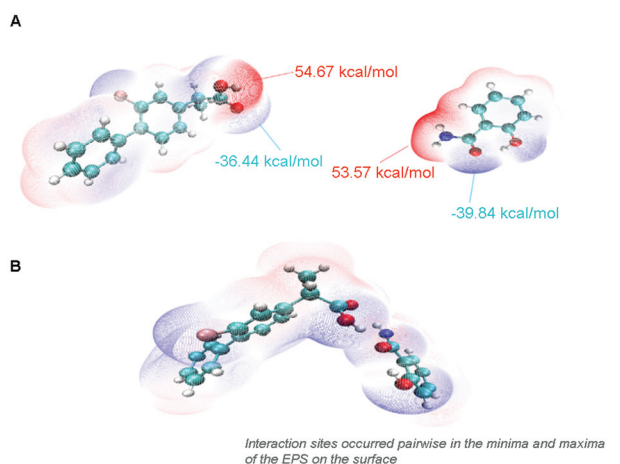


Fig. 7 (A) Electrostatic potential surface of different molecules in the co-crystal, and (B) interaction sites occurred pairwise in the minima and maxima of the ESP on the surface of the co-crystal FBP-2-OHBZA. ESP, electrostatic potential.

RDG Analysis

From the color-filled RDG isosurface in **Fig. 8**, we can identify different types of regions by simply examining their colors. As the color scale on the top shows, the bluer implies a stronger attractive interaction; in the current graph, the elliptical slab between oxygen and hydrogen atoms shows light blue color, so we can conclude that there is a hydrogen bond, but not very strong. The interaction region marked by a green circle can be identified as the vdW interaction region because the mapped color is green or light brown, which shows that the electron density in this region is low. The regions at the center of the two rings correspond to strong steric interaction since they are filled with red.

According to the RDG theory, a scatter diagram of $\text{sign}(\lambda_2)\rho$ versus RDG was drawn, from which the location, strength, and type of weak interactions were revealed. In **Fig. 8**, red fusiform regions were present in each ring of benzenes and reflected a strong steric effect, corresponding to a spike between +0.01 and +0.03. The existence of aromatic rings suggested a possible π - π stacking interaction. The RDG contour surface between the hydroxide radical in blue suggested a H-bond, corresponding to the leftmost. In the interior of the FBP molecule and 2-OHBZA, some RDG contour surfaces were observed with colors ranging from light green to earthy yellow, representing the existence of vdW forces and corresponding to a spike between -0.01 and +0.01.

The structures of FBP and 2-OHBZA were extracted to further study the π - π stacking interactions between FBP-2-OHBZA molecules and the minimal repeating stacking units in the co-crystal FBP-2-OHBZA. However, some differences were present among them. According to the π - π stacking interaction criteria, π - π stacking interactions always exist between two 2-OHBZA molecules in the co-crystal. However, π - π stacking interactions in 2-OHBZA were not continuous but on an interval in **Fig. 9B** because the centroid-centroid distance exceeded 3.8 Å. Therefore, we believed that the π - π

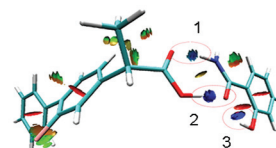
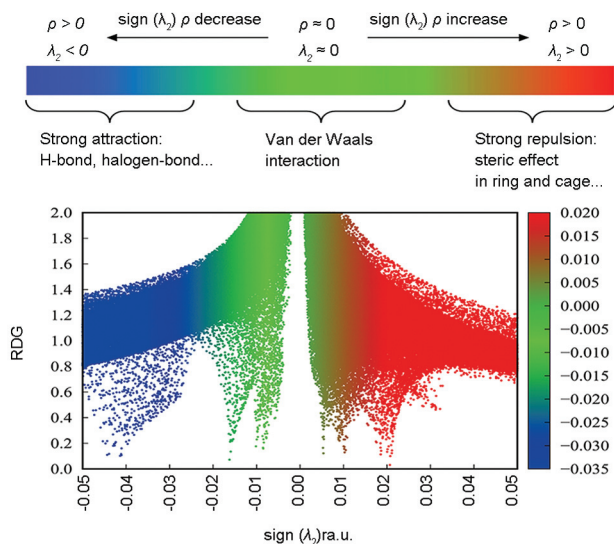


Fig. 8 RDG analysis of the co-crystal FBP-2-OHBZA. RDG, reduced density gradient.

interaction between FBP molecules still belongs to π - π stacking interactions, but it is a weak interaction.

Conclusion

FBP is widely used in the treatment of mild to moderate pain, rheumatoid arthritis, and osteoarthritis, and has a wide range of application values. It has been reported that the eutectic powder was prepared by grinding, but its complete single-crystal data have not been obtained. In this article, the surface potential energy parameters were calculated by DFT, and the co-crystal structure of FBP-2-OHBZA was studied using single-crystal X-ray crystallography. The experimental results show that FBP and 2-OHBZA in the crystalline state are composed of a stoichiometric ratio of 1:1. HS showed that the FBP molecule was connected to a 2-OHBZA molecule through O-H...O and N-H...O hydrogen bonds, and O-H...O hydrogen bonds were also formed in the 2-OHBZA molecule. RDG analysis showed that there was a strong π - π stacking interaction between 2-OHBZA molecules, and a weak interaction between FBP molecules. The combination of these two interactions determines the three-dimensional structure in which the eutectic aggregates infinitely in space. The maximum and minimum points of the surface potential energy calculated by ESP coincide with the eutectic binding sites shown by single crystal diffraction, and the hydrogen bond and π - π stacking interactions obtained by theoretical calculations are highly consistent with the eutectic data in this article.

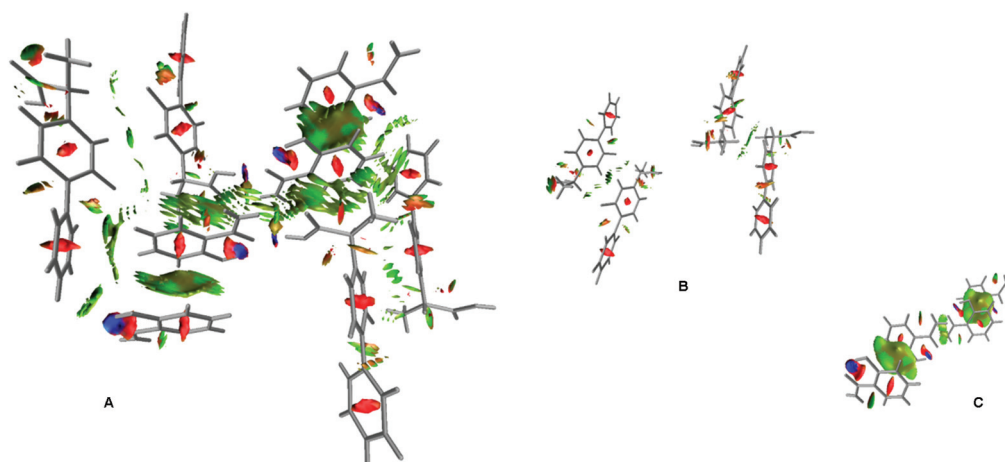


Fig. 9 Interactions of (A) FBP-2-OHBZA, (B) FBP, and (C) 2-OHBZA.

Conflict of Interest

The authors declare no conflict of interest.

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