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Bromhexine and its fumarate salt: Crystal structures, Hirshfeld surfaces and dissolution study

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1. Introduction

In the recent years, supramolecular chemistry together with crystal engineering has become one of the research hotspots in the field of pharmaceutical chemistry [1–4]. Preparation of multicomponent crystals including co-crystallization and salt formation promotes the design and synthesis of new solid type of Active Pharmaceutical Ingredient (API) with the desired physicochemical properties by exploring the advantages of supramolecular interactions [5–6]. Considerable researches have shown that both co-crystals and salts obtained by co-crystallization of the API and the GRAS (Generally Recognized as Safe) coformers have improved solid state properties of API such as better stability, solubility, bioavailability, and dissolution without altering the pharmacological activity of the API [7–15].

Bromhexine is an effective and safe expectorant drug, which is mainly used in patients with chronic bronchitis, asthma [16]. It has shown that bromhexine is also a potent inhibitor of TMPRSS2, a key protease in the transmission of SARS-CoV-2 [17–18]. Therefore, several clinical trials on the prevention or treatment of Novel Coronavirus Infectious Disease (COVID-19) with bromhexine are underway in the world to evaluate its prophylactic potential against SARS-CoV-2 [19–23].

However, the poor solubility of bromhexine gives rise to difficulties in pharmaceutical formulation for oral use, which may lead to variation in bioavailability [24]. To overcome these difficulties, salt or co-crystal strategy has been applied in bromhexine to develop novel salt or co-crystal with good solubility and stability bioavailability. Thus, screening experiments were performed for bromhexin co-crystallization with a series of carboxylic acid coformers by slow evaporation method. Finally, a new bromhexine crystal and its fumarate salt crystal have been obtained, which have not been found in the literature or Cambridge Crystallographic Data Centre (CCDC) so far [25–28]. The two crystals have been characterized by single-crystal and powder X-ray diffraction, IR spectroscopy, thermal analysis (TGA). Hirshfeld surfaces analysis has been carried out in order to analyze the intermolecular contacts. The equilibrium solubility and powder dissolution of the two crystals have been also evaluated. The fumarate of bromhexine exhibits better thermal stability and water solubility, compared with the free bromhexine base.

2. Experiment section

2.1. Materials and physical measurements

Bromhexin (> 99%) was gifted from Chia Tai Qingjiang Pharmaceutical Co., Ltd, Jiangsu, China. All the coformers and solvents were analytically pure purchased from Sinopharm Chemical Reagent and used as received without further purification.
Table 1

| Compound | 1 | 2 |
|----------|---|---|
| Formula  | C14 H23 Br2 N2 | C14 H23 Br2 N2 O4 |
| Formula weight | 376.14 | 492.21 |
| Crystal system | monoclinic | monoclinic |
| Space group | P 21/n | P 21/n |
| Z | 4 | 4 |
| a/Å | 6.5271(1) | 8.1069(12) |
| b/Å | 23.5874(2) | 26.277(4) |
| c/Å | 9.9502(1) | 9.4104(13) |
| a/° | 90 | 90 |
| β/° | 94.190(1) | 103.994(4) |
| y/° | 90 | 90 |
| V, Å³ | 1529.19(3) | 1945.1(5) |
| T/K | 293(2) | 193 |
| μ (mm⁻¹) | 6.596 | 3.790 |
| D calc (Mg m⁻³) | 1.634 | 1.681 |
| Cryst dimensions(mm) | 0.09 × 0.13 × 0.22 | 0.10 × 0.15 × 0.20 |
| No. of refinls collected | 3110 | 3554 |
| No. of unique refls | 2725 | 3502 |
| No. of params | 173 | 251 |
| Goodness of fit on F² | 1.095 | 1.207 |
| R1, wR2 (I > 2σ(I)) | 0.0418, 0.1162 | 0.0621, 0.1472 |
| R1, wR2 (all data) | 0.0478, 0.1261 | 0.0628, 0.1477 |
| CCDC No. | 2025907 | 2025908 |

With the samples prepared as KBr pellets, infrared spectra were performed using a Bruker-TENSOR27 FT-IR spectrometer in the range 4000-400 cm⁻¹. Thermo gravimetric analysis (TGA) were performed with a thermal analyzer TG209 F3 at a heating rate of 10°C /min under an atmosphere of N₂.

2.2. Crystals growth

Single crystal of bromhexine (Compound 1): 60 mg (0.160 mmol) bromhexine and 23 mg (0.160mmol) L-glutamine were dissolved in 10 ml ethanol and 2 ml distilled water by ultrasonic treatment for 20 min, then left for slow evaporation at room temperature after filtering. The colorless plate-like crystals were obtained after about 2 weeks with the total yield of 50.2%.

Fumarate of bromhexine (Compound 2): 100 mg (0.266 mmol) bromhexine and 31 mg (0.266 mmol) fumaric acid were dissolved in 20 ml acetonitrile and 2 ml methanol. The mixture was stirred at room temperature for 30min followed by filtering. The colorless block-like crystals were obtained after about 2 weeks for slow evaporation with the total yield of 45.0%.

2.3. X-Ray crystallography

Crystal structures of compounds 1–2 were determined by single-crystal X-ray diffraction, the date of compound 1 were collected at 293 K with Cu Kα radiation (λ = 1.54184 Å), while the date of compound 2 were collected at 193 K with Ga Kα radiation (λ = 1.34138 Å), using ω-scan method. The structures were solved by SHELXT 2015 program [29] in direct method and refined by SHELXL-2015 program [30], respectively. A summary of the crystallographic data for compounds 1–2 was provided in Table 1. Molecular graphics were prepared using DIAMOND [31] and Mercury program [32]. CCDC reference numbers 2025907 and 2025908 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

2.4. Powder X-ray diffraction (PXRD)

Room temperature PXRD analyses were performed using a Bruker D8 Discover diffractometer (Bruker, AXS) with Cu Kα radiation (λ = 1.5406 Å) at 40 kV and 40 mA. The data were collected over an angular range from 5° to 50° (2θ) in continuous scan mode with a step size of 0.02° (2θ) and a step time of 0.15 s. Mercury CSD 3.5.1 program was used to calculate PXRD patterns with the data of single crystal structures [33].

2.5. Hirshfeld surfaces analysis

Molecular Hirshfeld Analysis for both compounds was performed using the Crystal Explorer 3.1 software to investigate the nature of intermolecular interactions and their relative contributions in the crystals [34]. The distances from the Hirshfeld surface to the closest nucleus outside and inside the surface are defined as d_e and d_i, respectively. The normalized contact distance dnorm is based on d_e,d_i and the van der Waals radii of the atoms. The dnorm values can be mapped onto the Hirshfeld Surface, which facilitates easy comparison of intermolecular contacts relative to van der Waals radii by a simple red-white-blue color scheme (contacts shorter or longer than van der Waals contacts and equal to the sum of the van der Waals radii, are visualized as red, blue and white colours in the Hirshfeld surfaces respectively). [35]. In this study, a standard (high) surface resolution was used to generate the Hirshfeld surfaces of bromhexine molecules in compounds 1–2. The 3-D dnorm surfaces were mapped with a fixed color scale of 0.76 Å (red) to 2.4 Å (blue). Using standard 0.6 Å–2.6 Å view, the 2-D fingerprint plots were displayed with the d_e and d_i distance scale.

2.6. Solubility and dissolution experiments

To compare the equilibrium solubility values, an excess amount of bromhexine and its fumarate was added in 10 ml distilled water and the supersaturated solutions were shaken at 37°C. After 72 h, the suspensions were filtered and the concentrations of the drugs were analyzed by high-performance liquid chromatography (HPLC). For each dissolution experiment, powdered sample containing 480 mg of bromhexine or its equivalent in fumarate was added in 100 ml distilled water, and the resulting suspension stirred at 25°C and 500 rpm. At a predetermined time interval, each aliquot was taken filtered through a 0.45 μm membrane filter, and then the concentration of the drug was quantified by HPLC. The concentration of bromhexine was determined by Agilent 1260 HPLC equipped with a variable UV detector (set at 284 nm). A C18 column (100 mm × 4.6 mm, 5 μm) was used and the flow rate was set to 1.0 ml/min with the column temperature at 30°C. The mobile phase consisted of 0.1% phosphoric acid buffer (adjusted to pH 3.5 with trimethylamine): acetonitrile (10:90 v/v). Solubility and dissolution experiments for each sample were conducted three times to calculate the standard deviations.

3. Results and discussion

3.1. Crystal structural analysis

Compound 1 crystallizes in monoclinic space group P21/n with Z = 4 and the thermal ellipsoid plot at 50% probability is shown in Fig. 1a. In the molecular structure, the C–N–C bond angle is 111.07(2)° and the cyclohexane ring adopts the most stable chair configuration. Br1, Br2, N1 are almost coplanar with benzene ring, with slight deviations of 0.071(2) Å, 0.004(1) Å, 0.086(1) Å, respectively. In addition, the molecule is stabilized by intramolecular hydrogen bonds N1–H1B–N2 [N1–N2 distance of 2.855(4) Å, H1B–N2 distance of 2.115(3) Å, N1–H1B–N2 angle of 142.2(4)° (Table 2). A weak N1–H1A–Br1 interaction has been detected with the N1–Br1 distance of 3.098 Å significantly shorter than the sum of the van der Waals radii (3.40 Å) [36], formed intramolecular five-membered hydrogen bonds which have also been reported
Fig. 1. Molecular structure of compound 1 (a); One-dimensional chain structure of compound 1 (b); Two-dimensional network of compound 1 (c).
Table 2
Hydrogen bond parameters in compounds 1-2.

| compound | D-H (Å) | H···A (Å) | D···A (Å) | d(D-H···A) (Å) | Symmetry operation |
|-----------|---------|----------|----------|---------------|-------------------|
| N1-H1A...Br1 | 0.87(3) | 2.65(3) | 3.098(3) | 114(3) | x, y, z |
| N1-H1B...N2 | 0.87(3) | 2.12(3) | 2.855(4) | 142(4) | x, y, z |
| compound 2 | | | | | |
| N1-H1A...Br1 | 0.87(7) | 2.71(8) | 3.050(5) | 105(6) | x, y, z |
| N1-H1A...O2 | 0.87(7) | 2.14(7) | 2.854(6) | 139(7) | x,y,-1+z |
| N1-H1B...O3 | 0.90(7) | 2.17(7) | 3.011(6) | 155(6) | -1+x,y,-1+z |
| N2...H2...O1 | 0.90(6) | 1.84(6) | 2.692(5) | 159(5) | x, y, z |
| O4-H4...O2 | 0.840 | 1.710 | 2.534(5) | 167 | 1+x,y,z |
| C7-H7B...N1 | 0.990 | 2.560 | 2.926(7) | 102 | x, y, z |
| C9-H9...O4 | 0.95(5) | 2.46(5) | 3.267(6) | 142(4) | -1+x,y,z |

in the crystal structures of bromhexine hydrochloride [25] and other bromo-substituted aromatic amides [37]. As shown in Fig. 1b, the bromhexine molecules are connected in a head-to-tail fashion, resulting in the formation of a one dimensional zigzag chain through C-H...Br [H...Br distance of 2.966(1) Å] contacts. The adjacent chains are further linked by C-Br...Br halogen bonding contacts [Br...Br distance of 3.6670(7) Å] shown in Fig. 1c, belonged to type-I halogen-halogen interactions [38], with both C-Br...Br angles of 148.04(9)°.

Compound 2 also crystallizes in monoclinic space group P2₁/a with Z = 4 and the basic structure unit consists of one protonated bromhexine cation and one deprotonated fumaric acid anion, where one proton transfers from carbonylate of fumaric acid to N-methyl amino group of bromhexine [Fig. 2a]. The C-N–C bond angle is 111.6(4)° and the cyclohexane ring also adopts the most stable chair conformation, similar to those in compound 2. Bromhexine cations and fumaric acid anions are held together via N2+...H2...O1–...[N2+...O1– distance of 2.692(5) Å, H2–O1 distance of 1.836(6) Å, N2–H2–O1 angle of 158.7(5)°] along with N1–H1–O2 [N1–H1–O2 distance of 2.854(6) Å, H1–O2 distance of 2.144(7) Å, N1–H1–O2 angle of 138.7(5)°] hydrogen bond interactions to give infinite 1D chains (Fig. 2b). The 1D chains are further interacting by R₂̇(9) and R₂̇(8) supramolecular heterosynths through C9–H9...O4 [C9–O4 distance of 3.267(6) Å, H9–O4 distance of 2.462(5) Å, C9–H9–O4 angle of 141.9(4)°], O4–H4–O2 [O2–O4 distance of 2.534(5) Å, H4–O2 distance of 1.710 Å, O4–H4–O2 angle of 166.6(1)°] and N1–H1B–O3 [N1–O3 distance of 3.011(6) Å, H1B–O3 distance of 2.167(7) Å, N1–H1B–O3 angle of 155.2(6)°] hydrogen bonds thereby form a 2D layer along the (010) plane (Fig. 2c, Table 2). In addition, the weak π–π stacking interactions are detected with distance of 3.580 (3) Å, which also involved in stabilizing the crystal structure (Fig. 2d).

3.2. Fourier-transform infrared spectra (FT-IR) analysis

FT-IR spectroscopy is an effective tool used to differentiate distinct chemical structures and environments. In FT-IR spectra of compounds 1–2, obvious distinctions have been exhibited in the N–H stretching, N–H bending and carbonyl stretching vibrations frequencies (Supplementary Fig.S1 and Table S1). Compound 1 shows IR absorption frequencies at 3412 cm⁻¹ (asymmetric N–H stretching) and 3134 cm⁻¹ (symmetric N–H stretching) and at 1601 cm⁻¹ (N–H bending) without carbonyl stretching. Compound 1 shows N–H stretching at 3412 cm⁻¹, 3134 cm⁻¹ and N–H bending at 1601 cm⁻¹ attributed to aromatic amino groups, while no carbonyl stretching has been observed. On the other hand, compound 2 displays blue-shift of N–H stretching frequencies at 3444 cm⁻¹ and 3333 cm⁻¹, with N–H bending at 1633 cm⁻¹ due to changes in molecular conformations and hydrogen bonding. The N–H stretching vibration of the protonated aminomethyl group in compound 2 appears at 3232 cm⁻¹. The carbonyl stretching vibration for compound 2 appears at 1688 cm⁻¹, confirming the presence of carboxyl groups associated with the fumaric acid coformer. From comparison the spectrum with respect to fumaric acid, an increase in carboxyl acid stretching frequency has been observed for compound 2, also indicated the formation of salt.

3.3. Thermal analysis

The thermal behaviors of compounds 1–2 have been investigated and the TGA curves are shown in Fig.S2. Compound 1 undergoes one step of mass loss from 125°C with a continued weight loss completed by 300°C. Compound 2 undergoes three steps of mass loss, a first loss of 10% at approximately 152°C; the second step is a loss of 23% at approximately 172°C, while the third step is a loss of 44% at approximately 266°C. In addition, the melting points of compounds 1–2 have been tested with X-5 precise micro melting point tester. Compound 1 melted at about 60°C, while compound 2 melted at about 148°C. The results of thermal analysis confirm that compound 2 as a novel solid-state form improved the physical stabilities through higher decomposition and melting point.

3.4. Hirshfeld surface analysis

In order to provide further insight into the intermolecular interactions, Hirshfeld surfaces of bromhexine molecules in compounds 1–2 associated finger print plots have been calculated. The Hirshfeld surface mapped with d(norm) of bromhexine molecule in compound 1 is illustrated in Fig. 3a. Small red areas on the d(norm) Hirshfeld surfaces of compound 1 represent the C–H...Br interactions, while slight red area is in accordance with weaker Br...Br halogen bonding interactions. It’s noticeable that there are more deep red spots in the 3D-d(norm) surfaces of bromhexine molecule in compound 2 compared to that in compound 1, mainly due to close-contact N–H...O interactions from fumaric acid(Fig. 3b).

On the shape index surface of bromhexine molecule in compound 2, adjacent red and blue triangles have been observed, indicating the presence of aromatic stacking (Fig.S3). However, the π–π stacking interactions are absent in compound 1, because there is no evidence of adjacent bow-tie patterns on the shape index surfaces [39]. On the coincident curvedness surfaces of bromhexine molecule in compound 2, the relatively large and green flat regions, marked with oval in Fig.S3, also provide proof for the existence of aromatic stacking interactions.

The 2D finger print plots for the main intermolecular contacts of the two compounds are shown in Fig. 4. For compound 1, the H–H interactions exhibit the most significant contribution (52.3%) to the total Hirshfeld surfaces. The H...Br intermolecular interactions are seen as sharp spikes in the 2D fingerprint plots, accounting for 28.6% of the total Hirshfeld surfaces. Whereas in compound 2, the proportion of H–H interactions is decreased after salifica-
tion, account for 41.2% of the total Hirshfeld surfaces. However, close-contact O–H intermolecular interactions appear as a distinct spike in the 2D fingerprint plot, obviously increasing up to 16.3% of the total Hirshfeld surfaces. The other observed interactions are summarized in Table 3.

In summary, the results of Hirshfeld surface analysis indicate that there are stronger intermolecular forces in compound 2. It is found that forming the salt multicomponent crystal changed the bonding mode and intermolecular force of the two compounds.

3.5. Solubility and dissolution study

It has been reported that the solubility of bromhexine hydrochloride is increased by 5 fold at 15 mmol/L of methylated β-cyclodextrin [40], while the stability and safety of inclusion complexes need to be further studied [41]. It is also found that amino acid prodrugs can increase the solubility of bromhexine hydrochloride by about 3–5 times [42], but with complicated chemical synthesis and difficult purification. Therefore, development of new stable cocrystals/salts with simple processing and lower cost is still
Fig. 3. Hirshfeld surfaces mapped with $d_{norm}$ of bromhexin molecules in compound 1 (a) and 2 (b), respectively.

Fig. 4. 2D fingerprint plots of bromhexin molecules in compounds 1-2.
Bromhexine is a powerful expectorant and has been repurposed as a TMPRSS2 inhibitor also proposing for COVID-19 therapy. In this study, a new bromhexine crystal and its fumarate salt crystal have been obtained and characterized by single crystal X-ray diffraction, TGA and FT-IR spectroscopy. It is found that both compounds feature different crystal structures and proton transfer occurred from fumaric acid to bromhexine molecule in compound 2. The results of Hirshfeld surface analysis further confirm that there are stronger intermolecular forces in compound 2. Moreover, compound 2 exhibits higher thermostability and also shows an enhanced solubility in water and a better dissolution profile. By forming the salt multicomponent crystal, thermostability and solubility of compound 2 has been improved from the parent drug. Therefore, with a pharmaceutically acceptable coformer of fumaric acid, compound 2 is a promising candidate worthy of further research.

Declaration of Competing Interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents aconflict of interest in connection with the work submitted.

CRediT authorship contribution statement

Ya-an Zhang: Data curation, Formal analysis, Writing – original draft, Visualization. Cui-Min Yan: Methodology, Validation. Bai-Wang Sun: Conceptualization, Supervision, Funding acquisition. Lin-Xuan Wang: Investigation.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.130154.

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