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Original Research Article

Equimolar Complex Formation of Urea or Thiourea with 2-alkoxy-benzamides: Structural Factors Required for the Equimolar Complex Formation

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Abstract

Equimolar complex formation of either urea or thiourea with 2-ethoxy-benzamide (2-EB) and 2-methoxy-benzamide (2-MB) was investigated. Complex formation of urea and 2-MB was observed both by co-grinding method and by coprecipitation method. Molecular arrangement of the complex was determined by single crystal X-ray diffraction method as an equimolar complex. The crystal structure of the urea-2-MB complex, especially hydrogen bond networks, was quite different from that of thiourea-2-MB complex. In urea-2-MB equimolar complex, not only intermolecular hydrogen bond between urea and 2-MB but also hydrogen bond network between urea molecules played important roles to form the complex. When urea was co-ground with 2-EB, equimolar complex formation was not observed. Conformational change of guest molecules by the complexation was investigated in terms of intramolecular hydrogen bond length and the dihedral angles. Reduction of intramolecular hydrogen bond length of 2-MB and the conformational change to the flatter structure affected the equimolar complex formation.

Introduction

Thiourea and urea are known to form crystalline host-guest inclusion complexes having various crystal structures [1]. Thiourea inclusion complexes were known to form tunnel structures with the internal diameter of 5.8–7.1 Å [2]. Alkanes and alicyclic compounds have been candidates as the guest compound. The host-guest stoichiometry was usually between 3:1 and 6:1, depending on the size, shape and degree of saturation of the guest molecules [3]. Complexation of thiourea has been applied for stabilization and extraction of foods and medicinal drugs [3]. Thiourea inclusion complex of layered structure with the host-guest stoichiometry of 2:1 has also been reported [4]. Basicity and symmetrical location of amines in guest molecules could be a determinant factor for the formation of corrugated or laminar structure. In the case of conventional urea inclusion complex, three dimensional hydrogen-bonded array of urea formed hexagonal channel with the internal diameter of 5.5–5.8 Å [2, 3], within which organic molecules such as *n*-alkanes [3], fatty acids [5] and polymers [6] were densely packed through van der Waals forces. Most of the urea inclusion complex showed incommensurate structural properties [7, 8]. However, α,ω-dinitriles [9], di- and tricarboxylic acids [10] have been reported to form specific cocrystals with urea molecules.

Concerning about the preparation procedure, coprecipitation is a commonly used method to make host-guest inclusion complexes. However, some solvents were easily included in the place of guest [11] or interacted with guest and host molecules to form a complex [12]. Grinding is an alternative methodology to prepare the host-guest complexes more easily and rapidly without using solvent [13, 14].

Recently, a first example of grinding-induced equimolar complex formation between ethenzamide (2-ethoxy-benzamide; 2-EB) and thiourea has been reported and the crystal structure and molecular interaction between 2-EB and thiourea were demonstrated [15]. 2-Methoxy-benzamide (2-MB) has also been reported to form an equimolar complex with thiourea [15], though the crystal structure was not well demonstrated. In this

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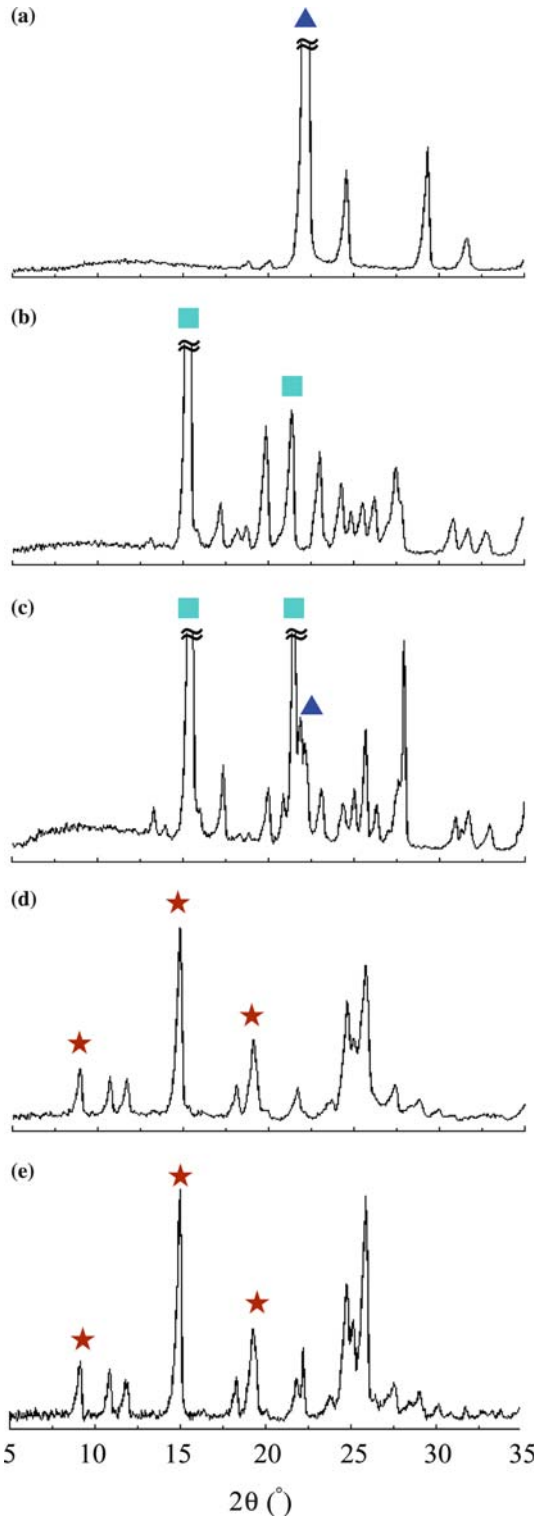


Figure 1. Powder X-ray diffraction patterns of urea/2-MB systems (molar ratio of urea/2-MB = 1/1). (a) Urea, (b) 2-MB, (c) PM, (d) GM (30 min), (e) sealed heated PM (125 °C for 3 h). The diffraction peaks due to urea, 2-MB and the complex were indicated by ▲, ■ and ★, respectively.

study, we prepared urea-2-alkoxy-benzamide equimolar complex both by grinding and by coprecipitation. The crystal structure was compared with that of the thiourea-2-alkoxy-benzamide complexes. Structural factors of guest molecules required for the equimolar complexation formation were discussed based on the conformational changes of 2-MB determined.

Experimental

Materials

Thiourea, urea and 2-EB were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). 2-MB was obtained from Lancaster Synthesis (Lancashire, UK). All other reagents were of analytical grade.

Preparation of urea-2-MB and –2-EB ground mixtures (GMs)

Urea was physically mixed with either 2-EB or 2-MB at a molar ratio of 1:1 in a glass vial by using a vortex mixer. The physical mixture (PM) was ground in a vibrational rod mill (CMT TI-200, CMT Co., Ltd., Japan) for 30 min to obtain the GM.

Preparation of sealed-heated sample

Urea and 2-MB PM (1.0 g) were sealed in a glass ampoule (25 ml) and heated in a gas chromatograph oven at 125 °C for 3 h.

Preparation of thiourea- and urea-2-MB single crystals

2-MB (0.8 g) was dissolved in 20 ml of ethanol and heated to 60 °C. After addition of 0.4 g of urea, the solution was stirred for 10 min. Then the solution was stored at 25 °C for 24 h to obtain the crystals. In the case of thiourea-2-MB complex, methanol was used instead of ethanol. The precipitated complex was collected on a paper filter and dried in air.

Powder X-ray diffraction (PXRD)

Powder X-ray diffraction was performed on a Rigaku Miniflex diffractometer (Rigaku Corporation, Japan) as following experimental conditions: Ni-filtered Cu-Kα, 25 °C, voltage 30 kV, current 15 mA, scanning speed 4° min^{-1}, $5° < 2\theta < 35°$.

Differential scanning calorimetry (DSC)

A DSC3100 differential scanning calorimeter (MAC Science Co, Japan) was used. About 5 mg of sample was loaded in a closed aluminum pan and measured at heating rate of 5 °C/min under nitrogen gas flow (50 ml/min).

Fourier transform-infrared spectroscopy (FT-IR)

Fourier transform-infrared spectroscopy measurements were carried out by KBr disc method. FT-IR spectra were recorded with JASCO 230 FT-IR spectrometer (JASCO Corporation, Japan).

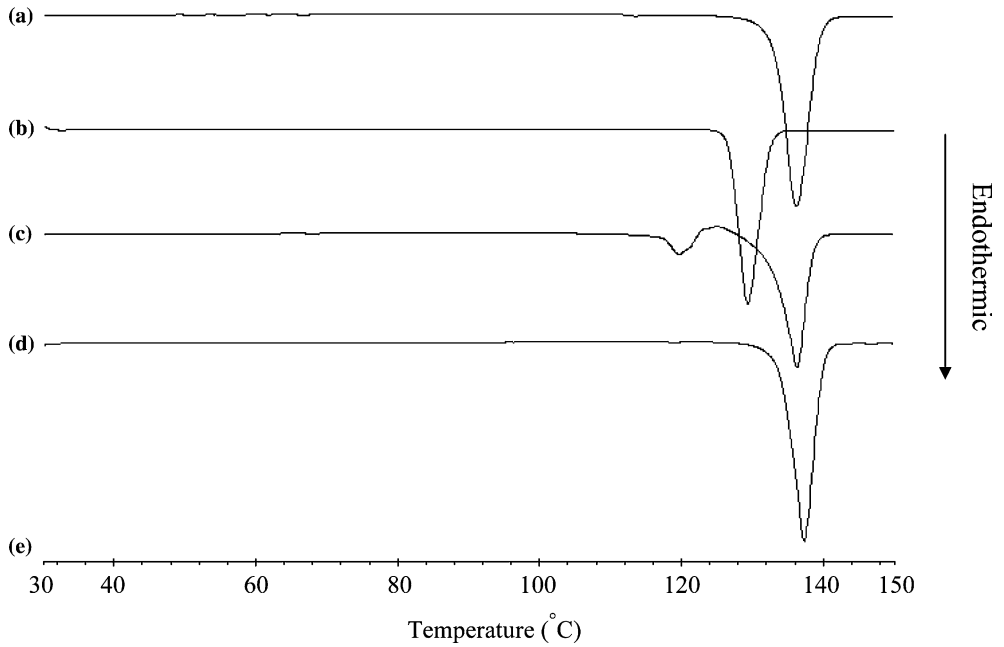


Figure 2. Differential scanning calorimetry curves of urea/2-MB systems (molar ratio of urea/2-MB = 1/1, heating rate: 5 °C/min, sealed pan). (a) Urea, (b) 2-MB, (c) PM, (d) GM (30 min).

Single crystal X-ray structural analysis

Measurements were made on a Bruker Smart 1000 CCD plate area detector with graphite monochromated MoKα radiation (Rigaku Corporation, Japan). The structure was solved by direct methods (SIR92) [16] and expanded using Fourier techniques (DIRDIF94) [17]. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined.

Crystallographic data of 2-MB, urea-2-MB and thiourea-2-MB have been deposited at the Cambridge Crystallographic Data Center in CIF format, CCDC No. 262857, 262858 and 262859, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033.

Results and discussion

Equimolar complex formation of urea-2-alkoxybenzamide induced by co-grinding

The equimolar complex formation has been demonstrated when thiourea was co-ground with 2-EB [15], even though thiourea has been known to form inclusion compounds with guest compounds as a hexagonal channel structure at the molar ratio of higher than 3:1 [3]. Urea is also a promising candidate to form the equimolar complex. In this study, two alkoxy-benzamides, 2-MB and 2-EB were used as guest compounds in order to investigate the formation of equimolar complex with urea.

When urea was co-ground with 2-MB at a molar ratio of 1:1, new PXRD peaks at $2\theta = 9.0$, 14.9 and 18.9° were observed as shown in Figure 1. PXRD peaks of intact urea and 2-MB crystals were gradually decreased with increasing grinding time and finally disappeared after the grinding for 30 min. Peak positions of new peaks were apparently different from those of the conventional urea inclusion complexes, indicating that urea formed the equimolar complex with 2-MB. Figure 2 shows DSC curves of the equimolar urea-2-MB PM and GM. Intact urea and 2-MB crystals showed endothermic peaks due to the fusion at 136 and 133 °C, respectively. The PM showed an endothermic peak due to the fusion of 2-MB at 119 °C, followed by a small exothermic peak and an endothermic peak around 136 °C. In the case of GM, only an endothermic peak was observed at 137 °C. As heat-induced complexation was observed from PXRD pattern of the sealed heated sample at 125 °C for 3 h (Figure 1e), the endothermic peaks observed in PM and GM around 136 °C were supposed to be due to the fusion of the equimolar complex, though it was difficult to differentiate from that of urea. Molecular interaction between urea and 2-MB in the GM was investigated by FT-IR spectroscopy (Figure 3). N–H asymmetric and symmetric vibration bands of 2-MB at 3412 cm^{-1} and 3192 cm^{-1} observed in intact crystals were shifted to higher wave number, 3460 cm^{-1} and 3228 cm^{-1} by grinding, respectively. The position of N–H vibration bands of urea was also changed by grinding, suggesting that the amide group of 2-MB forms hydrogen bonds towards the urea molecule.

In addition, urea was co-ground with 2-EB and PXRD patterns of the PM and GM were shown in Figure 4. In contrast to the urea-2-MB GM, PXRD pattern of urea-2-EB GM showed no change by the

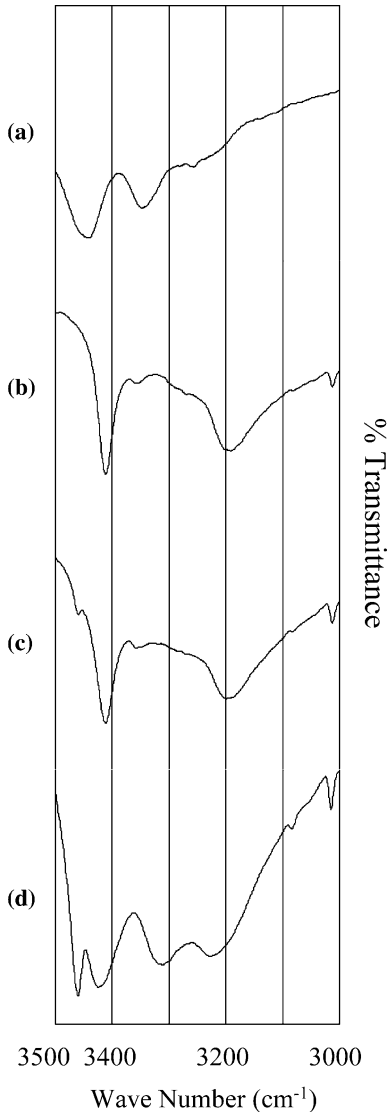


Figure 3. Changes in IR spectra of urea-2-MB systems (molar ratio of urea/2-MB = 1/1). (a) Urea, (b) 2-MB, (c) PM, (d) GM (30 min).

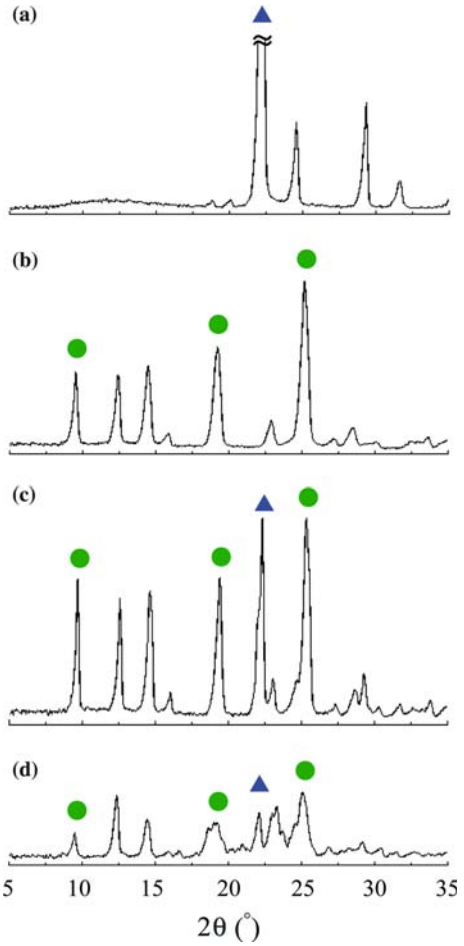


Figure 4. Powder X-ray diffraction patterns of urea-2-EB systems (molar ratio of urea/2-EB = 1/1). (a) Urea, (b) 2-EB, (c) PM, (d) GM (60 min). The diffraction peaks due to urea and 2-EB were indicated by ▲ and ●, respectively.

co-grinding compared to that of PM. PXRD peaks of intact urea and 2-EB crystals were still observed after the grinding of 60 min. Figure 5 shows the IR spectra of urea-2-EB PM and GM. No significant spectral changes were observed between them. Coprecipitation and sealed-heating methods were also applied to prepare urea-2-EB complex, however, the complex formation was not observed. The ethoxy group in 2-EB seemed to be not suitable for the molecular interaction between urea and 2-EB molecules to form equimolar complex.

Crystal structure of urea- and thiourea-2-MB equimolar complex

To determine the crystal structure of urea-2-MB equimolar complex induced by grinding, single crystals of the complexes were prepared. Thiourea-2-MB equimolar complex formation induced by grinding has already been reported [15], however, the crystal structure was not reported. In this study, single crystal of the thiourea-2-MB complex was also prepared to compare the crystal structure. View of the crystal packing and molecular unit of the urea-2-MB and thiourea-2-MB equimolar complexes were shown in Figures 6 and 7, respectively. PXRD patterns of urea-2-MB or thiourea-2-MB GM and the powdered single crystals were same as in the case of thiourea-2-EB [15] (data not shown). The structure confirmed equimolar complexes of urea-2-MB and thiourea-2-MB and the crystal structures were entirely different from those of the tunnel-type urea and thiourea inclusion complexes reported previously.

Selected structural parameters and hydrogen-bond lengths of 2-MB and the equimolar complexes with urea and thiourea were given in Tables 1 and 2. In urea-2-MB equimolar complex, it was found that 2-MB formed N3–H5 ··· O3 intramolecular hydrogen bonding (2.661 Å) and interacted with urea through N3–H6 ··· O1 (3.001 Å), N1–H1 ··· O2 (3.022 Å) and N2–H3 ··· O2 (3.032 Å) hydrogen bondings along the *a* axis. Furthermore, the existence of different kind of hydrogen bonding was observed, that is, urea molecules interacted each other through N2–H4 ··· O1 (2.978 Å)

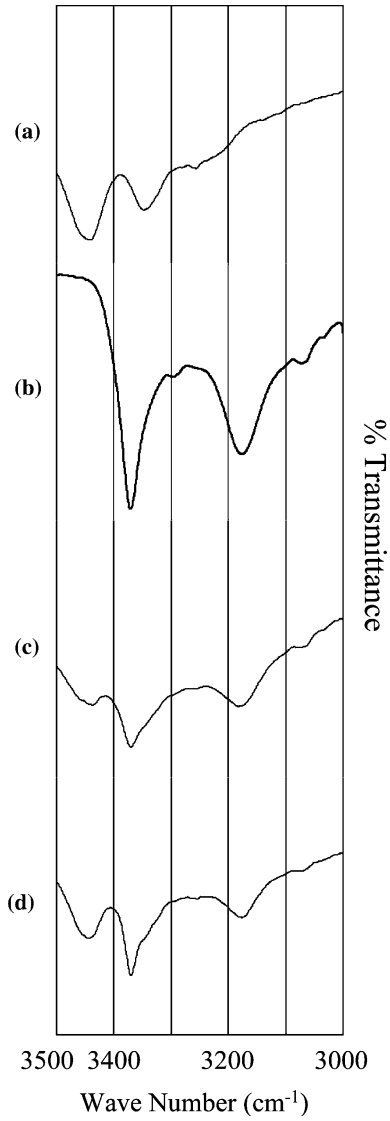


Figure 5. Changes in IR spectra of urea-2-EB systems (molar ratio of urea/ethenzamide = 1/1). (a) Urea, (b) ethenzamide, (c) PM, (d) GM (60 min).

and N1–H2 ··· O1 (2.997 Å) hydrogen bondings to form extended polymeric structure using 8-membered hydrogen-bonded ring motifs.

The urea hydrogen bonded network as well as intermolecular hydrogen bonds between urea and 2-MB was recognized to be important to form urea-drug equimolar complex. To form the tightly-packed hydrogen bond networks, ethoxy group of 2-EB would hamper the hydrogen bond formation and it seemed to be the reason why urea did not form equimolar complex with 2-EB.

Urea has been reported to form equimolar cocrystals with α, ω-dinitriles [9], maleic acid and phthalic acid [10]. In maleic acid and phthalic acid cocrystals, urea and drug molecules were arranged alternately. In the case of α, ω-dinitriles, the alternately-arranged molecules formed layered structure through N–H ··· O hydrogen bondings between urea molecules. Molecular cocrystals of urea with aromatic carboxylic acids have also been investigated by Smith *et al.* [18]. Crystal structure of urea-2-MB equimolar complex was different from that of the equimolar cocrystals with α,ω-dinitriles or dicarboxylic acids. The urea-2-MB complex was rather similar to urea-4-aminobenzoic acid cocrystals, which was reported by Smith *et al.* [18], though the stoichiometry of urea to 4-aminobenzoic acid was 1:2.

The molecular packing in thiourea-2-MB complex was significantly different compared to that of urea-2-MB as shown in Figures 6 and 7. In thiourea-2-MB equimolar complex, a 2-MB molecule formed N3–H5 ··· O2 intramolecular hydrogen bonding (2.638 Å) and interacted with thiourea molecules through N1–H2 ··· O1 (2.920 Å), N3–H6 ··· S1 (3.335 Å), and N2–H3 ··· O1 (3.061 Å) intermolecular hydrogen bondings. Thiourea-thiourea hydrogen bond networks formed in thiourea crystals, however, were not observed in the equimolar complex. N–H ··· S distance between thiourea molecules in the equimolar complex was either 3.445 or 3.479 Å, which was slightly longer than the distance observed in thiourea crystals (3.39 Å) [19]. Hydrogen bond network structure of thiourea molecules in the complex was also different from that in thiourea crystals which showed the 8-membered dimeric structure. Structural parameters and molecular arrangement of thiourea-2-MB complex were almost same to those of thiourea-2-EB complex reported previously [15]. Structural difference between them was that 2-MB was more closely interacted with thiourea to form the equimolar complex.

Conformational change of 2-MB by complexation

Structural factors of drugs affecting the grinding-induced complex formation with thiourea have been investigated and 2-alkoxy-benzamide structures were found to be required for the guest molecule to form the equimolar complex with thiourea [15]. Conformational changes of 2-MB molecule by the equimolar complex formation were investigated in terms of intramolecular hydrogen bond length and the dihedral angles. As shown in Table 2, the length of intramolecular hydrogen bond observed in 2-MB crystals (2.771 Å) changed to 2.638 and 2.661 Å in thiourea- and urea-2-MB equimolar complexes, respectively. Figure 8 shows the torsion angles of 2-MB in the equimolar complex with urea and thiourea. Dihedral angle of intact 2-MB crystals between C–O and C–N was determined as 30.4°. In both of the urea-2-MB and the thiourea-2-MB equimolar complexes, however, both of the dihedral angles were 4.5°. These results indicated that not only intermolecular hydrogen bond formation between thiourea or urea and guest molecule but also reduction of intramolecular hydrogen bond lengths and conformational change to the flatter structure occurred as a result of the equimolar complex formation.

For 2-MB molecules in the crystal, the distorted plain was maintained as shown in Figure 8. Stereoeffects

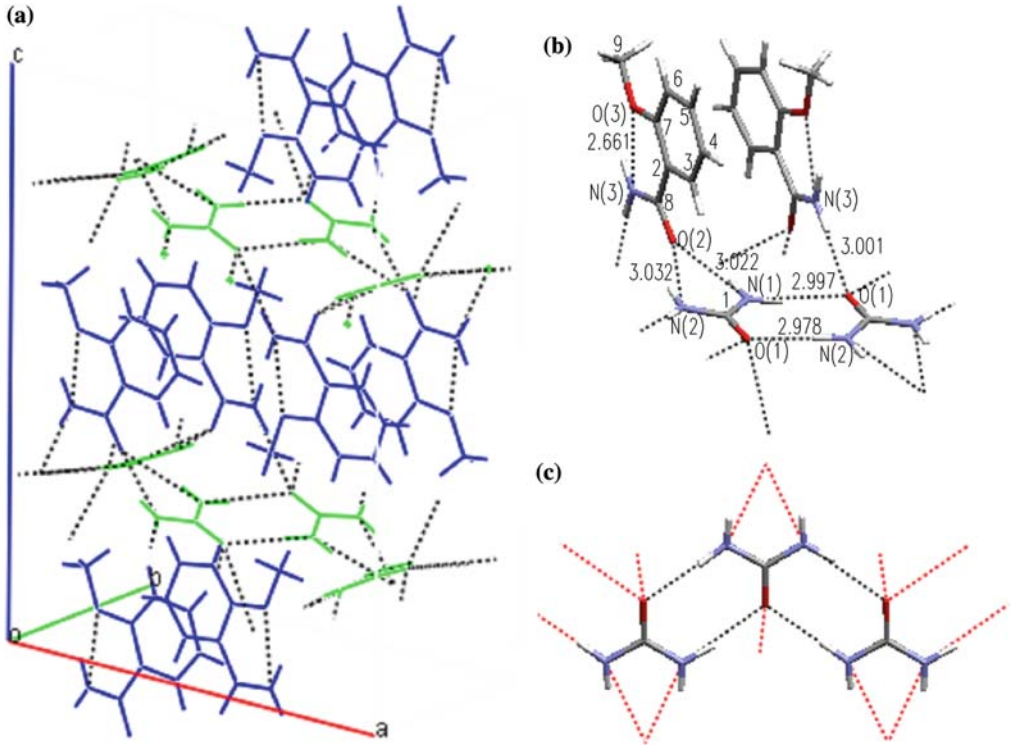


Figure 6. Structure and packing of urea-2-MB complex in the crystal lattice. (a) Packing of the complex in a crystal lattice. (b) Structure of the equimolar complex. (c) Intermolecular interaction between urea molecules.

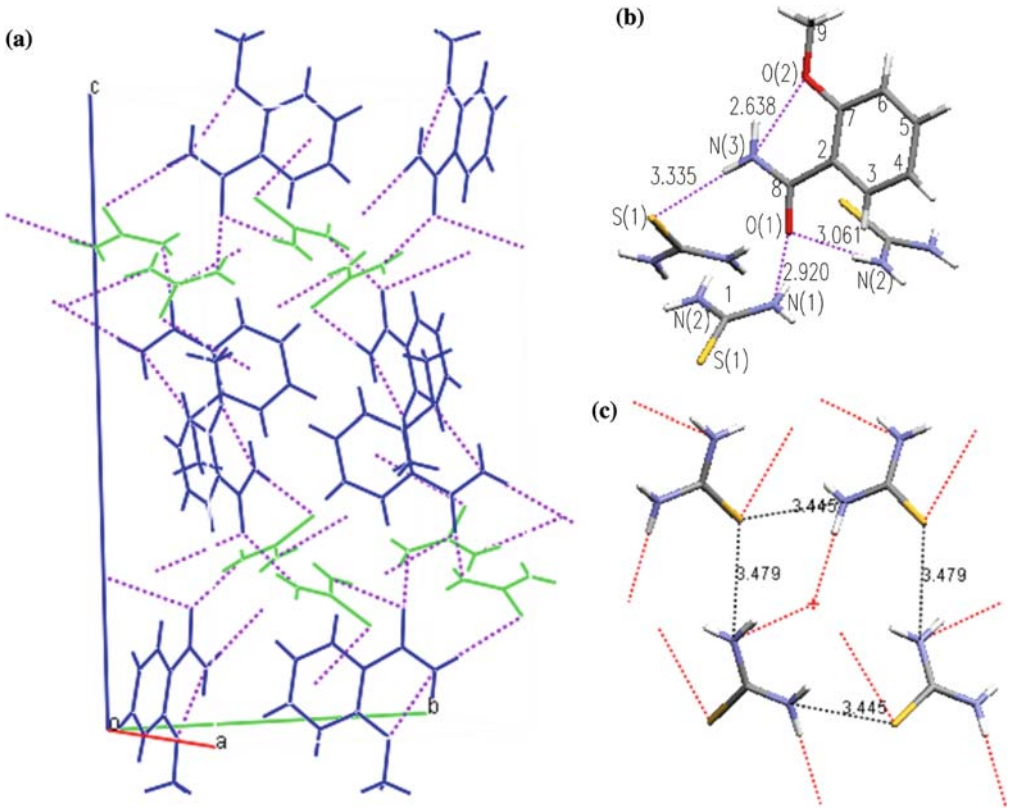


Figure 7. Structure and packing of thiourea-2-MB complex in the crystal lattice. (a) Packing of the complex in a crystal lattice. (b) Structure of the equimolar complex. (c) Intermolecular interaction between thiourea molecules.

Table 1. Crystal data of 2-MB and the equimolar complex with urea and thiourea

	2-MB	Urea-2-MB	Thiourea-2-MB
Empirical formula	$C_8H_9NO_2$	$C_9H_{13}N_3O_3$	$C_9H_{13}N_3O_2S_1$
Formula weight	151.16	211.22	227.28
Crystal color, habit	clear, prism	clear, prism	clear, prism
Crystal system	orthorhombic	orthorhombic	Orthorhombic
a/Å	5.197(2)	14.930(4)	10.042(3)
b/Å	11.095(4)	7.199(2)	10.878(3)
c/Å	13.374(5)	19.425(5)	20.399(5)
V/Å^3	771.2(5)	2087(1)	2228.3(10)
Space group	$P2_12_12_1$(#19)	Pbca (#61)	Pbca (#61)
Z	4	8	8
D_{calc}/g cm^{-3}	1.302	1.344	1.355
μ(MoKα)/cm^{-1}	0.94	1.03	2.75
Radiation	MoKα($\lambda = 0.71069$Å)	MoKα($\lambda = 0.71069$Å)	MoKα($\lambda = 0.71069$Å)
Monochromator	graphite	graphite	Graphite
Temperature/°C	26	26	−153.0
No. of observations	904 (I > 1.50 σ (I))	1261 (I > 1.50σ(I))	2185 (I > 2.00σ(I))
No. of variables	102	137	137
R	0.042	0.050	0.035
R_w	0.052	0.043	0.04
Goodness of fit indicator	0.88	1	1.3
Maximum peak in final diff. map/e Å^{-3}	0.16	0.22	0.27
Minimum peak in final diff. map/e Å^{-3}	−0.19	−0.22	−0.31

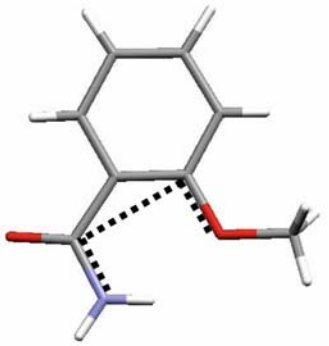
of the amide and methoxy groups, hydrogen-bond donating and accepting capabilities and crystal packing capabilities would be responsible for the twisting configuration of 2-MB molecules. Flattening of the 2-MB by the complexation simultaneously satisfies the directional demands of the hydrogen bonds formed between the urea and the 2-MB molecules and the close packing requirements of the crystal lattice. The shortening of the internal hydrogen bond and the issued flattening of 2-MB would be the result of the crystal packing. For further investigation of the complexation mechanism, calculation of stabilization energy should be helpful to know the structural restraint and stability difference.

Table 2. Hydrogen-bond lengths of 2-MB and the equimolar complex with urea and thiourea

Hydrogen-bonds	Bond length (Å)	Symmetry operations
2-MB		
N1-H1 ··· O2	2.771	
N1-H2 ··· O1	2.980(3)	$X+1$, Y, Z
N1-H1 ··· O1	2.951(2)	$X+1/2$, $-Y-1/2$, $-Z+1$
Urea/2-MB		
N3-H5 ··· O3	2.661	
N2-H4 ··· O1	2.978(3)	$-X+1$, $Y+1/2$, $-Z+1/2$
N1-H2 ··· O1	2.997(3)	$-X+1$, $Y-1/2$, $-Z+1/2$
N3-H6 ··· O1	3.001(2)	$X+1/2$, Y, $-Z+1/2$
N1-H1 ··· O2	3.022(2)	
N2-H3 ··· O2	3.032(2)	
Thiourea/2-MB		
N3-H5 ··· O2	2.638	
N1-H2 ··· O1	2.920(2)	$-X+1$, $Y+1/2$, $-Z+1/2$
N3-H6 ··· S1	3.335(1)	$-X+1/2$, $Y-1/2$, Z
N2-H3 ··· O1	3.061(2)	

Conclusions

In conclusion, urea could form an equimolar complex with 2-MB by grinding. Both the intermolecular hydrogen bond formation between urea and 2-MB and the hydrogen bond network formation between urea molecules played important roles for the equimolar complex formation. On the contrary, urea could not form an equimolar complex with 2-EB. The bulky ethoxy group would hamper the intermolecular hydrogen bond network formation between urea molecules and between urea and ethenzamide. Reduction of intramolecular hydrogen bond lengths and conformational change of 2-MB to the flatter structure affected the equimolar complex formation with urea. For further investigation, computational calculation would be helpful not only to know the complexation mechanism but also to predict chemical structure of drug molecules required for the complex formation. These findings would give new insights for supramolecular structures of drug-urea and drug-thiourea complexes.



Torsion angle between alcoxy C-O and amide C-N	(°)
in 2-methoxybenzamide crystal	30.4
in thiourea/2-methoxybenzamide complex	4.5
in urea/2-methoxybenzamide complex	4.5

2-methoxybenzamide intact

2-methoxybenzamide in Thiourea complex

2-methoxybenzamide in Urea complex

Figure 8. Torsion angles of 2-MB molecules calculated from the crystallographic data.

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