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Synthesis and Crystal Structure Studies of Mitomycin C Dihydrate

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Abstract

The absolute configuration of mitomycin C was determined by X-ray single crystal diffraction (CuKα), and a new crystalline dihydrate of mitomycin C had been prepared. The experiment result provides a definitive answer to the real absolute configuration of mitomycin C and may put an end to the dispute that baffles researchers for decades. At the same time, some contentious structures about the mitomycin C in *American Pharmacopoeia USP*36-*NF*31, *Chinese pharmacopoeia* 2015 *edtion* and numbers of literatures are marked. The absolute configuration is also verified by 1D (^{1}H and ^{13}C) and 2D (HSQC, HMBC, ^{1}H-^{1}H COSY and NOESY) NMR studies indirectly. Powder X-ray diffraction (PXRD) pattern of the mitomycin C dihydrate is similar to that calculated for it, which suggests that the purity of sample is excellent.

Keywords

Crystal Structure, Dihydrate, Absolute Configuration, Mitomycin C

1. Introduction

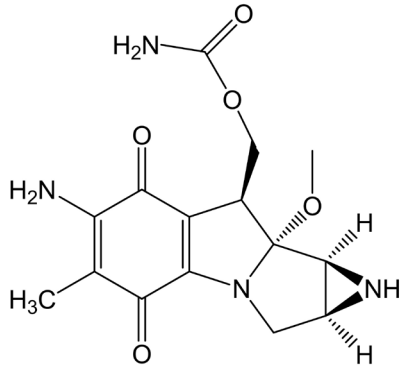
Mitomycin C, an antibiotic antitumor drug [1] [2] [3], was first extracted from Streptomyces species in 1958, and its chemical structure was determined in 1963 [4] [5] [6]. Surprisingly, although mitomycin C has been successfully used in clinical circumstance for more than thirty years [6] [7] [8] [9], there are still many arguments about its absolute configuration [5] [10] [11] (**Figure 1**).

*American Pharmacopoeia USP*36-*NF*31 and *Chinese pharmacopoeia* 2015 *edtion* define configuration B as the standard structure of mitomycin C; instead, *European Pharmacopoeia EP*7.0, *British Pharmacopeia BP*2017 and *Japanese Pharmacopeia JP*17 define configuration A. Then, we probed further and found

*These authors contributed equally to this work.

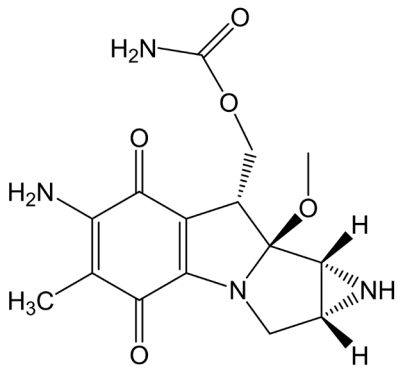
out that two drugs Mitosol and Mitozytrex, approved by FDA, both record the structure as configuration B (Table 1). In Cambridge Structural Database System, *S.K. Arora* [10] reported different kinds of single crystal of mitomycin C, but they failed to reach a mutual agreement. The reason contributed to this question may be due to limitation of early crystallography techniques, for example, they were inclined to use molybdenum target radiography, by which radiography is hardly to obtain believable absolute configuration of compound, rather than copper target.

In this paper, we determined the absolute configuration of mitomycin C by X-ray single crystal diffraction (CuKα), analyzed its structure by 1D and 2D NMR spectra, and prepared a new crystalline. Most importantly, the result of our experiment can provide a definitive answer and put an end to the dispute about the real absolute configuration of mitomycin C.



((1a*S*,8*S*,8a*R*,8b*S*)-6-amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-8-yl)methyl carbamate

Configuration A



((1a*R*,8*R*,8a*S*,8b*R*)-6-amino-8a-methoxy-5-methyl-4,7-dioxo-1,1a,2,4,7,8,8a,8b-octahydroazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-8-yl)methyl carbamate

Configuration B

Figure 1. Two configurations of mitomycin C reported from literatures.

Table 1. Some document record about the structure of mitomycin C.

Pharmacopoeia	Record Structure
*USP*36-*NF*31	B
*CP*2015	B
*EP*7.0	A
*BP*2017	A
*JP*17	A
Drug approved by FDA	
Mitosol (FDA2012)	A
Mitozytrex (FDA2002)	B
Literature	
S.K. Arora	B
K. Ogawa	B

2. Experimental

2.1. Reagents and Instruments

All reagents were obtained from commercial sources and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Unity 400 MHz spectrometer. 2D NMR were recorded by a Varian Unity 100 MHz NMR spectrometer and used to assist in structure elucidation. DMSO-d_6 was used as the solvent in both of 1D and 2D NMR spectra (DMSO-d_6: 2.49 ppm for ^{1}H and 39.9 ppm for ^{13}C). The powder X-ray diffraction (PXRD) pattern was recorded on crushed single crystals in the 2θ range of 6˚ - 55˚ with a CuKα radiation.

2.2. Preparation of Single Crystal

Dissolve mitomycin C (200 mg) in water (40 ml), then the solution was stirred in room temperature for 20 minutes [12]. After the violet insoluble substance was filtered, chloroform (10 ml) was added into the violet aqueous solution slowly. Keep the mixture in a sealed container stored in a dark place at room temperature and many purple-dark prismatic crystals appeared on the container wall after 3 days.

2.3. X-Ray Structure Determination

A purple-dark single crystal with approximate dimensions of 0.12 mm × 0.10 mm × 0.08 mm was selected. The data was collected by a Rigaku Saturn944 CCD diffractometer (CuKα radiation) in the ranges of $4.688^{\circ} \le \theta \le 72.379^{\circ}$, $-11 \le h \le 11$, $-15 \le k \le 15$ and $-16 \le l \le 16$. A total of 21,504 reflections were collected, of which 3386 were independent ($Rint = 0.0641$). The full-matrix least-squares refinement on F^2 (SHELXL-97) was performed with non-hydrogenatoms refined anisotropically. The final $R = 0.0534$ and $wR = 0.1133$ for 3186 observed reflections with ($F^2 \ge 2\sigma F^2$) and $R = 0.0546$ and $wR = 0.1154$ for all data.

3. Results and Discussion

3.1. NMR Spectra

The ^{1}H, ^{13}C, HSQC, HMBC, ^{1}H-^{1}H COSY NMR spectra allowed the assignments of all the protons to their bonding carbons (**Table 2**), which confirmed the planar structure of the molecule correct. There was a striking contrast between the signals of two protons linking to C_3 (C_{13}) in ^{1}H NMR spectrum (δH_3: 4.00, 3.35; δH_{13}: 4.53, 4.10) and this phenomenon could be ascribed to the chiral structure of C_2 and C_{10}.

The relative configuration of compound was assigned by NOESY spectra, in particular, by comparing the ^{1}H-^{1}H COSY (**Figure 2**) and NOESY (**Figure 3**) spectra, we observed that H_{15} had no ^{1}H-^{1}H COSY correlations with H1, H_2, H_3, H_{10} because of the long distance, but distinct NOESY correlations of H_{15} with H_1, H_2, H_3, H_{10} could be found. At the same time, NOESY correlations between H_{15}

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Chemical Shifts of mitomycin in DMSO-d_6 at 300 K.

Position	δ_H	δ_C	^{1}H-^{1}H COSY	HMBC	NOESY
C_1	2.80 s	35.45	N1	3	N1, 15
C_2	2.71 s	31.55	N1, 3	3	N1, 3, 15
C_3	4.01, 3.35 dd (12)	49.66	2	1, 2, 11	2, 15
C_4	-	155.31	-	10	-
C_5	-	176.87	-	10, 12	-
C_6	-	102.58	-	12	-
C_7	-	149.07	-	12	-
C_8	-	175.25	-	-	-
C_9	-	109.10	-	10	-
C_{10}	3.40 m	42.76	13	4, 5, 9, 11	15
C_{11}	-	105.75	-	N1, 15, 10, 3	-
C_{12}	1.67 s	8.38	-	5, 6, 7	-
C_{13}	4.55, 4.1 tt	60.90	10	-	-
C_{14}	-	156.60	-	-	-
C_{15}	3.11 s	49.17	-	11	1, 2, 3, 10
N_1	1.80 t	-	1, 2	-	1, 2
N_3	7.05 s	-	-	-	-
N_4	6.45, 6.70 ss	-	-	-	-

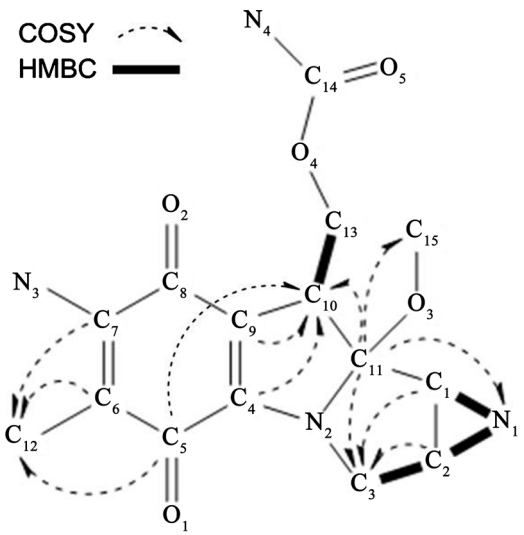


Figure 2. Key COSY and HMBC correlations of mitomycin C.

and H_{13} or H_{N1} could not be observed. All this information suggested that H_{15} and H_1, H_2, H_3 are on the same side of the mitomycin C, in other words, C_{15} is on the other side of C13 and N_1 which could be ascribed to the bond of C_{11}-O_3 had different orientation with C_{10}-C_{13} and C_1-N_1.

Unfortunately, the 1D and 2D NMR spectra did not provide enough information to ascertain the absolute configuration of mitomycin C [13] [14]. Thus, a single crystal of mitomycin C was prepared.

3.2. Description of the Single Crystal Structure

The absolute configuration of the title compound is shown in Figure 4. These configurations at C_{10}, C_{11}, C_1 and C_2 are, respectively, (S), (R), (S) and (S) in our crystal, which structure is in accordance with configurations A, but is exactly opposite to configurations B (Figure 5).

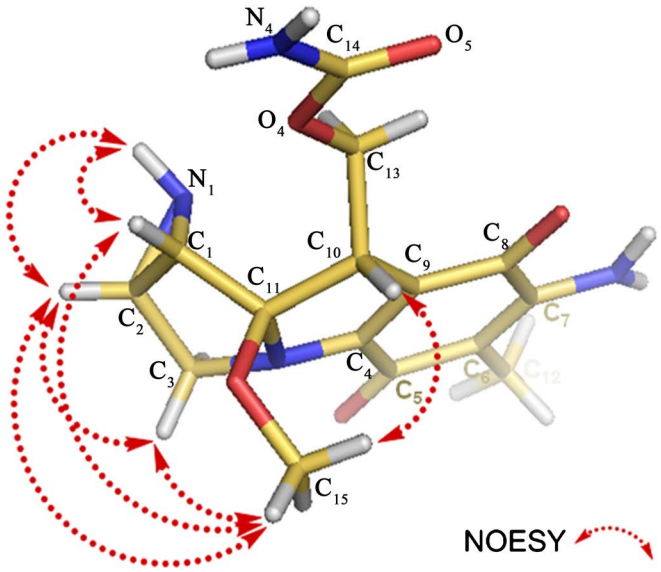


Figure 3. Key NOESY correlations and X-ray crystallographic structure of mytomycin C.

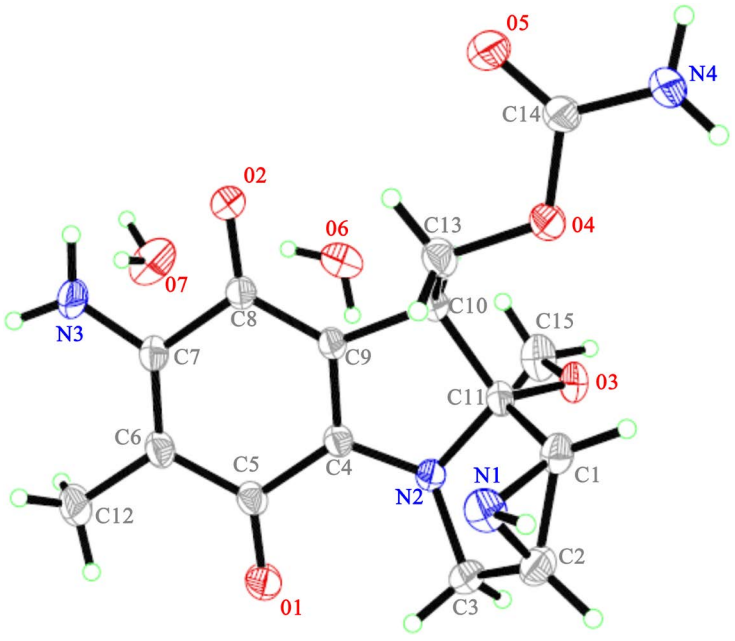


Figure 4. The X-ray crystal structure of mitomycin C dihydrate (CCDC 993490), with displacement ellipsoids drawn at the 30% probability level.

The distance from C_{13} to plane B (Figure 6) is 1.1847(0.0029) Å, and the angle of plane A to B, B to C, C to D and D to A are, respectively, 2.19 (0.02)˚, 46.25 (0.07)˚, 81.18 (0.07)˚ and 54.99 (0.09)˚. Torsion angles in carbamoyloxymethyl side chain (C_{14}-O_4-C_{13}-C_{10}) is 115.87 (0.23)˚, and in methoxyl group (C_{15}-O_3-C_{11}-N_2) is 56.36 (0.27)˚. This structure is demarcated by the benzoquinone cycle, carbamoyloxymethyl side chain on one side, and two water molecules on other side (Figure 3). The selected bond lengths and bond angles are given in Figure 6.

We can observe an interesting phenomenon from Figure 7 that water molecules become the center of this unit cell, which are surrounded by the order array of mitomycin C. The interacting with each other by hydrogen bonds (Table 3) of these mitomycin C forming a construction of "well", at the same time these water molecules are inlaid in the "well" steady because of the hydrogen bonds between water and mitomycin C.

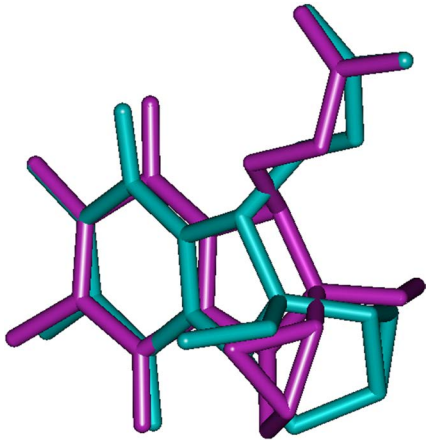


Figure 5. The molecule overlap of configuration A (purple) and configuration B (green).

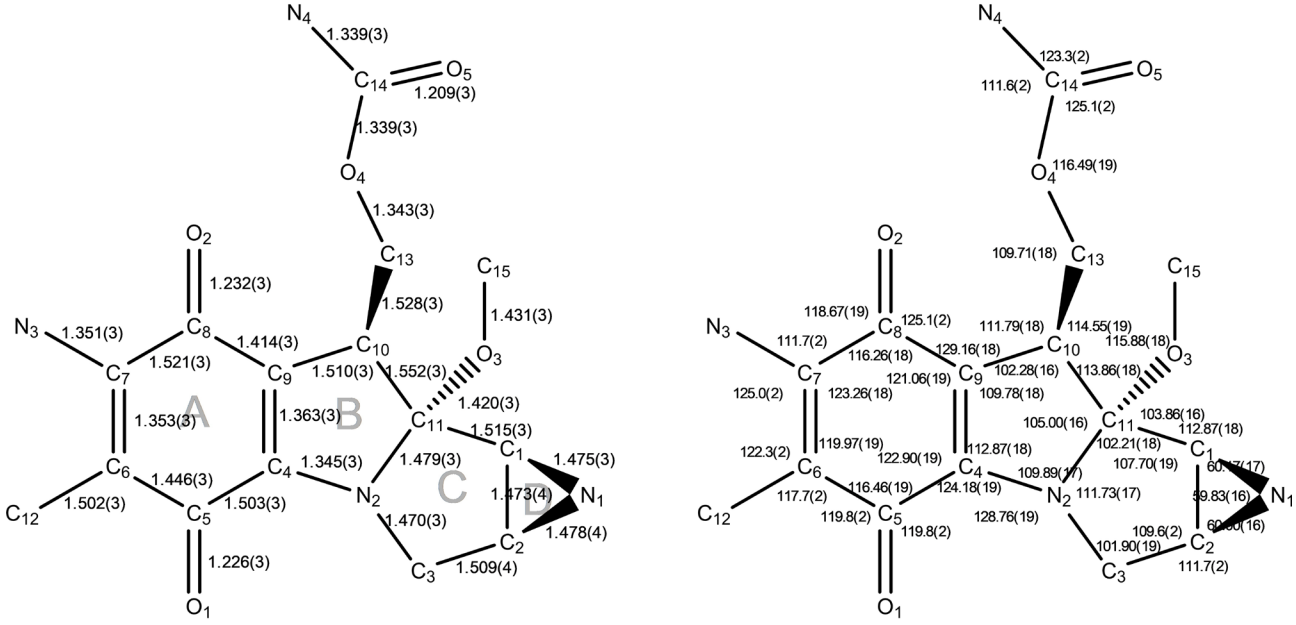


Figure 6. Bond lengths/Å and angles/˚ with their estimated standard deviations in parentheses.

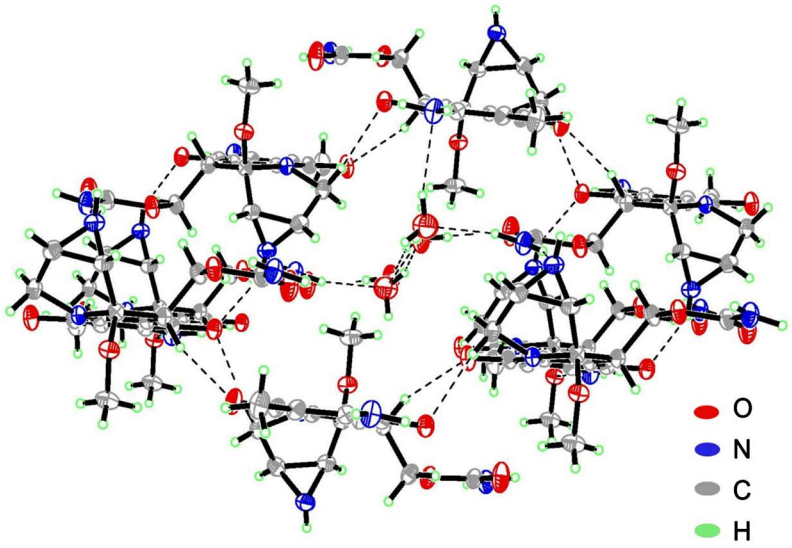


Figure 7. Molecule packing diagram of mitomycin C dihydrate in a unit cell.

Table 3. Hydrogen Bond Lengths (Å) and Bond Angles (˚).

D–H···A	d (D–H)	d (H···A)	d (D···A)	∠DHA
C (3)-H (3C)···O (2)[a]	0.97	2.47	3.423 (3)	167.9
C (3)-H (3D) ·· ·O (1)	0.97	2.46	2.999 (3)	114.9
C (10)-H (10)···O (1)[b]	0.98	2.48	3.393 (3)	154.8
C (13)-H (13B)···N (1)	0.97	2.61	3.231 (3)	121.7
N (1)-H (1A)···O (2)[c]	0.90 (4)	2.15 (4)	2.975 (3)	152 (3)
N (3)-H (3A)···O (3)[d]	0.89 (3)	2.03 (3)	2.903 (3)	168 (3)
N (4)-H (4A)···N (1)[c]	0.85 (4)	2.30 (4)	3.046 (3)	146 (3)
N (4)-H (4B) ···O (7)[b]	0.90 (5)	2.17 (5)	3.051 (4)	165 (4)
O (6)-H (6A)···O (7)	0.83 (3)	2.07 (4)	2.870 (5)	160 (5)
O (6)-H (6B)···O (5)[a]	0.84 (3)	1.93 (4)	2.758 (3)	166 (5)
O (7)-H (7A)···N (3)	0.91 (3)	2.35 (3)	3.225 (4)	160 (5)
O (7)-H (7B)···O (6)[e]	0.88 (3)	1.89 (4)	2.703 (4)	153 (6)

Symmetry codes: (a) −x, y + 1/2, −z + 3/2; (b) −x, y − 1/2, −z + 3/2; (c) x − 1/2, −y + 1/2, −z + 2; (d) x + 1, y, z; (e) x + 1/2, −y + 1/2, −z + 1.

3.3. Powder X-Ray Diffraction (PXRD) Analysis

The mitomycin C dihydrate is also characterized by Powder X-ray diffraction (PXRD). As shown in **Figure 8**, the PXRD pattern is almost consistent with its simulated spectra, indicating the sample has good purity.

4. Conclusion

In this research, it has been found that the absolute configuration of mitomycin C at C_{10}, C_{11}, C_1 and C_2 is, respectively, (S), (R), (S) and (S). This conclusion is confirmed by X-ray single crystal diffraction with Cu radiation, which could give

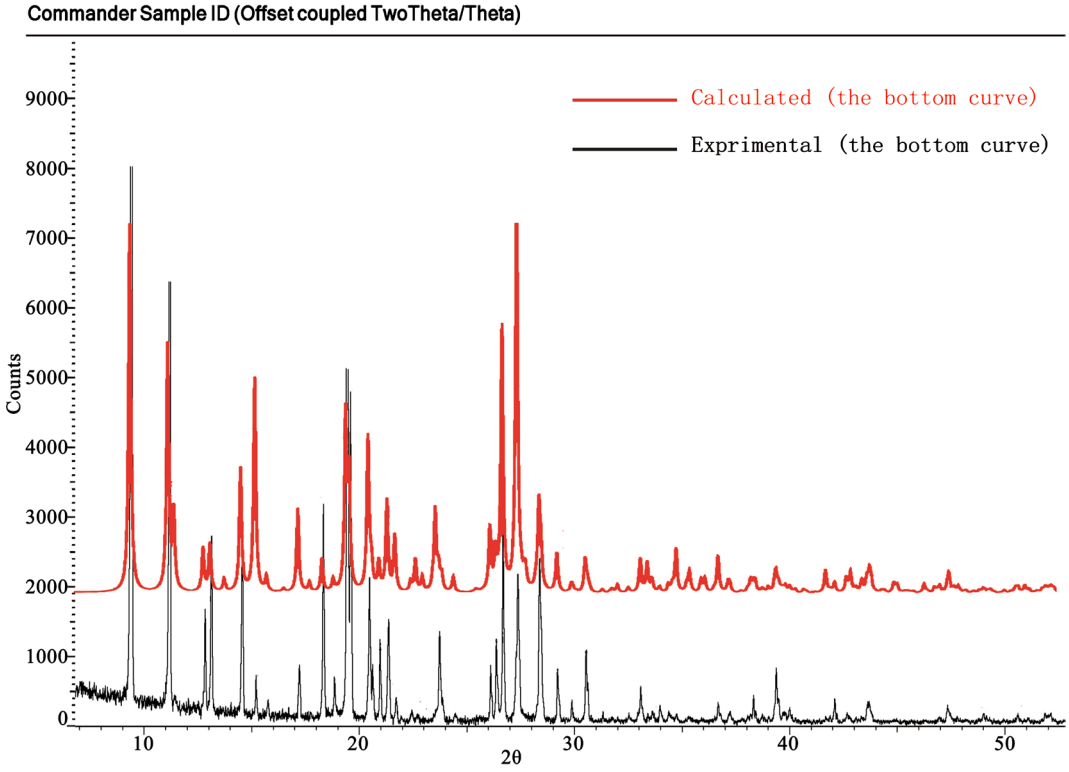


Figure 8. Experimental and calculated PXRD patterns.

a unified end to the argument about true absolute configuration of mitomycin C. In addition, the structure of mitomycin C also is confirmed and described in detail by 1D and 2D NMR spectra. What's more, a novel crystal morphology called mitomycin C dihydrate is obtained, which has the advantages of simple preparation and stable quality, and shows a promise to be a new drug crystal morphology.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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