Supplementary Information

Revealing the critical role of templates' functional groups ordering on template-directing crystallization of pyrazinamide

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1. Determination of PZA concentration.

In order to find the optimal experimental conditions of supersaturation in pyrazinamide template-induced crystallization, comparative experiments were carried on. Here, we listed the polymorphic outcomes of PZA with different amount of hydrochlorothiazide and different supersaturations of PZA in water in Table S1. As seen in Table S1, α form preferentially crystalized from water in the absence of template. The crystallization of γ form, though concomitantly with α form, is only possible when the supersaturation is extremely high (i.e., S=3.5). In contrast, the pure γ form can be easily crystallized in the presence of hydrochlorothiazide template, and the greater amounts of template the lower degree of supersaturation needed. It was found that pure γ form can be obtained at supersaturation low to 2.22 when the 10% template was added in supersaturated solution. For other six sulfonamides templates, pure γ form can be obtained only when the supersaturation reached at 2.99. In all, our experimental results indicated that template-induced pyrazinamide crystallization could be worked in supersaturation regions of S > 1.50, which depended on the type of template. Parambil and his coworker[1] reported similar conclusion, in which carbamazepine template-induced polymorphic domain was determined.

Table S1. Polymorphic Outcomes of PZA with different amount of hydrochlorothiazide and different supersaturation of PZA in water ($S = (c-c_0)/c_0$).

The amounts of templates	0.50	1.50	2.22	2.99	3.50
0	α	α	α	α	α+γ
1%	α	α	α	α+γ	γ
5%	α	α	α+γ	γ	γ
10%	α	α	γ	γ	γ

For methanol and ethanol, template-induced experiments were conducted in the supersaturation region from 0.90 to 3.50 (Tables S2 and S3) at different mounts of template. It was evident that hydrochlorothiazide template effectively regulating the crystallization of γ form in water does not alter crystallization outcomes in methanol and ethanol. Accounting for the above results of crystallization experiments, we thus set the supersaturation to be 2.99 in which template can regulate the preferential nucleation of pyrazinamide γ forms in water, and also the effect of template loading amounts can be studied.

Table S2. Polymorphic Outcomes of PZA with different amount of hydrochlorothiazide and different supersaturation of PZA in methanol ($S = (c-c_0)/c_0$).

The amounts of templates	0.90	1.10	1.69	2.99
0	δ	γ+β+δ	γ+β	γ+β
1%	δ	γ+β+δ	γ+β	γ+β
5%	δ	γ+β+δ	γ+β	γ+β
10%	δ	γ+β+δ	γ+β	γ+β

Table S3. Polymorphic Outcomes of PZA with different amount of hydrochlorothiazide and different supersaturation of PZA in ethanol ($S = (c-c_0)/c_0$).

The amounts of templates	0.90	1.05	1.87	2.99
0	δ	γ+δ	γ+β+δ	γ+β
1%	δ	γ+δ	γ+β+δ	γ+β
5%	δ	γ+δ	γ+β+δ	γ+β
10%	δ	γ+δ	γ+β+δ	γ+β

2. Determination of effective crystal face of template

For both templates, the crystal shapes are prepared into thin flakes (Figure S2 and S5). XRPD pattern of hydrochlorothiazide indicated that crystal faces perpendicular to [0 0 1] direction are the preferential facets. Combined with crystal habit shown in figure S2, the biggest face of used hydrochlorothiazide is (0 0 2). Figure 3 shows the (0 0 2) face with the sulfonamide groups pointing towards the surface. Sulfonamide groups can form hydrogen bonds with the carbonyl groups of pyrazinamide which promoted the nucleation of γ form.[2] Therefore, the facet perpendicular to [0 0 1] direction is the face who interacted with pyrazinamide molecules directly and induced the nucleation of γ form of pyrazinamide.

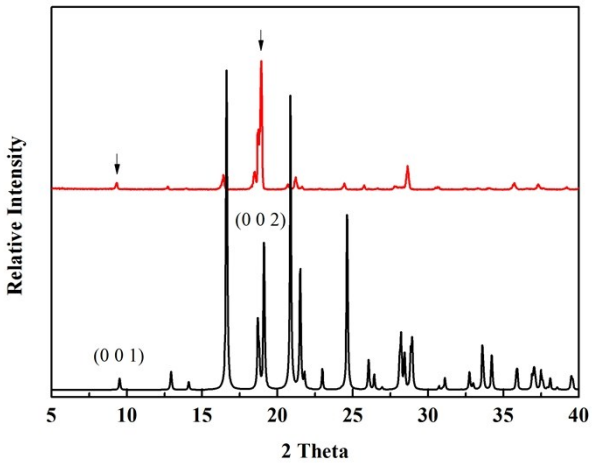

Figure S1. X-ray powder diffraction patterns of hydrochlorothiazide (red line), along with the simulated PXRD pattern (black line).

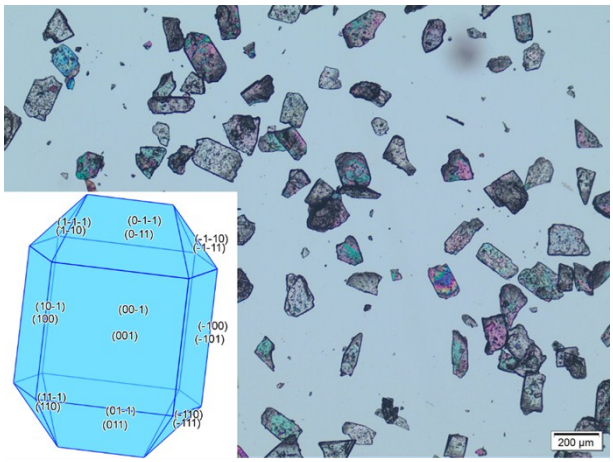

Figure S2. Microscope images of hydrochlorothiazide, imbedded image is BFDH morphology of hydrochlorothiazide calculated by Mercury.

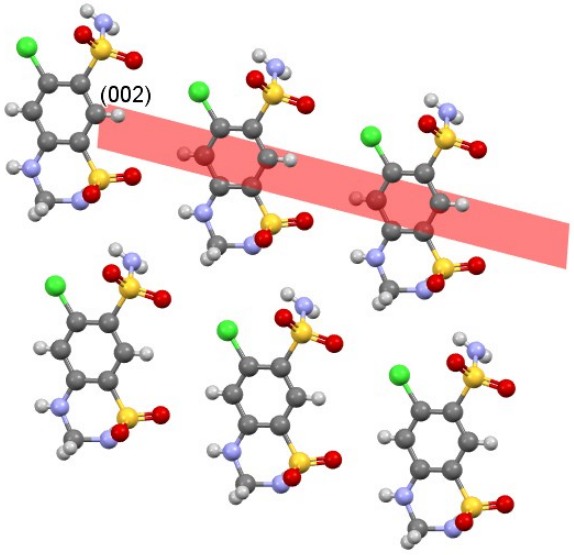

Figure S3. View of the (0 0 2) face of hydrochlorothiazide (view along b axis).

The situation in sulfathiazole is similar with that in hydrochlorothiazide. The sulfathiazole crystals used in this manuscript are very thin flakes with facet perpendicular to [1 0 0] being the biggest crystal face (Figure S4 and S5). As shown in Figure S6, the exposed functional groups on face (4 0 0) were found to be again sulfonamide groups. Thus, the vital facet which can modulate the nucleation outcomes of PZA is the face (4 0 0).

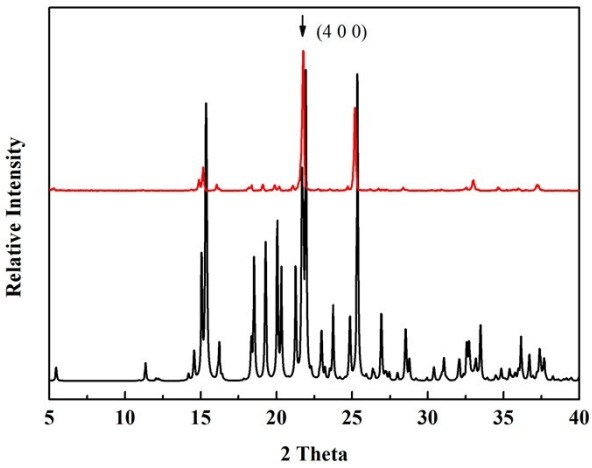

Figure S4. X-ray powder diffraction patterns of sulfathiazole (red line), along with the simulated PXRD pattern (black line).

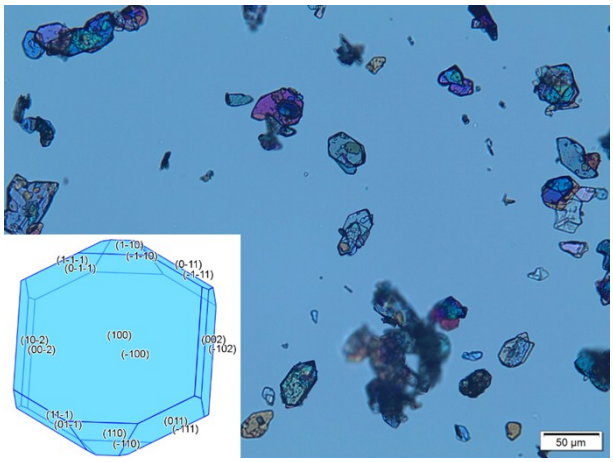

Figure S5. Microscope images of sulfathiazole, imbedded image is BFDH morphology of sulfathiazole calculated by Mercury.

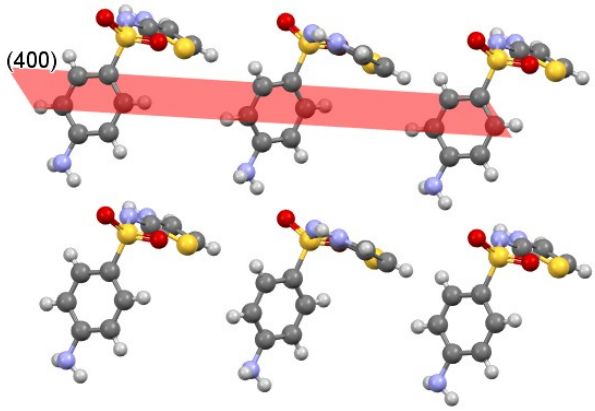

Figure S6. View of the (4 0 0) face of sulfathiazole (view along b axis).

3. Dissolution measurement of templates

The amounts of dissolution throughout crystallization experiments of the amorphous and crystalline forms of sulfathiazole and hydrochlorothiazide in water were determined by using gravimetric method.[3] The dissolve ability was analyzed during the experimental process in which

the solution was cooled from 45 °C to 10 °C with a cooling rate of 0.58 °C /min and during the cooling crystallization, the samples remained undisturbed. In the experiment, all measurements of mass were performed on an analytic balance (AB204-N, Mettler-Toledo, Switzerland) with uncertainty of ±0.0001 g. The measured amounts of dissolution in water was listed in Table S1.

To establish the pyrazinamide polymorph control and selection is due to the solid template effect not the soluble part, we conducted the same experimental process described in manuscript with template whose adding amount was the amounts of dissolution in Table S1. All the obtained PZA crystals were determined by X-ray Powder Diffraction. The results show that all the crystals were α form (Fig. S6) which indicated that the polymorph control and selection is due to solid template effect.

Table S4. Amounts of dissolution of the amorphous and crystalline forms of sulfathiazole and hydrochlorothiazide in water during the experimental process (from 45 °C to 10 °C with a cooling rate of 0.58 °C /min).

Amounts of dissolution (g/g)	Amorphous	Crystalline
Hydrochlorothiazide	0.00036-0.0044	0.00032-0.00036
Sulfathiazide	0.00044-0.00048	0.00040-0.00046

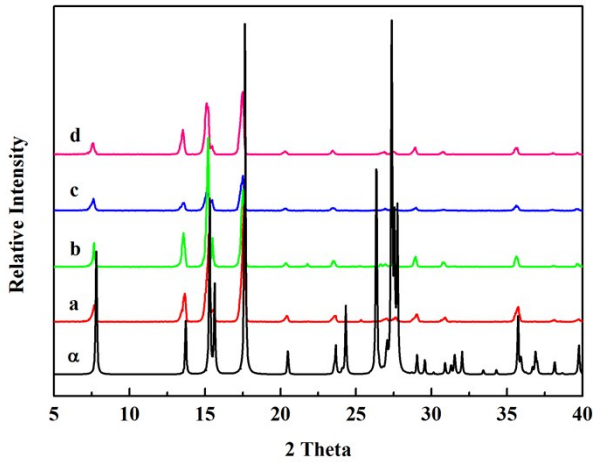

Fig. S7 X-ray powder diffraction patterns of obtained PZA crystals with a: soluble crystalline hydrochlorothiazide; b: with soluble amorphous hydrochlorothiazide; c: with soluble crystalline sulfathiazole; d: with soluble amorphous sulfathiazole, along with the simulated PXRD pattern of α form of PZA.

Table S5. Crystallographic Parameters of γ form of PZA, Allopurinol and Hydrochlorothiazide.

	Refcode	Space Group	a	b	c	Alpha	Beta	Gamma	*Z'*
Pyrazinamide γ	PYRZIN05	Pa	3.73	7.185	10.72	106.73	90	90	1
Allopurinol	ALOPUR	P21/c	3.683	14.685	10.318	90	97.47	90	1
Hydrochlorothiazide	HCSBTZ	P21	7.419	8.521	10.003	90	111.332	90	1

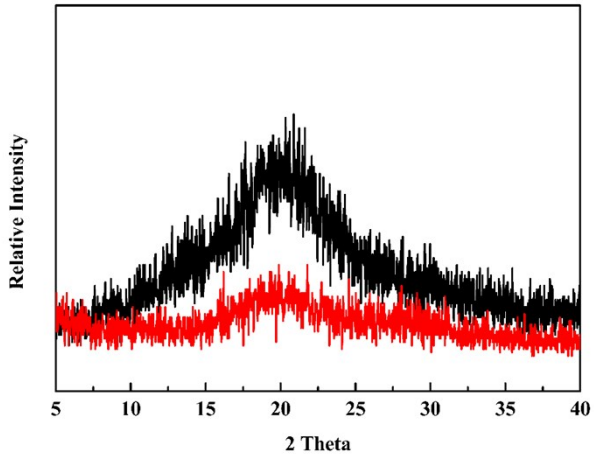

Fig. S8 X-ray powder diffraction patterns of amorphous hydrochlorothiazide (black line) and amorphous sulfathiazole (red line).

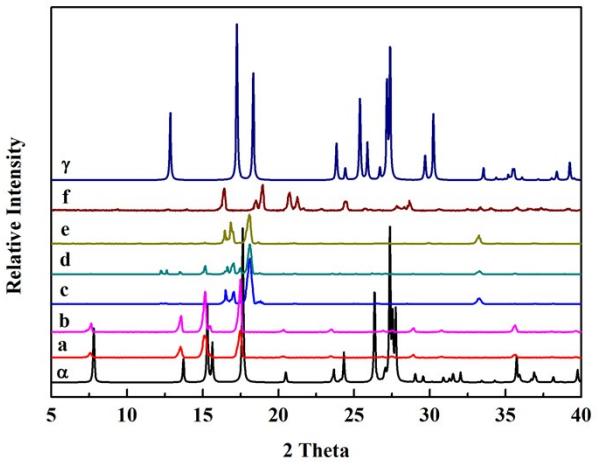

Fig. S9 X-ray powder diffraction patterns of obtained PZA crystals with hydrochlorothiazide (a: without templates; b: with amorphous templates; c: with physical mixture of crystalline templates: amorphous template = 1:4; d: with physical mixture of crystalline templates: amorphous template = 3:2; e: with crystalline templates; f: crystalline hydrochlorothiazide.), along with the simulated PXRD pattern of α form and γ form of PZA.

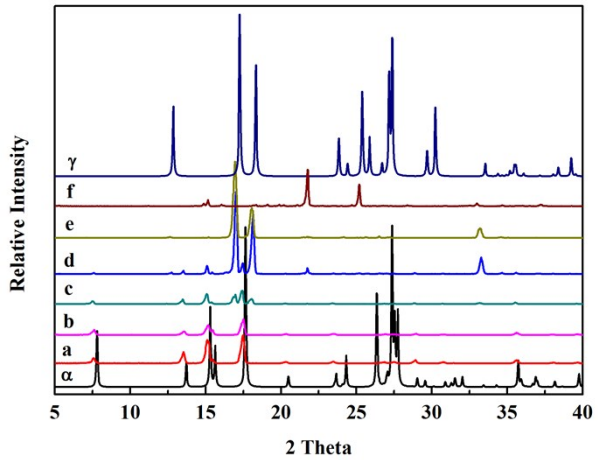

Fig. S10 X-ray powder diffraction patterns of obtained PZA crystals with sulfathiazole (a: without templates; b: with amorphous templates; c: with physical mixture of crystalline templates: amorphous template = 1:4; d: with physical mixture of crystalline templates: amorphous template = 3:2; e: with crystalline templates; f: crystalline sulfathiazole.), along with the simulated PXRD pattern of α form and γ form of PZA.

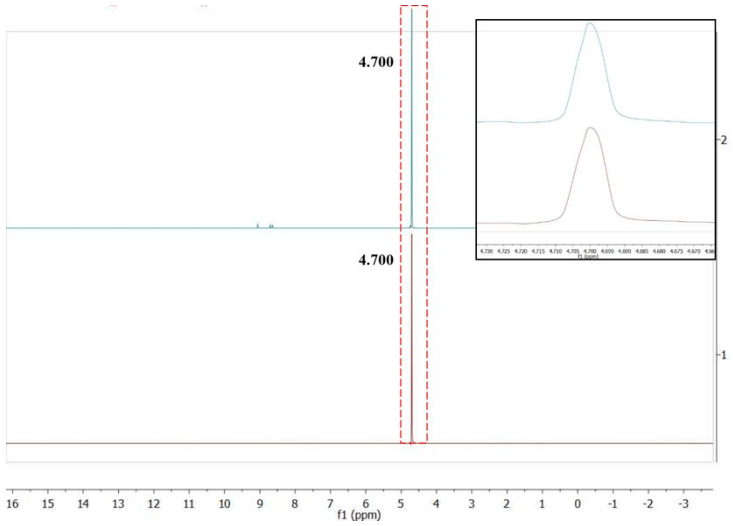

Fig. S11 ^{1}H NMR spectra of water-d_2 and pyrazinamide-water-d_2 solution. (The signals in red dashed box are from hydrogens.)

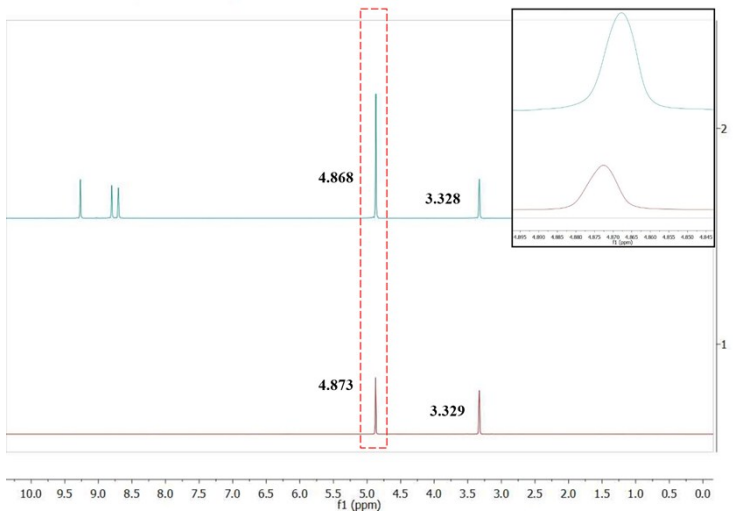

Fig. S12 ^{1}H NMR spectra of methanol-d_4 and pyrazinamide-methanol-d_4 solution. (The signals in red dashed box are from hydroxyl hydrogen.)

References:

[1] J. V. Parambil, S. K. Poornachary, R. B. H. Tan, J. Y. Y. Heng, J. Cryst. Growth, 2017, 469, 84–90.

[2] K. Zhang, S. Xu, S. Liu, W. Tang, X. Fu, J. Gong, Cryst. Growth Des., 2018, 18 (9), 4874-4879.

[3] K. Zhang, H. Shen, S. Xu, H. Zhang, M. Zhu, P. Shi, X. Fu, Y. Yang, J. Gong, J. Chem. Thermodyn., 2017, 112, 204-212.