Electronic Supplementary Information

Two polymorphs of Remdesivir: Crystal Structure, solubility, and pharmacokinetic study

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EXPERIMENTAL SECTION

Materials

Remdesivir is supplied by Zhejiang Ausun Pharmaceutical Co. Ltd. (Taizhou, China), purity >99% (by HPLC analysis). Roswell Park Memorial Institute (RPMI) 1640 cell culture medium for COS-7 and Dulbecco's modified Eagle medium (DMEM) for Vero-E6 were purchased from Bristol-Myers Squibb Trading Co. Ltd (New York, NY, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma (St. Louis, MO, USA). All the other chemicals were obtained directly from commercial sources and used as received.

Preparation of RDV-I and RDV-II

Preparation of polymorph I (**RDV-I**): Remdesivir was dissolved in a mixture of MeOH and CH_2Cl_2 at 50 °C, and then cooled down to the 0 °C to yield the single crystals of **RDV-I**. Preparation of polymorph II (**RDV-II**): Remdesivir was dissolved in MeCN at 90 °C, and then cooled to room temperature to give single crystals of **RDV-II**.

Powder X-ray Diffraction

The powder X-ray diffraction (PXRD) results were obtained on a Rigaku D/Max-2550 powder diffractometer (Rigaku Corporation, Tokyo, Japan) with CuKα radiation(λ=1.54059 Å) , operating at 40 kV and 250 mA. The scans were run from 3.0 to 40.0° (2θ), with an increasing step size of 0.02° (2θ) and scanning rate of 5 °/min. Data were processed using the MDI-Jade version 9.0 software.

Single-crystal X-ray Diffraction

Bruker D8 Quest CCD diffractometer (Bruker, Ettlingen, Germany) with Ga Kα (λ = 1.3414

Å) and Bruker Apex-II CCD diffractometer (Bruker, Ettlingen, Germany) with Mo Kα (λ = 0.7107 Å) radiation was used to collect the crystal data of **RDV-I** and **RDV-II**. The SAINT V8.38A program was used on data reduction. The absorption correction was applied with the use of semi-empirical methods of the SADABS program [1]. The crystal structures were solved by direct methods and refined with full-matrix least-squares methods with anisotropic thermal parameters for all non-hydrogen atoms on F^2 using SHELXL-2016 [2]. Hydrogen atoms were placed in the position of calculation and were refined isotropically using a riding model. Crystallographic data for the crystal structures have been deposited in the Cambridge Crystallographic Data Centre (CCDC) with supplementary numbers of 2022369 (**RDV-I**) and 2022370 (**RDV-II**). These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/data_request/cif. A summary of the key crystallographic data is listed in Table 1.

Refinment of RDV-I crystal structre: The two pendant alkane chains attached bonded to C22A and C22B displayed conformational disorder with their relative ratios of 0.45/0.55 and 0.47/0.53 refined for the disordered domains. The structure is twinned about (1 0 0) lattice direction and the twin matrix was suggested by ROTAX program while the HKLF 5 type file was prepared using MAKE HKLF5 in WinGX suite[3]. The ratios of two twinned domains were fined to 0.58/0.42.

Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (TA DSC Q100) was used to perform thermal analysis on the samples of RDV-I and RDV-II. Powder sample of approximately 2.4 mg was placed in an aluminum pan and heated at a rate of 10 °C min^{-1} under a nitrogen flow of 50 $mLmin^{-1}$ with a

temperature range of 20-200 °C.

FT-IR and Raman Spectroscopy

A VECTOR-22 Fourier Infrared Spectrometer was used to collect the FT-IR spectra in the range of 4000–400 cm^{-1}. Raman spectra were obtained using a LabRAM HR Evolution Raman spectrometer.

Solubility and Dissolution Measurement

To investigate the solubility of **RDV-I** and **RDV-II**, Thermo Scientific Evolution 300 UV–Vis spectrometer (Thermo Scientific, Waltham, MA) was used. The concentrations of **RDV-I** and **RDV-II** were calculated by the standard curve (λ_{max} = 245 nm). A beaker contains 200 mL distilled water (pH 7) was equilibrated at 37 °C, then approximately 100 mg of samples were added upon filtration through 100 mesh sieve. The mixture was stirred at 150 rpm with a magnetic stirrer. 4 mL of dissolved sample was withdrawn at specific time intervals for 240 min and replaced with an equal volume of the fresh medium to maintain a constant total volume. Each filtered aliquot was assayed by UV analysis at 245 nm. To ensure the accuracy of the experimental data, all experiments were repeated three times.

The solubility of **RDV-I**, **RDV-II** in pH 7 distilled water at 37 °C was measured by adding excess drug (about 200 mg) in 20 mL water in a 25 mL glass bottle. The bottles were then vortexed in the water bath at 100 rpm and kept at 37 °C (± 0.2 °C). The supernatant was collected after 24 h, then filtered with 0.22 μm nylon filters and measured by a UV spectrometer.

The in *vivo* experiments were performed in accordance with the CAPN (China Animal Protection Law), which were approved by the Zhejiang University Institutional Animal Care and Use Committee in China (approval ID: ZJU20190018). Six week old (20-25 g) male ICR mice

that were provided from the Zhejiang Chinese Medical University were used for in *vivo* experiments. A pathogen-free environment under controlled humidity and temperature was used for maintaining the mice.

In *Vivo* Pharmacokinetic Study

For pharmacokinetic experiments, ICR mice were randomly divided into two groups (8 for each group) for **RDV-I**, **RDV-II**. The drugs were orally administered with a dosage of 8 mg/kg. Blood was collected from the orbital sinus with the presence of a heparinized syringe at 15, 30, 45, 60, 75, 90, 105, and 120 min after drug administration. Blood was first centrifuged at 10000 rpm for 5 min, then 0.16 mL of the plasma was extracted, then trifluoroacetic acid was used to acid precipitate protein, and NaOH to neutralize the solution. The mixture was diluted using MeCN: H_2O (5:5 v/v). After 10 min of precipitating, supernatant fluids were collected by centrifugation at 10000 rpm for 5 min and filtered with syringe through a 0.22 μm hydrophilic membrane filter and measured by HPLC method. HPLC assay: The analytical column was an Agilent ZORBAX SB C18 column (4.6 mm × 150 mm, 5 μm. The mobile phase was MeCN: H_2O (5:5 v/v), the flow rate of mobile phase was 0.8 mL min^{-1} and the UV detector was at 245 nm.

In *Vivo* Biosafety Estimation

Mice were randomly divided into three groups (3 for each group) for **RDV-I**, **RDV-II**, and control. The drugs were orally administered daily with a dose of 4 mg/kg (suspension) for one day. For each mouse, 0.3 mL blood was collected and analyzed using an automatic analyzer (Hitachi 7020, Japan).

Table S1. Relevant crystallographic data for **RDV-I** and **II**.

Crystal data	**RDV-I**	**RDV-II**
CCDC No.	2022369	2022370
Chemical formula	$C_{27}H_{35}N_6O_8P$	$C_{27}H_{35}N_6O_8P$
M_r	602.58	602.58
Crystal system	triclinic	monoclinic
Space group	*P*1	$P2_1$
Temperature (K)	170(2)	170(2)
a (Å)	8.5565(11)	10.5286(17)
b (Å)	10.5456(16)	12.809(2)
c (Å)	17.147(2)	11.1106(19)
α (°)	96.105(4)	90
β (°)	99.219(4)	100.022(5)
γ (°)	94.937(4)	90
V (Å^3)	1510.1(4)	1475.6(4)
Z	2	2
D_c/(g cm^{-3})	1.34139	1.356
Radiation type	GaKα	MoKα
μ(mm^{-1})	0.837	0.152
F(000)	636	636
T_{min}, T_{max}	0.5372, 0.7508	0.4799, 0.7455
Measured, independent, and observed reflections	24511, 24511, 9621	28620, 6455, 5990

Flack parameter	0.09(4)	−0.01(4)
R_{int}	0.1356	0.0606
$R[F^2>2\sigma(F^2)]$, $wR(F^2)$, S	0.0885, 0.2506, 0.998	0.0572, 0.1538,1.077
No. of parameters	860	384
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	−0.339, 0.299	−0.332, 0.961

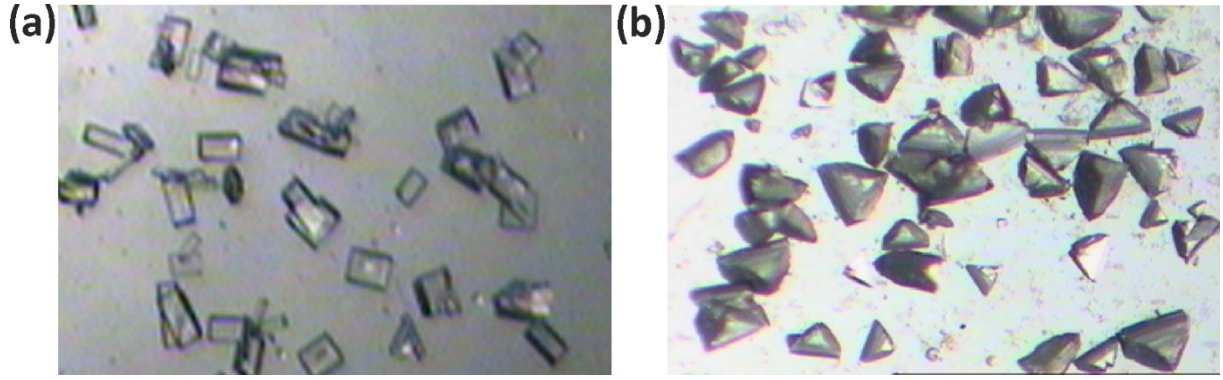

Fig. S1 (a) Optical microscope graphs of **RDV-I** (a) and **RDV-II** (b) crystals.

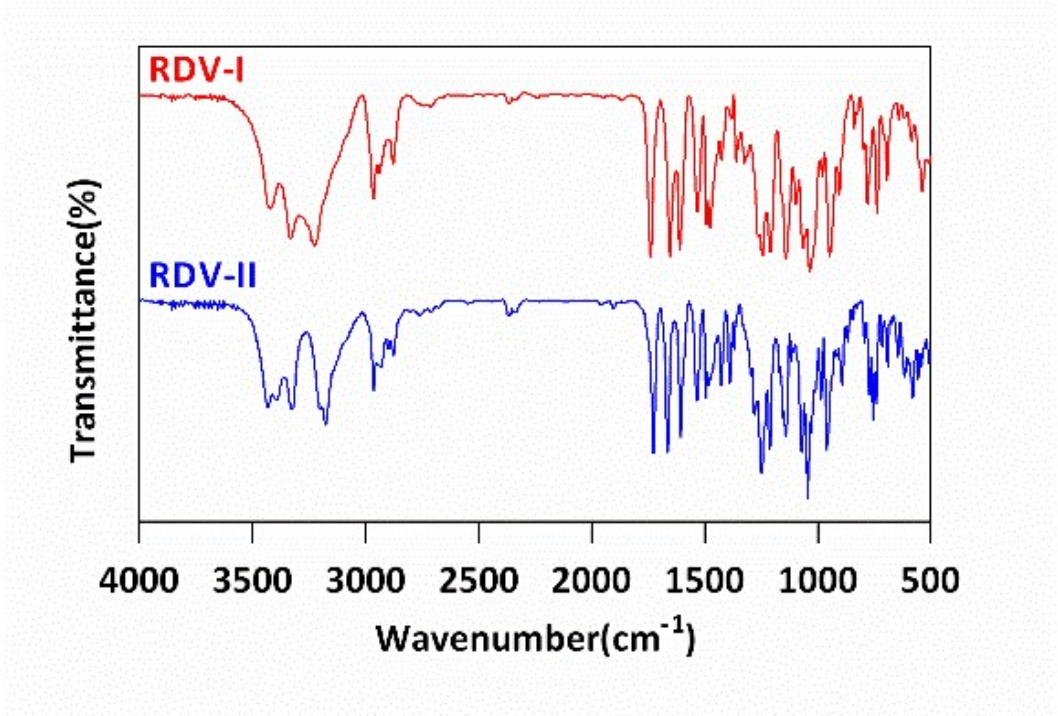

Fig. S2 FT-IR spectra of **RDV-I** and **RDV-II**. O−H stretching modes: 3422 cm^{-1} for RDV-I and 3431 cm^{-1} for RDV-II; N−H vibrational modes: 3329/3223 cm^{-1} for RDV-I and 3322/3174 cm^{-1} for RDV-II.

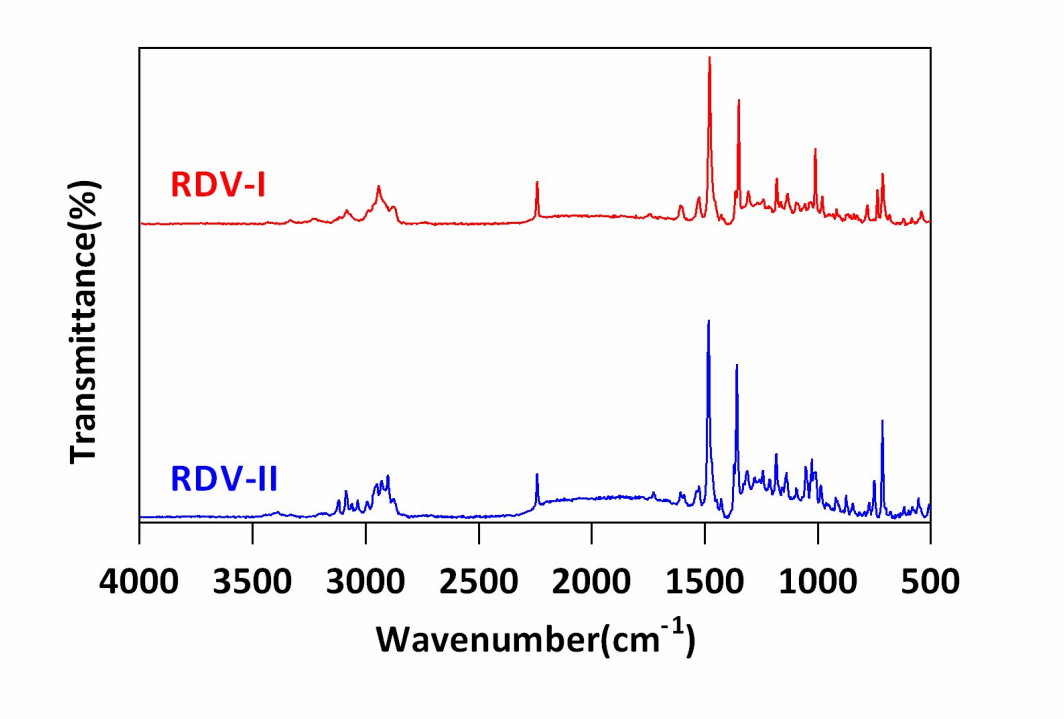

Fig. S3 The Raman spectra of **RDV-I** and **RDV-II**. O−H stretching vibration: 3380 ± 100 cm^{-1}; N−H stretching vibration: 3300-3500 cm^{-1}.

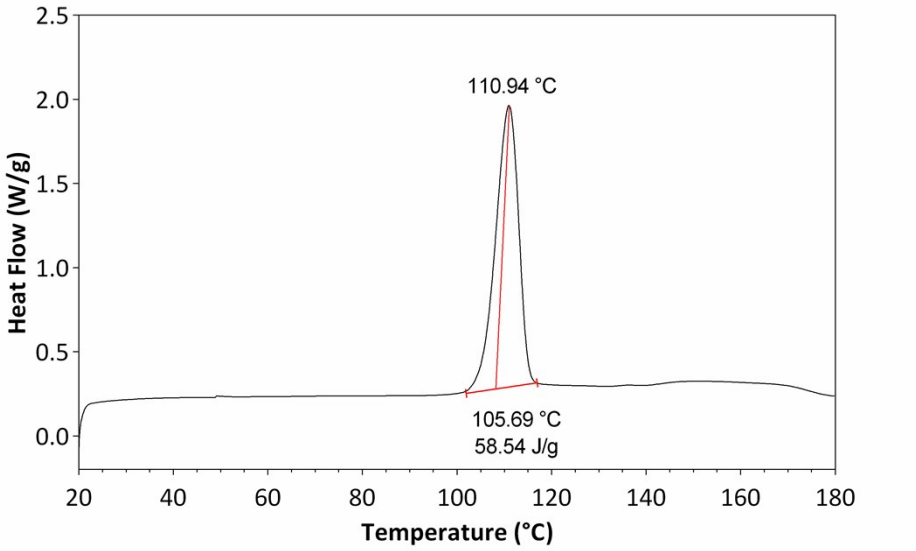

Fig. S4 The differential scanning calorimetry (DSC) plots for **RDV-I**.

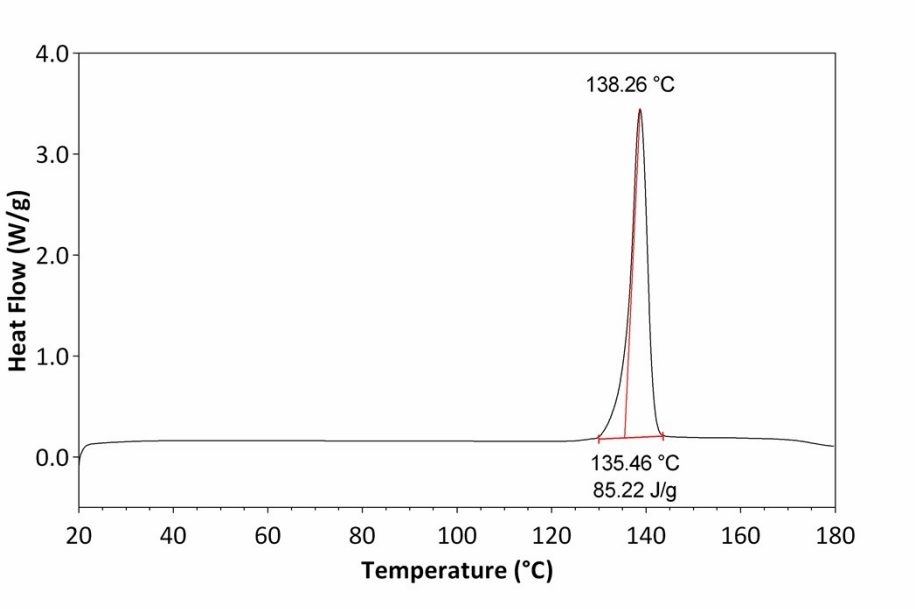

Fig. S5 The differential scanning calorimetry (DSC) plots for **RDV-II**.

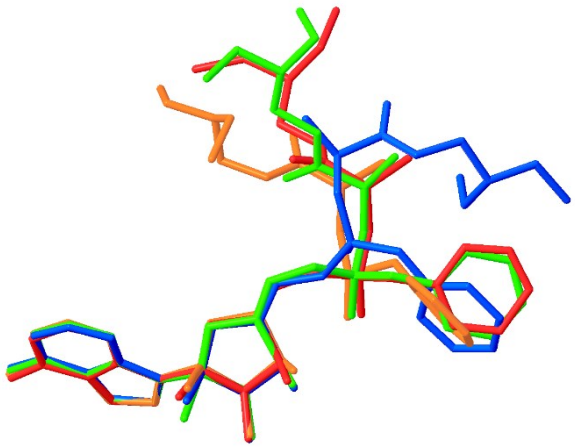

Fig. S6 A superposition of the molecular conformations of **RDV-I**, **RDV-II** and **RDV-III**. Molecular conformation of **RDV-I**, **RDV-II** and **RDV-III** were shown in red/orange, blue and green, respectively.

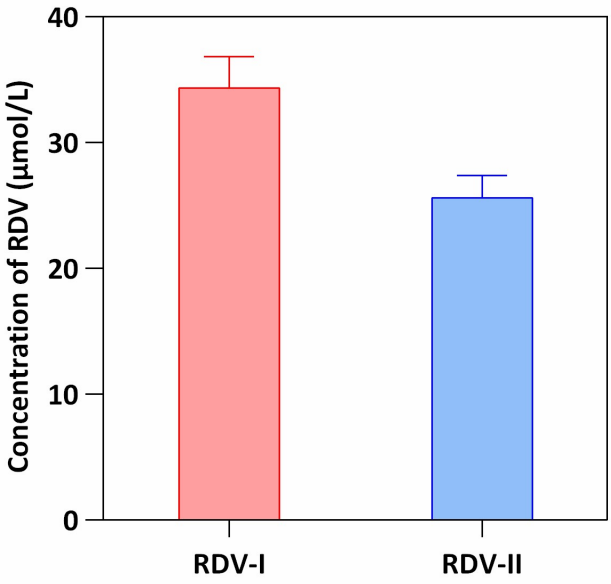

Fig. S7 Solubility data of **RDV-I** and **RDV-II** upon equilibrium for 24 h.

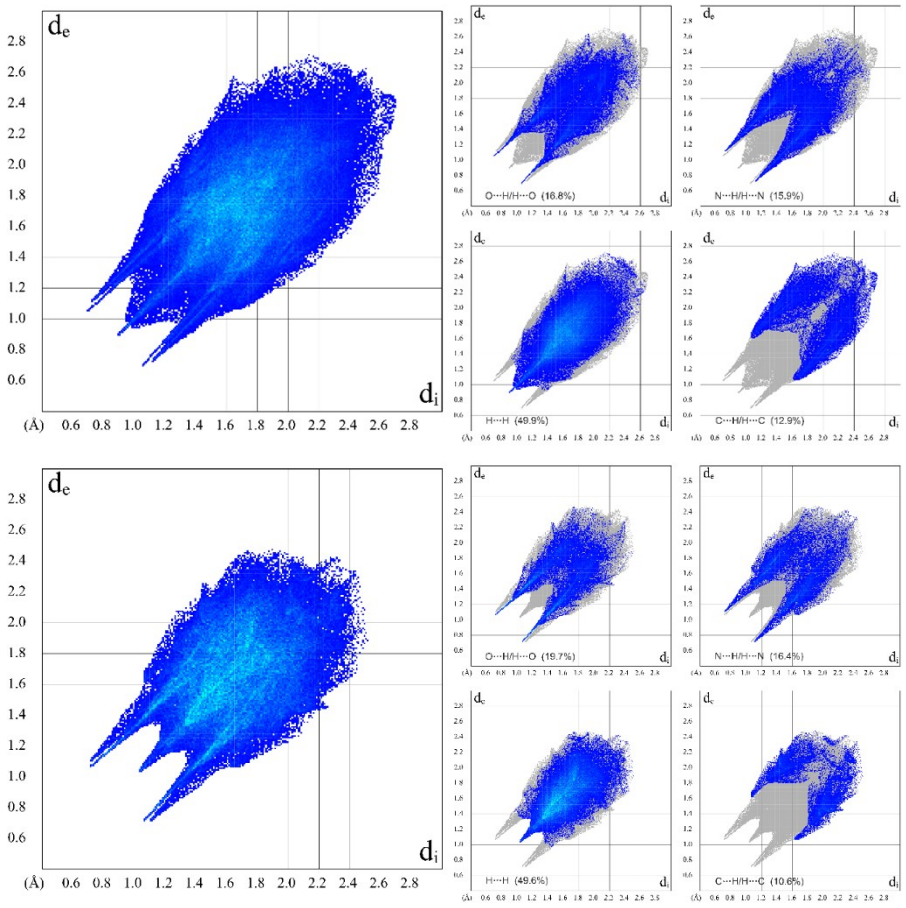

Fig. S8 Fingerprint plots of **RDV-I** (up), **RDV-II** (bottom).

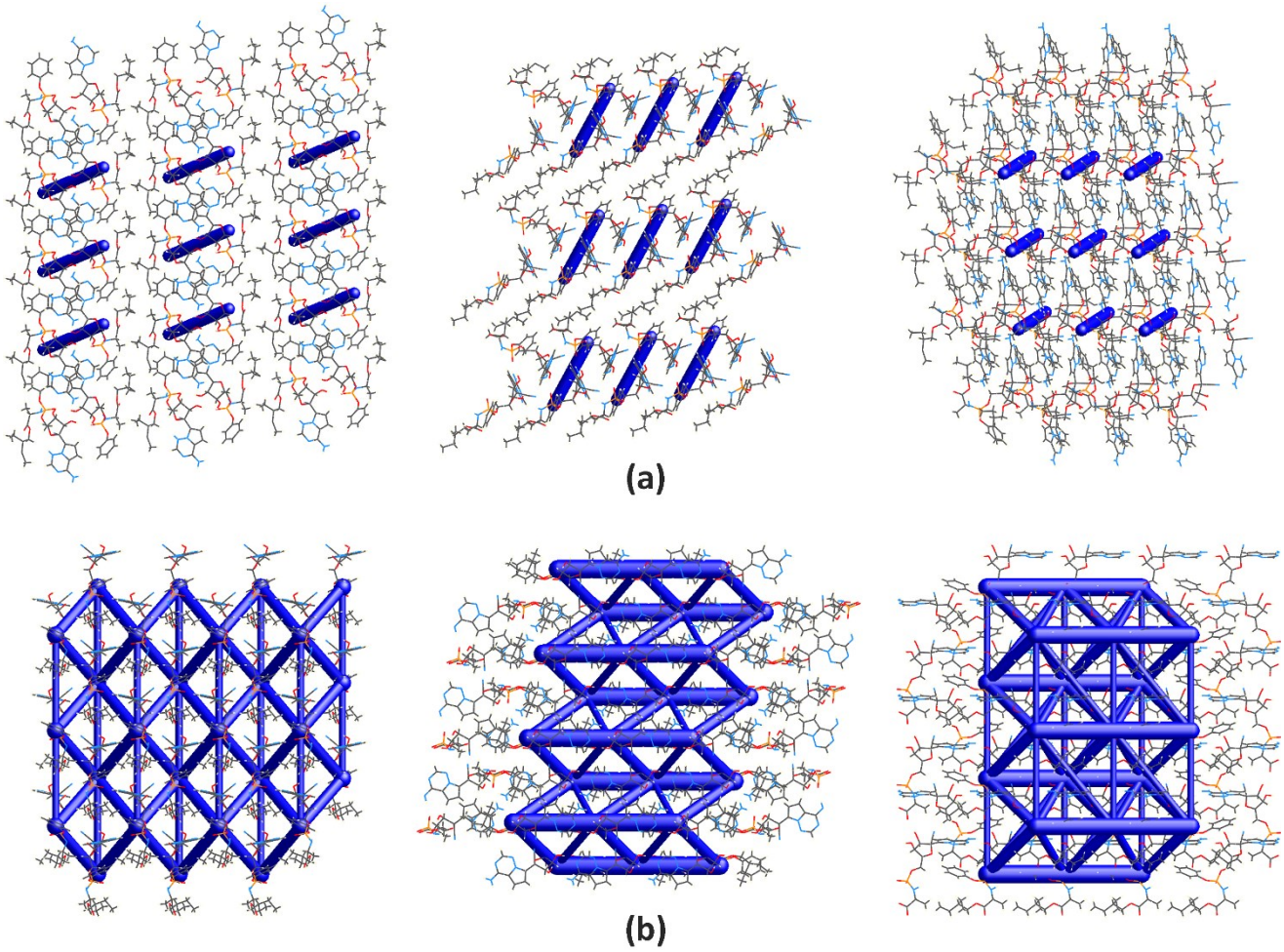

Fig. S9 Energy frameworks for the crystal structures of (a) **RDV-I** and (b) **RDV-II** viewed along the down *a* axis (left), *b* axis (middle), and *c* axis(right). The energy scale factor is 150, and the interaction energies with magnitudes smaller than 10 kJ/mol have been omitted.

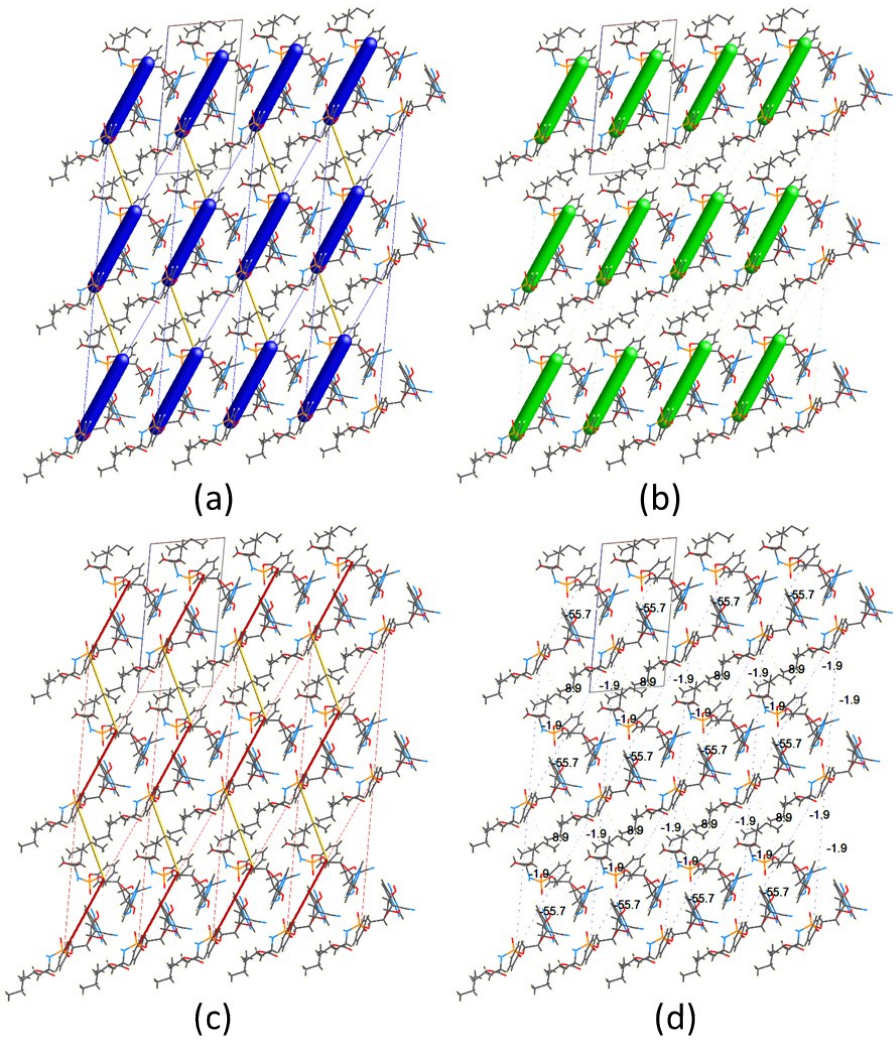

Fig. S10 Energy frameworks for the crystal structures of **RDV-I** viewed along the down *b* axis. The energy scale factor is 150. The blue cylinder (a), green cylinder (b), red cylinder (c) are the total energy, dispersion energy and coulomb energy respectively. The dotted number is the value of total energy (d).

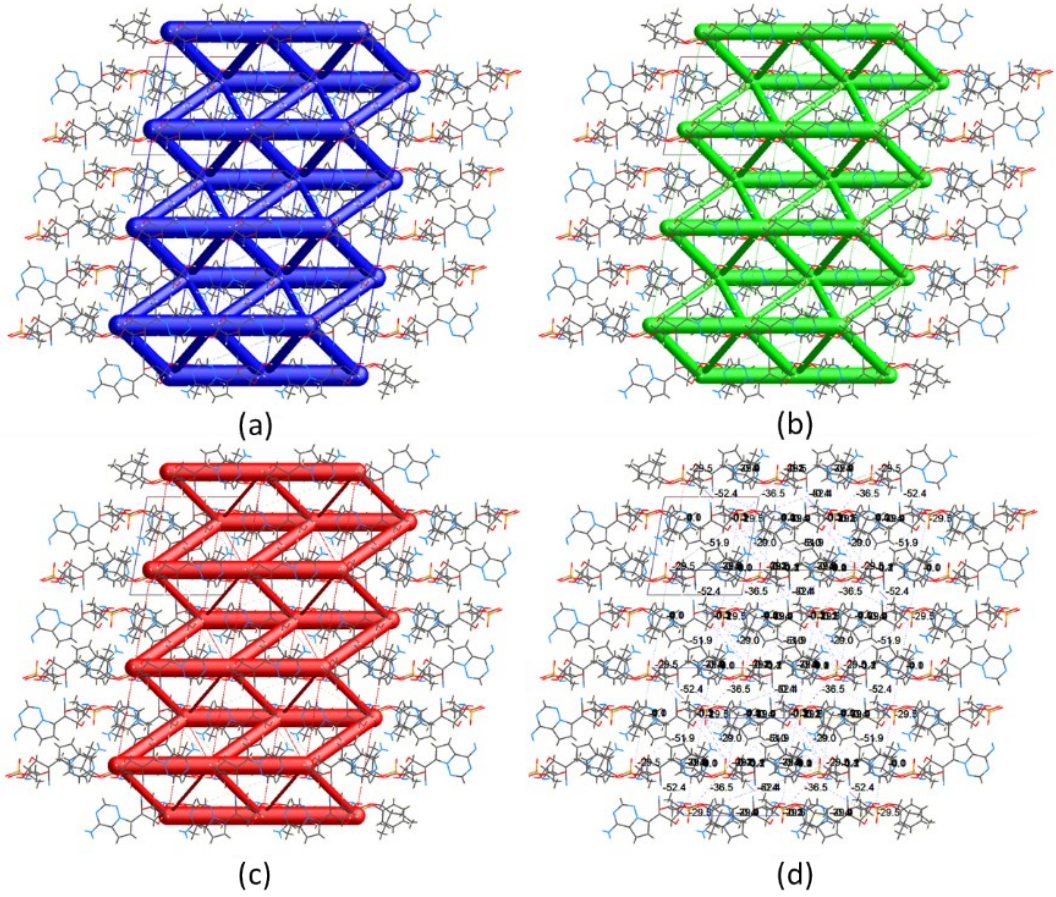

Fig. S11 Energy frameworks for the crystal structures of **RDV-II** viewed along the down *b* axis. The energy scale factor is 150. The blue cylinder (a), green cylinder (b), red cylinder (c) are the total energy, dispersion energy and coulomb energy respectively. The dotted number is the value of total energy (d).

1 Bruker *APEX2, SAINT and SADABS. Bruker ACS Inc., Madison, Wisconsin, USA.* **2014**.

2 Sheldrick, G. Crystal structure refinement with SHELXL. *Acta Crystallogr., Sect. C* **2015**, *71*, 3−8.

3 Farrugia, L. J. WinGX suite for smallmolecule single-crystal crystallography. *J. Appl. Cryst.* **1999**, *32*, 837−838.