Four Novel Pharmaceutical Cocrystals of Oxyresveratrol, Including a 2:3 Cocrystal with Betaine

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Regular Article

Cocrystal engineering can alter the physicochemical properties of a drug and generate a superior drug candidate for formulation design. Oxyresveratrol (ORV) exhibits a poor solubility in aqueous environments, thereby resulting in a poor bioavailability. Extensive cocrystal screening of ORV with 67 cocrystal formers (coformers) bearing various functional groups was therefore conducted using grinding, liquid-assisted grinding, solvent evaporation, and slurry methods. Six cocrystals (ORV with betaine (BTN), L-proline (PRL), isonicotinamide, nicotinamide, urea, and ethyl maltol) were found, including four novel cocrystals. Powder X-ray diffraction, low frequency Raman spectroscopy, and thermal analysis revealed unique crystal forms in all obtained samples. Conventional Raman and infrared data differentiated the cocrystals by the presence or absence of a hydrogen bond interacting with the aromatic ring of ORV. The crystal structures were then elucidated by single-crystal X-ray diffraction. Two new cocrystals consisting of ORV:BTN (2:3) and ORV:PRL:H2O (1:2:1) were identified, and their crystal structures were solved. We report novel cocrystal-line solids of ORV with improved aqueous solubilities and the unique cage-like crystal structures.

Key words cocrystal; oxyresveratrol; cocrystal screening; solubilized formulation; cage-like structure
and tend to yield high crystallinities and thermodynamically stable cocrystals.\textsuperscript{21,25} Recently, the nano-spot method has been described as a specific and verified tool for exploring possible cocrystals from small masses (approx. 10 ng) of microcrystals precipitated on a hydrophobized glass plate. Due to this low consumption of the API, it is particularly useful in drug screening and discovery.\textsuperscript{13,26}

Oxysresveratrol (ORV) is well-known as a polyphenolic stilbene plant extract from the heartwood of \textit{Artocarpus lakoocha} Roxburgh (Moraceae). It is commonly used in Thai traditional therapies,\textsuperscript{27} and has been revealed to possess a number of pharmacological effects, such as antioxidant/anti-inflammatory,\textsuperscript{28,29} tyrosinase enzyme inhibition,\textsuperscript{30} and neuroprotective effects,\textsuperscript{31} in addition to antiviral activities against herpes simplex virus (HSV-1 and HSV-2),\textsuperscript{32,33} varicella zoster virus,\textsuperscript{34} and influenza virus.\textsuperscript{35}

Previously, ORV cocrystals with citric acid and glutaric acid have been reported by Suzuki \textit{et al.},\textsuperscript{36} while Ouiyangkul \textit{et al.} identified ORV cocrystals with nicotinamide and proline,\textsuperscript{37} and solved their single crystal structures.\textsuperscript{38} Although studies investigating molecular crystals are key to achieving the desired modifications of physicochemical properties and dosage forms, it remains a poorly understood area. Thus, the purpose of the current study is to explore new ORV cocrystals comprehensively by rationalizing the experimental process for a screening using 67 coformers with a range of chemical structures (\textit{i.e.}, 20 acids, 24 amino acids, 10 amides, and 13 saccharides, supplementary Table S1). Screening and scale-up were conducted using the grinding (GM), LAG, solvent evaporation (SE), and Slu methods, and the obtained cocrystals were characterized by powder and single crystal X-ray diffraction, Raman and IR spectroscopy, and thermal analysis. Single crystal structures were also solved for two cocrystals, and the solubilities of the obtained cocrystals were investigated.

**Experimental**

**Materials** ORV anhydrate (chemical purity 99.25%) and ORV dihydrate were obtained from the Natural Products for Ageing and Chronic Disease Research Unit and Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University (Bangkok, Thailand). A total of 67 coformers, consisting of acids, amides, amino acids, saccharides, were used for the screening studies. These coformers were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). ORV was used as an API, while the other compounds were used as coformers. Ethyl acetate (AcOEt), chloroform (CHCl\textsubscript{3}), ethanol (EtOH, >99.5%), methanol (MeOH), n-hexane, acetone, sodium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, sodium chloride, and hydrochloric acid were purchased from Tokyo Chemical Co., Ltd. (Tokyo, Japan). A total of 67 coformers, consisting of acids, amides, amino acids, saccharides, were used for the screening studies. These coformers were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). ORV was used as an API, while the other compounds were used as coformers. Ethyl acetate (AcOEt), chloroform (CHCl\textsubscript{3}), ethanol (EtOH, >99.5%), methanol (MeOH), n-hexane, acetone, sodium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, sodium chloride, and hydrochloric acid were purchased from Tokyo Chemical Co., Ltd. (Tokyo, Japan). A total of 67 coformers, consisting of acids, amides, amino acids, saccharides, were used for the screening studies. These coformers were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). ORV was used as an API, while the other compounds were used as coformers. Ethyl acetate (AcOEt), chloroform (CHCl\textsubscript{3}), ethanol (EtOH, >99.5%), methanol (MeOH), n-hexane, acetone, sodium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, sodium chloride, and hydrochloric acid were purchased from Tokyo Chemical Co., Ltd. (Tokyo, Japan). A total of 67 coformers, consisting of acids, amides, amino acids, saccharides, were used for the screening studies. These coformers were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). ORV was used as an API, while the other compounds were used as coformers. Ethyl acetate (AcOEt), chloroform (CHCl\textsubscript{3}), ethanol (EtOH, >99.5%), methanol (MeOH), n-hexane, acetone, sodium dihydrogen phosphate, anhydrous disodium hydrogen phosphate, sodium chloride, and hydrochloric acid were purchased from Tokyo Chemical Co., Ltd. (Tokyo, Japan).

**Cocrystal Preparation** For the screening, ORV and the desired coformer were weighed in an equimolar ratio (1:1, total mass 100 mg) and pre-mixed in an agate mortar to obtain a physical mixture (PM). The SE or Slu method was additionally chosen for further scale-up to permit full characterization and dissolution studies, depending on the sample yield and purity.

**Cocrystal Screening by the LAG and GM Methods** Each PM was transferred to a 2.0 mL sample tube with two grinding balls (5 mmϕ, zirconia type), and the desired solvent was added (20 µL, AcOEt and CHCl\textsubscript{3} were selected as good and poor solubility solvents for the coformers, respectively). No solvent was added in the case of the GM. Then, each mixture was ground for 24 h using a multi-sample vibrating ball mill (MSC-100, BMS, Tokyo, Japan) under a rotational speed of 1500 rpm and a temperature of 25 °C.

**Liquid/Liquid Diffusion Method** Each PM was completely dissolved in the desired solvent (50 mL of AcOEt, EtOH, EtOH–water, or acetone) depending on the coformer, and then evaporated at 40 °C using a rotary evaporator under reduced pressure (50mmHg/Torr) until a clear solution was obtained.

**Scale-up by the Solvent Evaporation (SE) Method** A single crystal of the cocrystal of ORV with PRL was obtained serendipitously. The PM of ORV and PRL (100 mg, 1:1 ratio) was dissolved in MeOH and allowed to crystallize statically under ambient conditions. The sample bottle was wrapped with filter paper to allow the solvent to evaporate slowly, and the crystals formed as a precipitate at the bottom of the bottle.

**Differential thermal analysis (TG-DTA)** was performed using a Thermo Plus EVO2 TG8121 instrument (Rigaku Corporation, Tokyo, Japan). The TG-DTA curves were obtained at a heating rate of 5 °C/min under a flow of nitrogen gas.

**Conventional Region (CV) and Low-Frequency Region (LF) Raman Spectroscopy** A microscopic Raman spectrometer (Workstation, Kaiser Optical Systems, Inc., CA, U.S.A.) was used for measurement of the conventional region (CV) spectra (200–2400 cm\textsuperscript{-1}). A Performance BallProbe attached to an all-in-one Raman spectrometer (MarQMetrix Inc., WA, U.S.A.) was also used. The laser wavelength was set at 785 nm, the exposure times were 5 or 0.1 s, and three data accumulations were used.

A low frequency (LF) Raman spectroscopy unit (THz-Raman Micro, Coherent Inc., CA, U.S.A.) was used for measurement in the low wavenumber region (200 to 200 cm\textsuperscript{-1}). Measurements were carried out at a laser power of 58 mW with a laser wavelength of 976 nm, an exposure time of 5 s,
and an accumulation count of three. A THz-Raman® probe system comprising a TR-PROBE (Coherent Inc.) attached to an all-in-one Raman spectrometer (MarqMetrix Inc.) with high laser power (300 mW) was also used with a laser wavelength of 808 nm, an exposure time of 0.1 s, and an accumulation count of three.

**Fourier Transform IR Spectroscopy (FT-IR)**

The ATR FT-IR spectra were acquired using a MIRacleA instrument (S.T.JAPAN Inc., Tokyo, Japan) with an accumulation of 100 scans between 4000 and 600 cm\(^{-1}\). An empty cell was used to record the background, and labSolutions IR software (Shimadzu, Kyoto, Japan) was used to process the data.

**Single-Crystal X-Ray Diffraction Measurements (SCXRD)**

X-ray single crystal structural analyses were performed using a RAXIS RAPID diffractometer equipped with a MicroMax-007HF X-ray source (Rigaku, Tokyo, Japan) with multilayer mirror monochromated Cu Kα radiation (\(λ = 1.54187\) Å). The tube voltage and applied current were 40 kV and 30 mA, respectively. The single crystals were immersed in oil and then flash-cooled to 93 K under a flow of nitrogen gas. The single crystal sizes of ORV-BTN and ORV-PRL were 0.220 \(×\) 0.100 \(×\) 0.080 and 0.220 \(×\) 0.190 \(×\) 0.170 mm, respectively. The crystal structures were solved by a direct method using SHELXT version 2014/4.42 software \(^{39}\) and refined using SHELXL version 2014/7.43 software (full-matrix least-squares refinement). \(^{40}\)

**Dissolution Testing**

To evaluate the physical properties and solubilities of the cocrystals, dissolution tests were performed using a RAXIS RAPID diffractometer equipped with a MicroMax-007HF X-ray source (Rigaku, Tokyo, Japan) with multilayer mirror monochromated Cu Kα radiation (\(λ = 1.54187\) Å). The tube voltage and applied current were 40 kV and 30 mA, respectively. The single crystals were immersed in oil and then flash-cooled to 93 K under a flow of nitrogen gas. The single crystal sizes of ORV-BTN and ORV-PRL were 0.220 \(×\) 0.100 \(×\) 0.080 and 0.220 \(×\) 0.190 \(×\) 0.170 mm, respectively. The crystal structures were solved by a direct method using SHELXT version 2014/4.42 software \(^{39}\) and refined using SHELXL version 2014/7.43 software (full-matrix least-squares refinement). \(^{40}\)

**Table 1. Screening of Cocrystallization Procedure by PXRD**

| API     | Coformers     | Betaine (BTN) | Isonicotinamide (INI) | Nicotinamide (NIC) | Urea (URE) | L-Proline (PRL) | Ethyl maltol (ETM) |
|---------|---------------|---------------|-----------------------|--------------------|------------|-----------------|-------------------|
| ORV     | AcOEt         | ○             | ×                     | ○                  | ○          | ○               | ○                 |
|         | CHCl₃         | ○             | ×                     | ×                  | ○          | ○               | ○                 |
| SE      | AcOEt         | -             | -                     | -                  | -          | -               | -                 |
|         | EtOH (>99.5%) | ○             | ○                     | ○                  | ○          | ○               | ○                 |
|         | EtOH·H₂O      | -             | -                     | -                  | -          | -               | -                 |
|         | Acetone       | -             | -                     | -                  | -          | -               | -                 |
| Slurry  | AcOEt         | 24 h          | ×                     | ○                  | ×          | ×               | ×                 |
|         |               | 72 h          | ×                     | -                  | -          | ×               | -                 |
|         | EtOH          | 24 h          | ×                     | ○                  | ×          | ○               | ×                 |
|         |               | 72 h          | ○                     | -                  | -          | ○               | ○                 |
|         | CHCl₃         | 24 h          | ×                     | ○                  | ×          | ○               | ×                 |
|         |               | 72 h          | ×                     | -                  | -          | ×               | -                 |
|         | H₂O           | 24 h          | ○                     | -                  | -          | ○               | ○                 |

○: New crystalline form, ×: no change, —: not conducted, *1: This paper.
performed by comparison of the obtained cocrystals to ORV anhydrate. More specifically, a sample of the ORV powder (300 mg) was sieved (150 µm) to obtain uniform particles. Lactose monohydrate (600 mg) was vortex-mixed with ORV to improve the dispersibility of ORV in the test solution. The resulting mixture was then added to a pH 6.8 dissolution medium (300 mL, JP 2, the Japanese Pharmacopoeia) using the dissolution tester (NTR-3000, Toyama Industry Co., Ltd., Osaka, Japan). The tests were performed according to the Japanese Pharmacopoeia, 17th Edition using the paddle method with a rotation speed of 100 rpm and a temperature of 37 ± 0.5 °C. Aliquots (5 mL) were taken after 5, 10, 15, 20, 25, 30, 60, 90, and 120 min, after which 5 mL of the dissolution medium were added after each sampling. They were filtered by 0.45 µm filters and diluted them between the standard concentrations, under 10 µg/mL. The absorbance of each sample solution at 325 nm was measured using an UV-visible spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). The concentration at each time point was calculated in µg/mL, and all measurements were carried out in triplicate.

Results and Discussion
Cocrystal Screening A total of 67 multicomponent crystalline forms consisting of ORV and various guest molecules successfully generated six unique solid phases including four novel cocrystals (ORV with betaine (BTN), isonicotinamide (INI), urea (URE), and ethyl maltol (ETM)) by various screening experiments (Table 1). Notably, ETM is the first saccharide coformer to be isolated as a cocrystal with ORV. The six coformers were obtained from the amino acid, amide, and saccharide groups, which indicates that there is a limited systemic solution to predict which coformers bind to ORV. The four novel cocrystals were identified using PXRD, LF and CV Raman scattering, IR spectroscopy, and thermal analysis, which were employed as complementary characterization methods for confirming a cocrystal hit (supplementary Figs. S1–S5). The single crystal structures of 2 of the cocrystals were also determined, as discussed below.

Following the investigations of the various cocrystal screening methods (Table 1), the LAG approach (with the addition of AcOEt) produced all six cocrystals including the novel four cocrystals, while the SE method using the same solvent gave only one cocrystal. However, when EtOH was used as the solvent in the SE method, the all six cocrystals were produced. Generally, all the approaches are able to randomly generate ORV cocrystals under the appropriate conditions, as outlined in Table 1. However, selection of the appropriate solvent for each method was essential, as different solvents were required to obtain the cocrystals using the LAG, SE, and Slu methods.

In the Slu method, the potentially successful rate was found to be limited under specific conditions. More specifically, EtOH was the most appropriate solvent for the six cocrystals, including the four novel cocrystals. However, the processing time was also found to be a key parameter since this was associated with the energy input into the system to induce the intermolecular hydrogen bonding interactions between the API and the coformer. A longer processing time facilitated cocrystal formation with BTN, URE, and ETM in EtOH; however, in CHCl₃, no cocrystal formation was observed with BTN and URE, despite increasing the processing time from 24 to 72 h. Conversely, the Slu method was previously reported to be an efficient screening technique, and in our study the solution-mediated process under ambient conditions required a processing time between 12 h and 8 d to obtain cocrystal transformation. In addition, water was selected in the context of green chemistry, and it was found that cocrystals could be formed using such an aqueous Slu method wherein amino acid and saccharide coformers were employed.

The SE and Slu methods were previously reported to be appropriate techniques for scale-up; however, the most appropriate solvent must still be chosen to optimize cocrystal formation under a low solvent volume and a short processing time. We found that the SE method was more feasible for scale-up from a standpoint in purity and crystallinity of cocrystals between SE and Slu methods in this study. It should be noted here that the Slu method was demonstrated for solely ORV-BTN because the SE method was significantly more time consuming and produced extremely small crystals.

Diverse preparation methods, grinding, SE, Slu, and thermal analysis, were conducted to explore new cocrystals of ORV in various experimental conditions including solvents. Since different cocrystals were obtained by each method, we concluded that comprehensive experimental screening as methods is also a rational design to find new cocrystals.

Characteristic PXRD, LF Raman, and melting point data were obtained for the ORV-BTN sample produced by LAG with AcOEt and CHCl₃, by solvent evaporation with EtOH, and by the Slu method, and the obtained results were compared to those of ORV and the individual coformers (Fig. 1). PXRD was employed as the primary analytical tool for cocrystal screening, as it can easily distinguish new crystal forms through the unique diffraction peaks. As shown, the strong characteristic peaks of the APIs were replaced by a new series of peaks originating from the anhydrate and dehydrate forms of ORV, thereby confirming the presence of new crystal formations.

In the LF region (0–200 cm⁻¹), Raman spectroscopy provides information related to non-covalent intermolecular interactions, such as hydrogen bonds and van der Waals forces (dipole–dipole interactions), which are specific to the lattice vibrations of different molecular crystals forms. Thus, it is a powerful tool to rapidly differentiate crystal forms, such as polymorphs and cocrystals, at a molecular level, despite them possessing the same chemical structures. We found that for the prepared cocrystals, new peaks appeared, while the peaks derived from the APIs disappeared, thereby suggesting the formation of new crystalline complexes (supplementary Fig. S1).

Although more complicated data were obtained from the LF Raman measurements, the individual crystal forms of the LF Raman data agree well with those of the PXRD measurements. On the other hand, CV Raman and FT-IR spectroscopy are complementary tools that are used for characterizing crystal forms. We found that from the CV Raman spectra, it was difficult to detect evidence of structural changes in the processed samples compared to a PM of ORV with the APIs (supplementary Figs. S2 and S4). Although CV Raman spectroscopy is a sensitive analytical method for detecting different vibration modes with symmetry interactions, such as C–C bonds, it is not specific for cocrystallization. Cocrystals are generally formed via hydrogen bonds, even though the C–H–·–N and π···π stacking interactions with the aromatic rings have an influence on the physicochemical properties of the cocrystals,
such as their solubility and release behavior. This implies that the stretching vibration of the aromatic ring at 1600 cm$^{-1}$ should be monitored,\textsuperscript{45,46} as in the cases of the ORV-INI and ORV-NIC systems. Our results therefore indicate that LF Raman spectroscopy is a more appropriate tool than CV Raman for the determination of cocrystallization.\textsuperscript{44}

The changes in intermolecular hydrogen bonds, which are caused by variations in the electron cloud densities of different functional groups, can be determined by IR spectroscopy,\textsuperscript{47} and this is crucial since hydrogen bonding is directly associated with cocrystal formation. More specifically, the fingerprint region of 600–1500 cm$^{-1}$ shows a unique pattern for each
compound, while in the region of 2300–3800 cm\(^{-1}\), a shift to
to less intense and broader peaks was observed for the signals
attributed to the hydroxyl group of ORV (3300–3400 cm\(^{-1}\)) (Fig.
2). This result indicates that intermolecular hydrogen bond
interactions form between the carboxyl and hydroxyl groups
of BTN and ORV, respectively. Meanwhile, peak shifts were
also detected in the other regions, which were dependent on
the coformer structure, and this result suggested that the en-
vironment around the phenol ring of ORV and the carboxylic
and ammonium groups of the various coformers had changed
due to cocrystal formation.

Subsequently, the thermal behaviors of the ORVs, PM, and

| Structural parameters | Parameter values |
|------------------------|------------------|
|                        | 2ORV-3BTN        | ORV-2PRL-H\(_2\)O |
| SC-XRD                 |                  |                  |
| Empirical formula      | C\(_2\)H\(_8\)N\(_2\)O\(_4\) | C\(_2\)H\(_8\)N\(_2\)O\(_6\) |
| Formula weight         | 839.93           | 492.52           |
| Crystal system         | triclinic        | orthorhombic     |
| Space group            | P-1z             | P212121          |
| a (Å)                  | 8.61190(16)      | 9.9286(2)        |
| b (Å)                  | 9.20006(17)      | 10.6103(2)       |
| c (Å)                  | 26.4842(5)       | 22.6160(5)       |
| α (°)                  | 86.653(6)        | 90               |
| β (°)                  | 88.239(6)        | 90               |
| γ (°)                  | 83.569(6)        | 90               |
| Volume (Å\(^3\))       | 2080.97(7)       | 2382.47(8)       |
| Z                       | 2                | 4                |
| R\(_{1}\) (I > 2σ(I)) | 0.0528           | 0.0259           |
| wR\(_{2}\) (All reflections) | 0.1343          | 0.0682           |
| Flack                   | -                | 0.016(18)        |
| GoF                     | 1.045            | -                |
| PXRD (2θ)              | 6.6, 9.9, 13.3, 13.9, 14.4, 14.9, 15.1, 15.9, 16.5, 16.7, 16.8, 17.0, 17.7, 19.1, 19.9, 20.5, 20.8, 21.2, 21.6, 22.0, 22.4, 22.7, 22.9, 23.2, 23.5, 24.0, 24.7, 25.7, 26.1, 27.1, 27.5, 28.2, 28.9, 29.2, 29.6 | 9.5, 11.3, 12.1, 12.5, 13.2, 14.2, 14.9, 15.4, 16.7, 17.0, 17.6, 18.0, 18.3, 18.8, 19.2, 19.6, 19.9, 20.4, 21.0, 21.2, 22.1, 22.7, 23.9, 24.3, 24.6, 24.9, 25.7, 26.2, 26.6, 26.9, 27.1, 27.5, 27.7, 28.2, 28.7, 29.1 |
| LF Raman (cm\(^{-1}\)) | 220.0, 590.0, 894.0, 1132.2, 192.7 | 176.8, 394.6, 77.0, 100.2, 188.4 |
| CV Raman (cm\(^{-1}\)) | 247, 264, 306, 383, 468, 521, 543, 596, 634, 683, 741, 779, 836, 871, 902, 1066, 1099, 1150, 1225, 1259, 1316, 1353, 1449, 1511, 1596, 1624 | 238, 262, 465, 519, 634, 741, 838, 871, 906, 994, 1101, 1154, 1261, 1318, 1351, 1593, 1614, 1624 |
| IR (cm\(^{-1}\))       | 611, 692, 732, 823, 837, 896, 931, 975, 1010, 1095, 1116, 1159, 1172, 1209, 1232, 1303, 1390, 1469, 1483, 1516, 1587, 1654, 3170 | 682, 742, 808, 833, 858, 870, 910, 925, 972, 979, 993, 1010, 1047, 1105, 1153, 1176, 1201, 1242, 1284, 1313, 1338, 1396, 1473, 1521, 1595, 1608, 1629, 2563, 2974 |
| Melting point (°C)     | 195.3            | 203.2            |
the new crystals were studied using TG-DTA. The endothermic peaks observed clearly differed from those of ORV, their coformers. The endothermic peaks are at where the eutectic temperature of ORV and the coformers suggesting the formation of new crystal forms. Although different cocrystals tend to possess different melting points due to the variations in crystal packing and lattice energies, we found that the melting points of the cocrystals fell between those of the starting materials, with the exception of ORV-ETM, for which the melting point was lower than the corresponding values for its two components (mp: ORV = 197.1 °C, INI = 156.3 °C, NIC = 126 °C, URE = 133.9 °C, BTN = 301 °C, PRL = 221 °C, and ETM = 88.8 °C).10,48 It should also be noted that the thermal traces of ORV-BTN and ORV-PRL were not clear, indicating that a 1:1 ratio for cocystal screening was not suitable.

### Crystal Structural Analysis

The lattice parameters of the newly obtained ORV-BTN and the previously reported ORV-PRL49 are listed in Table 2. ORV-BTN was triclinic and consisted of a ORV/BTN ratio of 2:3, wherein two different conformations of the ORV molecule were arranged in a unit cell. In contrast, ORV-PRL was orthorhombic and comprised a ORV/PRL/H$_2$O ratio of 1:2:1, as previously reported. The both calculated PXRD patterns from single crystals were identical to the bulk cocrystals (supplementary Fig. S6).

### Crystal Structure of ORV-BTN

For this study, the two ORV and three BTN molecules that constitute the unit lattice are defined in the SXRD data as follows: ORV-1 (C1 to O18) and ORV-2 (C19 to O36); BTN-1 (C37 to O44), BTN-2 (C45 to O52), and BTN-3 (C53 to O60). Among them, ORV-2 was disordered so that there were two alignments, namely the A-series and B-series for C19–C26 and O34 (occupancy A:B = 0.7527:0.2472). The carboxylic groups of BTN-1 and BTN-3 were also disordered, wherein O43 and O44 (occupancy A:B = 0.8935:0.1065) and C53, O59, and O60 (occupancy A:B = 0.7729:0.2271) were attributed to the A and B series alignments, respectively (Table 3).

Among the four phenolic hydroxyl groups of ORV-1, O15 was associated with the carboxylic oxygen atom of BTN-1 (O44), and O17 was associated with the hydroxyl group of ORV-2 (O34) and the carboxylic oxygen atom of BTN-3 (O59, O60). In addition, O18 was associated with the hydroxyl group of ORV-2 (O33) and the carboxylic oxygen atom of BTN-3 (O60), while O16 was hydrogen-bonded to the hydroxyl group (O60), while O18 was associated with the hydroxyl group (O33) and the carboxylic oxygen atom of BTN-3 (O59, O60). In addition, O18 was associated with the hydroxyl group (O60), while O16 was hydrogen-bonded to the hydroxyl group (O60). In addition, O18 was associated with the hydroxyl group (O33) and the carboxylic oxygen atom of BTN-3 (O59, O60). In addition, O18 was associated with the hydroxyl group (O60), while O16 was hydrogen-bonded to the hydroxyl group (O60).

It should also be noted that cage-like structures have been reported for sulfadimidine and 4-aminosalicylic acid in multi component crystals of organic compounds, which include co-crystals.49,50 The two ORV co-crystals reported in this paper can therefore be regarded as members of such cage-like structural groups.

### Dissolution Studies

The solubility and dissolution rate are key parameters when considering the control of drug absorption. In terms of cocystal dissociation and dissolution, these properties are controlled by the dissolution of the coformer and the supersaturated state of the API.51 Herein, it was found that all cocrystals exhibited an enhanced solubility.

### Table 3. Hydrogen-bond Geometries (Å, °) for ORV-BTN

| Donor | D–H···A | Acceptor | D–H distance | H···A distance | D···A distance | D–H···A angle |
|-------|---------|----------|--------------|----------------|----------------|---------------|
| ORV1  | O15-H15···O44A | BTN1    | 0.88 (3)     | 1.73 (3)       | 2.614 (3)      | 177 (3)       |
| ORV1  | O15-H15···O44B | BTN1    | 0.88 (3)     | 1.79 (3)       | 2.629 (17)     | 158 (2)       |
| ORV1  | O16-H16···O52  | BTN2    | 0.92 (3)     | 1.76 (3)       | 2.6772 (19)    | 174 (2)       |
| ORV1  | O17–H17···O59A | BTN3    | 0.843 (12)   | 1.888 (16)     | 2.696 (4)      | 160 (3)       |
| ORV1  | O17–H17···O60A | BTN3    | 0.843 (12)   | 2.38 (3)       | 2.904 (4)      | 121 (3)       |
| ORV1  | O17–H17···O60B | BTN3    | 0.843 (12)   | 1.96 (3)       | 2.569 (15)     | 128 (3)       |
| ORV1  | O18–H18···O60A | BTN3    | 0.89 (4)     | 1.59 (4)       | 2.448 (4)      | 161 (4)       |
| ORV1  | O18–H18···O60B | BTN3    | 0.89 (4)     | 2.10 (4)       | 2.965 (13)     | 166 (3)       |
| ORV2  | O33–H33···O18A | ORV1    | 0.84 (3)     | 1.76 (3)       | 2.553 (3)      | 155 (3)       |
| ORV2  | O34A–H34A···O17B | ORV1 | 0.84 (3)     | 1.76 (3)       | 2.553 (3)      | 155 (3)       |
| ORV2  | O34B–H34B···O17A | ORV1 | 0.84 (8)     | 2.20 (10)      | 2.943 (7)      | 147 (10)      |
| ORV2  | O35–H35···O43A | BTN1    | 0.844 (18)   | 1.827 (16)     | 2.656 (4)      | 167 (3)       |
| ORV2  | O35–H35···O43B | BTN1    | 0.844 (18)   | 2.04 (4)       | 2.87 (4)       | 167 (3)       |
| ORV2  | O35–H35···O44B | BTN1    | 0.844 (18)   | 2.14 (4)       | 2.809 (17)     | 136 (3)       |
| ORV2  | O36–H36···O52  | BTN2    | 0.91 (3)     | 1.77 (3)       | 2.681 (2)      | 176 (3)       |

Symmetry codes: (i) $x, y, z + 1$; (ii) $x, y, z + 1$; (iii) $x - 1, y, z$; (iv) $x - 1, y - 1, z + 1$; (v) $x - 1, y - 1, z - 1$; (vi) $x, y + 1, z$.

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126 °C, URE 88.8 °C).10,48 It should also be noted that cage-like structures have been reported for sulfadimidine and 4-aminosalicylic acid in multi component crystals of organic compounds, which include co-crystals.49,50 The two ORV co-crystals reported in this paper can therefore be regarded as members of such cage-like structural groups.

### Comparison with Cage Structures

Both cocrystals appear to contain coformers in a rectangular cage consisting of ORV molecules (supplementary Fig. S7), wherein each cage structure is unique. More specifically, the packing of the ORV-BTN cocrystal consists of two ORV molecules coordinating in an L-shape; the molecules face one another to form a cage, and three BTN molecules fit into the L-shape to form a single unit. This single crystal unit is formed from stacks that are offset from their neighbors by 1/3 of the longest unit side. On the other hand, the structure of the ORV-PRL cocrystal consists of multiple molecules, which interact in a complex manner with alternate 90° packing to form a unit lattice. From certain angles, it appears that cages form, wherein two ORV molecules coordinating in an L-shape are displaced by more than half a molecule in the long axis direction. This produces holes in the cage, which are filled with PRL and water molecules. The cage was aligned with neighboring cages along the long axis.

It should also be noted that cage-like structures have been reported for sulfadimidine and 4-aminosalicylic acid in multi component crystals of organic compounds, which include co-crystals.49,50 The two ORV co-crystals reported in this paper can therefore be regarded as members of such cage-like structural groups.

### Dissolution Studies

The solubility and dissolution rate are key parameters when considering the control of drug absorption. In terms of cocystal dissociation and dissolution, these properties are controlled by the dissolution of the coformer and the supersaturated state of the API.51 Herein, it was found that all cocrystals exhibited an enhanced solubility.
ity over 120 min compared to ORV anhydrate (Fig. 4), while the solubility of ORV cocrystals with citric acid and glutaric acid were 965 and 1058 µg/mL at 120 min of dissolution study, respectively. Moreover, the cocrystal drugs obtained from ORV-PRL and ORV-BTN were superior to ORV anhydrate, exhibiting approximately 1.3–1.4 times faster dissolution rates.

Fig. 3. Unit Cells and Crystal Structures of the (a), (b) ORV-BTN, and (c), (d) ORV-PRL Cocrystals
(Color figure can be accessed in the online version.)

Fig. 4. Dissolution Profiles of ORV Anhydrate and Six Cocrystals at pH 6.8 (n = 3)
(Color figure can be accessed in the online version.)
and higher solubilities.

Conclusion

Using a range of screening methods, including grinding, liquid-assisted grinding, slow evaporation, and slurry techniques, 67 coformers of ORV were screened, resulting in the detection of six cocrystals, four of which were novel for ORV. The formation of cocrystals was confirmed by PXRD measurements, LF/CV Raman spectroscopy, IR spectroscopy, and thermal analysis. Furthermore, single crystal XRD measurements demonstrated that the cocrystal formed between ORV and BTN possessed a cage structure with an ORV:BTN stoichiometric ratio of 2:3. All cocrystals exhibited improved solubilities and dissolution rates compared to the original API. Cocrystal engineering was therefore demonstrated to be advantageous in the design of new drugs exhibiting improved physicochemical properties. Currently we are attempting to improve the bioavailability of ORV by administrating these cocrystals.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Materials

The online version of this article contains supplementary materials.

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