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Article

Versatile Salts as a Strategy to Modify the Biopharmaceutical Properties of Venlafaxine and a Potential Hypoglycemic Effect Study

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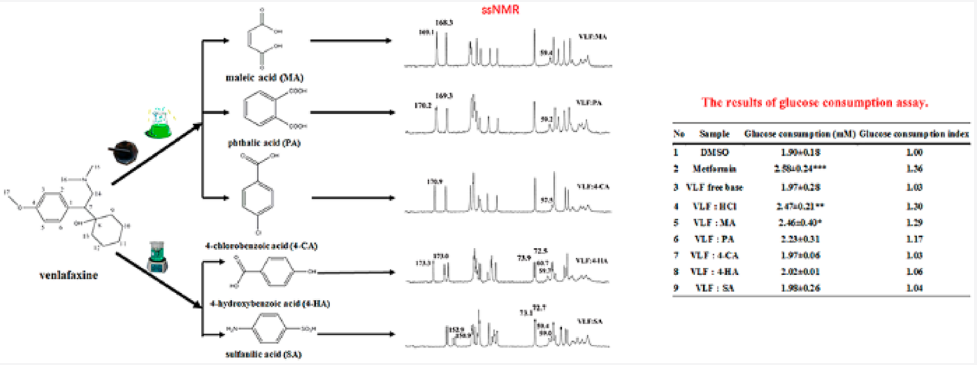

The results of glucose consumption assay.

No	Sample	Glucose consumption (mM)	Glucose consumption index
1	DMSO	1.90±0.18	1.00
2	Metformin	2.88±0.24***	1.56
3	VLF free base	1.97±0.28	1.03
4	VLF : HCl	2.47±0.21**	1.30
5	VLF : MA	2.46±0.40*	1.29
6	VLF : PA	2.23±0.31	1.17
7	VLF : 4-CA	1.97±0.06	1.03
8	VLF : 4-HA	2.02±0.01	1.06
9	VLF : SA	1.98±0.26	1.04

ABSTRACT: Venlafaxine (VLF) is a widely prescribed antidepressant thought to have little effect on glucose metabolism, but some clinical reports showed that a VLF overdose may cause hypoglycemia. The raw material of VLF hydrochloride is metastable and may cause problems during the manufacturing and storage process. Here, salt formation was employed to modify the biopharmaceutical properties, and the hypoglycemic potential of these salt forms was also investigated. Four organic acid salts with phthalic acid (PA), 4-chlorobenzoic acid (4-CA), 4-hydroxybenzoic acid (4-HA), and sulfanilic acid (SA) were obtained for the first time. These products were fully characterized by single crystal and powder X-ray diffraction, solid-state nuclear magnetic resonance, differential scanning calorimetry, and thermogravimetric analysis. Stability measurements revealed that most of these forms held superior phase stability against temperature, moisture, and illumination. All salts show significantly improved solubility relative to VLF. The glucose consumption test was also performed in vitro, VLF:HCl could significantly increase glucose consumption and have a higher glucose consumption index. VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA demonstrated no hypoglycemia side effect.

1. INTRODUCTION

More than 80% of marketed drugs are formulated in the solid form given the ease and advantage of preparation, manufacturing, processing, administration, and storage.[1] Investigators typically carry out a systematical study of different solid forms such as polymorphs, hydrates/solvates, salts, and amorphous forms of an active pharmaceutical ingredient (API) to modify its physicochemical properties including crystallinity, melting point, solubility, dissolution, stability, and bioavailability without changing its core chemical structure.[2,3] Among them, salt formation is one of the most traditional and straightforward methods in the pharmaceutical industry.

Venlafaxine (1-[2-(dimethylamino)-1-(4-methoxyphenyl)-ethyl]cyclohexanol, VLF, CAS: 93413-69-5, Figure 1) is a widely prescribed antidepressant (Effexor) that selectively inhibits the reuptake of serotonin and norepinephine.[4,5] Recent studies have shown that venlafaxine possesses anti-inflammatory activities,[6] alleviating neuropathic pain,[7−9] stimulates neurogenesis,[10,11] and disrupts larval behavior in zebrafish.[12] Venlafaxine may exist in diverse solid forms. The racemate crystal structure of venlafaxine was reported by Tessler et al. in 2004.[13] Three polymorphs of venlafaxine were reported by van Eupen,[14] and one venlafaxine besylate monohydrate was reported by Corvalan.[15] Synthesis and structure of venlafaxine hydrochloride,[16,17] venlafaxine hydrobromide salt,[18] and venlafaxine saccharin salts[19] were reported.

Venlafaxine is extremely insoluble, so it is orally administered in its hydrochloride form. Venlafaxine hydrochloride has high solubility. Because of its short half-life, venlafaxine is administered 2−3 times daily to maintain an effective therapeutic concentration. Thus, a set of molecular salts of

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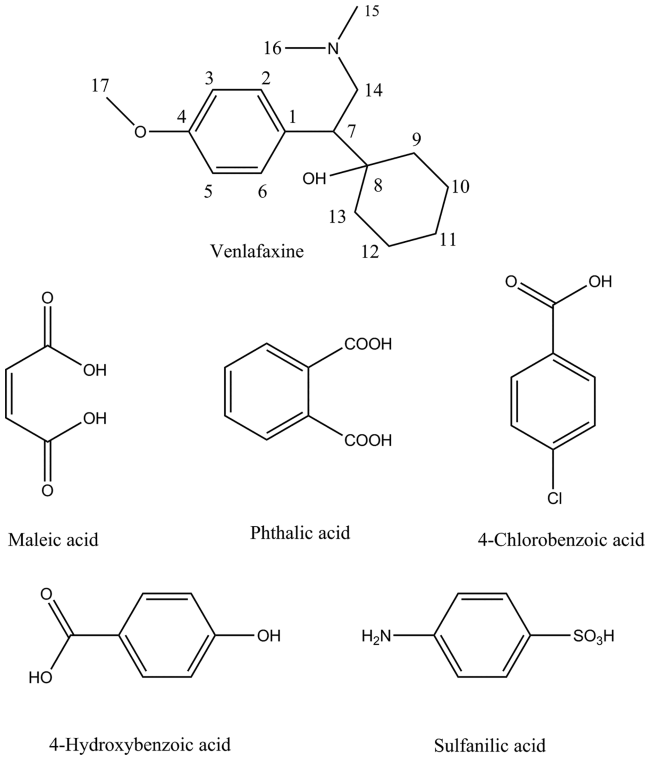

Figure 1. Molecular structures of the venlafaxine and the list of organic acids used in this study.

coumaric, ferulic, oxalic, salicylic, fumaric, and citric acid were obtained.[20] We had already prepared the venlafaxine maleate and characterized it.[21] To propose alternative forms, improve the manufacturing and extend the range of solid forms, eliminate or alleviate the side effect, and find new applications of venlafaxine,[22] we had related supramolecular synthesis and prepared four new venlafaxine salts with coformers with phthalic acid (PA), 4-chlorobenzoic acid (4-CA), 4-hydroxybenzoic acid (4-HA), and sulfanilic acid (SA). These forms were comprehensively characterized by X-ray diffraction. In addition, their physicochemical and biopharmaceutical properties were characterized using different techniques including solid state nuclear magnetic resonance (ssNMR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and solubility measurements. Stability of the salts was evaluated in different conditions. The new contents of spatial structure analyses, ssNMR, and solubility studies of venlafaxine maleate (VLF:MA) were also described here.

Venlafaxine, a serotonin norepinephine reuptake inhibitor, is thought to have little effect on glucose metabolism, but there are reports that venlafaxine hydrochloride overdose causes hypoglycemia.[23−25] In order to screen the bioactivities of the new salts of venlafaxine, we investigated glucose consumption using human liver cells.

Finally, this new salt of venlafaxine constitutes an interesting strategy to modify the biopharmaceutical properties and stability of venlafaxine. VLF:HCl could significantly increase glucose consumption and have a higher glucose consumption index. VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA demonstrated no hypoglycemia side effect, which means that further investigations are required to discover the hypoglycemic mechanism of the salts of venlafaxine hydrochloride.

2. MATERIALS AND METHODS

2.1. Materials. Venlafaxine hydrochloride raw material with a purity of 99.9%, according to high pressure liquid chromatography (HPLC), was supplied by Wuhan Yinhe Chemical Co., Ltd. (Hubei, China). Maleic acid, phthalic acid, 4-chlorobenzoic acid, 4-hydroxybenzoic acid, sulfanilic acid, and sodium hydroxide were purchased from Beijing Chemical Reagent Co. (Beijing, China). All analytical grade solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification.

Table 1. Crystallographic Data and Structure Refinement Details

	VLF:MA[21]	VLF:PA	VLF:4-CA
empirical formula	$(C_{17}H_{28}NO_2) + \cdot(C_4H_3O_4)^-$	$(C_{17}H_{28}NO_2) + \cdot(C_8H_5O_4)^-$	$(C_{17}H_{28}NO_2) + \cdot(C_7H_4ClO_2)^-$
molecular weight	393.47	443.51	433.95
crystal size (mm)	0.32 × 0.14 × 0.05	0.15 × 0.10 × 0.03	0.57 × 0.56 × 0.16
temperature (K)	295(2)	295(2)	295(2)
description	plate	plate	block
crystal system	monoclinic	triclinic	orthorhombic
space group	$P2_1/a$	$P\bar{1}$	$Pna2_1$
a (Å)	8.543(3)	8.907(4)	15.5718(3)
b (Å)	23.859(7)	10.562(5)	14.4294(3)
c (Å)	10.469(4)	12.638(6)	10.37708(17)
α (deg)	90	85.44(4)	90
β (deg)	100.608(12)	85.46(4)	90
γ (deg)	90	83.30(4)	90
Z	4	2	4
volume (Å^3)	2097.4(13)	1174.2(10)	2331.65(8)
density (g/cm^3)	1.246	1.252	1.236
independent reflections	4005	4412	3548
reflections with $I > 2\sigma(I)$	3704	3064	3176
R_{int}	0.0451	0.1076	0.0306
final R,wR (F^2) values [$I > 2\sigma(I)$]	0.0523, 0.1472	0.0876, 0.2186	0.0335, 0.0822
goodness-of-fit on F^2	1.017	1.092	1.031
completeness	0.977	0.971	0.997
CCDC no.	1960401	1960399	1960400

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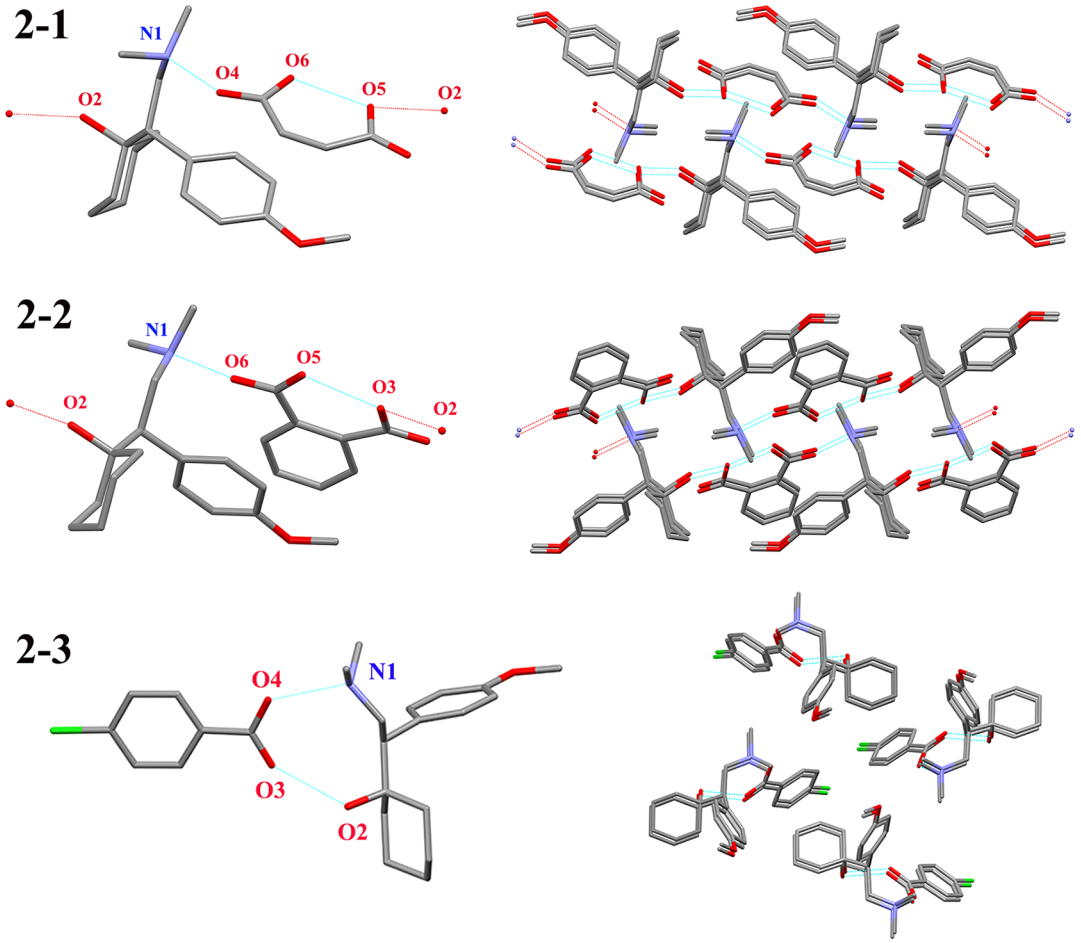

Figure 2. Crystal structure and the 1D, 2D salt bond, and H-bond contacts of venlafaxine salts.

2.2. Preparation of Venlafaxine. To prepare free base of venlafaxine,[26] venlafaxine chloride and NaOH were mixed at a definite stoichiometric ratio (1:1) in water. The resulting mixture was sufficiently stirred at room temperature for 40 min to make sure that the reaction was completed. Then, ethyl acetate was added to extract the free base, and the organic phase was evaporated slowly under decompression conditions. The powder was recrystallized in hexane to acquire venlafaxine crystal at room temperature for a week. The cell parameters are a = 8.4135(12) Å, b = 8.8669(12) Å, c = 21.7900(30) Å, β = 92.307(6)°, as described in the literature[14] (CCDC refcode: OCALAG02).

2.3. Salt Synthesis. Venlafaxine salts were obtained using different preparation methods as described below.

2.3.1. Liquid Assisted Grinding Method. VLF:MA, VLF:PA, and VLF:4-CA were prepared by liquid assisted grinding (LAG). Equimolar amounts of venlafaxine and organic acid were ground using a mortar and pestle for 10−15 min with the addition of a few drops of ethyl acetate/ethanol. The resulting powders were collected for characterization.

2.3.2. Slurry Method. VLF:4-HA and VLF:SA were prepared by a slurry method. Equimolar amounts of venlafaxine and organic acid were suspended in ethyl acetate. The suspension was agitated at 600g for 24 h at room temperature, and the agglomerates were filtered and dried in a 40 °C oven for 12 h.

2.4. Preparation of Single Crystals. VLF:MA, VLF:PA, and VLF:4-CA were carried out by dissolving venlafaxine and the acid coformers in 1:1 molar ratios in the minimum amount of the appropriate solvent (ethyl acetate or ethanol). The solutions were allowed to evaporate slowly at room temperature for about 30 days until crystals suitable for single crystal X-ray diffraction experiments were obtained.

2.5. Single Crystal X-ray Diffraction (SXRD). The SXRD data of the Three crystals were collected using an microMax 002+ system equipped with a Cu fine-focus sealed tube (λ = 1.54187 Å) at 295 K. The crystallographic data are listed in Table 1. The data were processed using CrystalClear software (Rigaku, 2009). The structure was solved by direct methods and refined with full matrix least-squares on F^2 with anisotropic displacement parameters of non-H atoms using the SHELXS-2016.[27] All non-hydrogen atoms were refined with anisotropic displacement parameters. Especially, H atoms of N−H or O−H were located from the difference electron density maps, whereas other H atoms were placed in calculated positions by the riding model in idealized geometries.

2.6. Powder X-ray Diffraction (PXRD). Powder X-ray diffraction (PXRD) studies of microcrystalline samples were performed in Bragg−Brentano geometry on a D/max-2550 (Rigaku, Japan) X-ray diffractometer with graphite monochomator (40 kV, 150 mA, CuKα, λ = 1.54187 Å). A scan speed of 8°/min with a step size of 0.02° in the 2θ range 3−40° was used. The simulated powder patterns were calculated from the single crystal data using Mercury 3.3.

2.7. Solid State NMR Measurements. ^{13}C Cross-polarization/magic angle spinning (CP/MAS) spectra of all samples were collected on a Bruker AVANCE III-500 spectrometer equipped with a 4 mm double resonance MAS probe. A total sideband suppression (TOSS) frame was embedded in the standard CP pulse program to remove the spinning sidebands. ^{13}C CP/MAS TOSS NMR experiments were performed at an 8 kHz MAS spinning frequency with a 2 ms contact time. The recycle delay parameters for MA, PA, 4-CA, and 4-HA were 500, 40, 60, and 120 s, and for other samples 8 s, respectively. The ^{13}C chemical shifts were externally referenced to tetramethylsilane (0 ppm).

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2.8. Thermal Analysis. The thermal behaviors of differential scanning calorimetry (DSC) of venlafaxine salts were investigated using DSC1 Instruments (Mettler Toledo, Greifensee, Switzerland), Sample measurements were performed at a heating rate of 10 °C min^{-1}.

The thermal behaviors of TGA were investigated using a Mettler-Toledo TGA/DSC STARe system (Mettler Toledo, Greifensee, Switzerland). Samples were heated from 30 to 500 °C in aluminum oxide cells at a heating rate of 10 °C min^{-1} under a nitrogen gas flow of 50 mL min^{-1}. The data were analyzed by using STARe software.

2.9. Stability Studies. For conventional stability studies, powdered samples of venlafaxine salts were stored in a drug stability test instrument (SHH-150SD) at three conditions, temperature (60 ± 1 °C), humidity (90 ± 5%, 25 °C), and light (4500 lx ±500 lx, 25 °C), Periodically, samples were removed from the instrument and then measured by the HPLC, PXRD, and DSC method.

2.10. Solubility Studies. Solubility and the dissolution profiles of venlafaxine salts were investigated in various solutions. Excess amounts of venlafaxine salts were added into distilled water in a flask. The salts were placed at 37 °C for 48 h. An aliquot was centrifuged at 10000*g* for 10 min. The content of venlafaxine salts in supernatants, defined as solubility in this study, was assayed by HPLC. The concentration was determined by HPLC based on a calibration curve method using an Agilent 1260 HPLC (Agilent Technologies, USA). Samples were separated by using a Linksil-ODS 5 μm (150 mm × 4.6 mm) column. The mobile phase consisted of acetonitrile and 0.1 mol L^{-1} potassium dihydrogen phosphate water solution (32:68, v/v), and the flow rate was 1.0 mL min^{-1} with simultaneous multichannel UV detection at 225 nm. The column temperature was set to 35 °C, and the injection volume was 5 μL.

2.11. Glucose Consumption Assay. HL-7702 cells were cultured in 1604 medium plus 20% FBS and 1% antibiotics in an atmosphere of 5% CO_2 at 37 °C. Then, cells were trypsinized and seeded onto 96-well plates with 1.0 × 10^5 cells per well. Before experiments, cells were allowed to grow to about 70−80% confluence. The cells were starved overnight in medium containing 0.5% FBS before drug treatment. For basal glucose consumption assay, the cells were treated with DMSO or 100 μM venlafaxine salts or 5 mM metformin for 12 h, with each treatment in quintuplet. Glucose levels in the supernatant of medium were assayed with glucose assay kit (based on glucose oxidase method). Glucose consumption was calculated as the glucose level of the fresh medium minus glucose level of the cultured medium. The glucose consumption index, defined as the ratio of drug to DMSO glucose consumption, was used to evaluate hypoglycemic activity *in vitro*.

3. RESULTS AND DISCUSSION

3.1. Single Crystal X-ray Diffraction. *3.1.1. VLF:MA Salt (1:1).* The crystal structure was determined in space group $P2_1/a$. Each asymmetric unit contains one molecule each of VLF and MA. VLF^+ and MA^- ions are bonded through ionic bond $N_1-H_1\cdots O_4$ (2.684 Å, 157.18°). The salt extended via ionic bond and intramolecular hydrogen bond $O_6-H_6\cdots O_5$ (2.418 Å, 166.67°) and intermolecular hydrogen bonds $O_2-H_2\cdots O_5$ (2.874 Å, 160.82°), forming into a 1D molecular chain infinitely extended along the [001] direction (Figure 2-1). The plane of MA is parallel to the benzene ring in venlafaxine.

3.1.2. VLF:PA Salt (1:1). The structure was determined in space group $P\bar{1}$. The crystal structure contains one VLF molecule and one PA molecule in the asymmetric unit. The pack mode was similar to the VLF:MA salt. VLF^+ and PA^- ions were bonded through ionic bond $N_1-H_1\cdots O_6$ (2.693 Å, 149.57°). The salt extended via ionic bond and intramolecular hydrogen bond $O_3-H_3\cdots O_5$ (2.594 Å, 167.01°) and intermolecular hydrogen bonds $O_2-H_2\cdots O_3$ (2.826 Å, 163.79°) into a 1D chain along the [010] direction (Figure 2-2).

3.1.3. VLF:4-CA Salt (1:1). The structure was determined in space group *Pna*21. The crystal structure contained one VLF molecule and one 4-CA molecule in the asymmetric unit. Different from VLF-MA and VLF-PA, VLF and 4-CA were bonded through an ionic bond $N_1-H_1\cdots O_4$ (2.565 Å, 163.18°) and intermolecular hydrogen bonds $O_2-H_{2A}\cdots O_3$ (2.750 Å, 175.77°) into a $R_2^2(10)$ ring (Figure 2-3).

The final data collection parameters and refinement statistics for all structures are summarized in Table 1. The CIF files for each refinement can be retrieved from the Cambridge Structural Database (CSD) (CCDC numbers 1960399−1960401). The ionic bonds and hydrogen-bond geometrical parameters are given in Tables S1.

Only VLF:4-CA was crystallized in the chiral space group $Pna2_1$, and the next two salts were all crystallized as enantiomer. In the molecules, the plane of ring 1 (C_1, C_2, C_3, C_4, C_5, C_6) and the least-squares plane of ring 2 (C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}) were almost perpendicular (95.0°, 86.2°, 90.0° for VLF: MA, VLF:PA, VLF:4-CA, respectively). The torsion angle of the side-chain substituents also had conformational differences in salts. The torsion angle of $C_8-C_7-C_{14}-N_1$ was 161.6°, 165.5°, 137.2°, and the torsion angle of $C_2-C_1-C_7-C_8$ was −102.9°, −79.2°, −96.3°, respectively. The molecule overlay is shown in Figure 3.

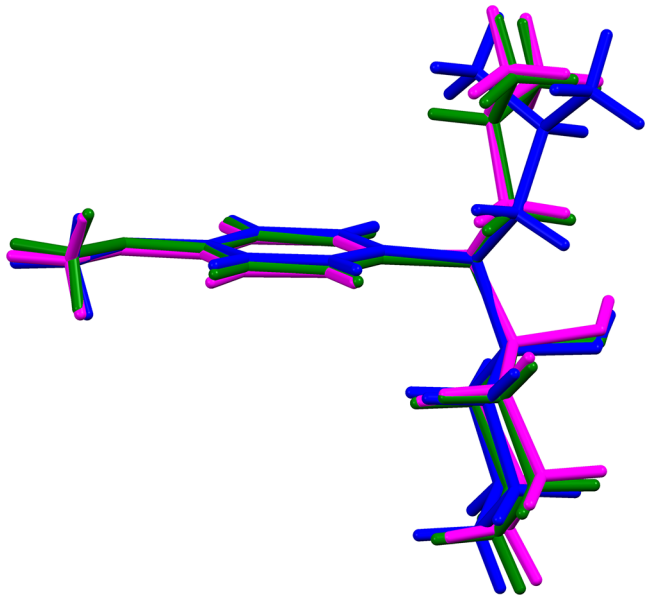

Figure 3. Overlay of molecular conformations (magenta, VLF:MA; green, VLF:PA; blue, VLF:4-CA).

3.2. Powder X-ray Diffraction. Each crystalline form of a given substance will produce a characteristic PXRD pattern. Figure 4 shows the PXRD patterns of venlafaxine, organic acids and venlafaxine salts, respectively. The experimental powder X-ray diffraction of VLF:PA, and VLF:4-CA demonstrated good agreement with their single crystal simulated patterns, which confirms the purity and homogeneity of the salts in a single phase. According to the salt forming reaction, the PXRD patterns of four salts were different with the raw materials and the physical mixture, indicating toward formation of the new solid phase. The main powder XRD peaks of salts and their raw materials are shown in Supporting Information (Table S2).

3.3. ^{13}C Solid-State NMR Analysis. Solid-state NMR is another often employed technique for analysis of solid forms of

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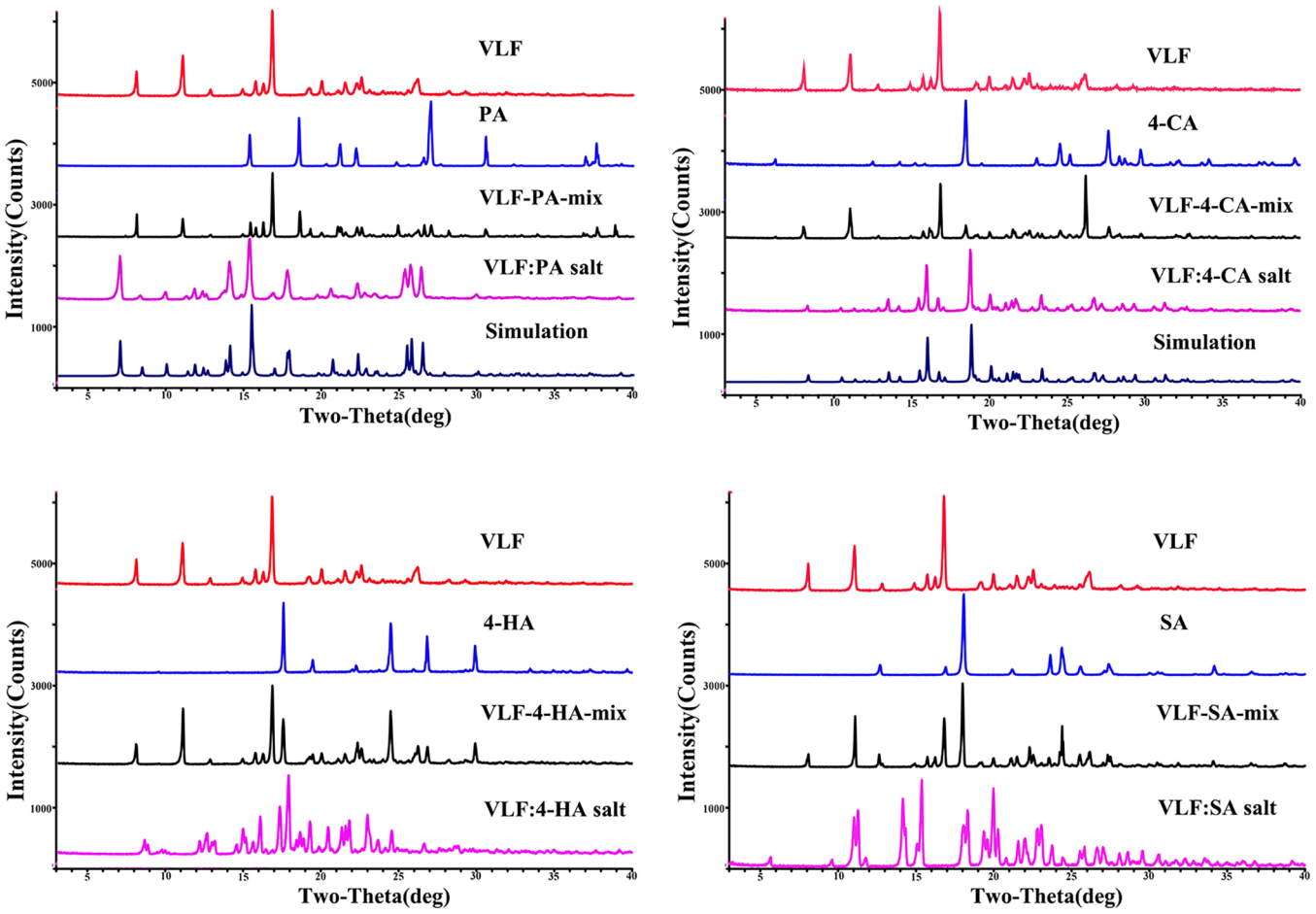

Figure 4. Powder X-ray diffraction patterns of venlafaxine salts and the raw materials.

pharmaceuticals. The ^{13}C CP/MAS TOSS NMR spectra of venlafaxine salts and their starting materials are shown in Figure 5. The ^{13}C chemical shift assignments of venlafaxine, in form I, can be found in ref 14. Each chemically distinct carbon in the form is represented by one single resonance, indicating that the number of molecules per asymmetric unit (Z') of this structure should be equal to 1,[28] which can be confirmed by the reported crystal structure. Similarly, the $Z' = 1$ information for VLF:MA, VLF:PA, and VLF:4-CA can also be learned from their ^{13}C spectra, which are all in line with the solved structures in this contribution. For VLF:4-HA and VLF:SA, the signal splitting at several carbon sites (e.g., 73.9/72.5 and 73.1/72.7 ppm for their C8 signals, respectively) can be observed, supporting that their Z' values are both equal to 2.

Recently, Grepioni et al. reported six venlafaxine salts along with their solid state ^{13}C spectra.[20] After salt formation, the chemical shifts of carboxylic signal are altered 3.9, −3.1, 1.9, 0.7, 1.7, and 2.0 ppm, respectively. It seems that the magnitude and regularity of these chemical shift changes cannot be used as a clear indicator of salt formation. In this study, similar phenomena were observed for VLF:MA (from 172.8 to 169.1 ppm), VLF:PA (from 173.1 to 170.2 ppm), VLF:4-CA (from 173.1 to 170.9 ppm), and VLF:4-HA (from 171.8 to 173.3/173.0 ppm). Meanwhile, we noticed that the ^{13}C chemical shift changes of the N bonded C14 site may be used as such an indicator, because this methylene ^{13}C signal is less affected by molecular packing changes, and the protonation of the dimethylamino N site should be the main contributor for the chemical environmental altering. The proton transfer from a salt former to the dimethylamino group leads to a larger ^{15}N chemical shift, i.e., a reduced shielding effect on the N site.[20] Then, an enhanced shielding effect on the N bonded C14 site (smaller ^{13}C chemical shift) is desired. These data are summarized in Table 2. For the two forms of venlafaxine hydrochloride, similar results, C14 signals at 60 ppm, have been reported.[29]

SA molecules exist in the zwitterion form in the solid state.[30] For SA, the signals at 134.0 and 145.6 ppm can be assigned to $-NH_3^+$ and $-SO_3^-$ bonded C, respectively. In VLF:SA, the intermolecular proton transfer should occur from the $-NH_3^+$ group of SA to dimethylamino N of VLF, which causes a significant chemical shift change on the $-NH_3^+/-NH_2$ bonded C from 134.0 to 150.9/152.9 ppm. The ionization state change of SA also causes a large high-field shift on the $-SO_3^-$ bonded C signal.

3.4. Thermal Analysis Method. Figure 6 shows the DSC thermograms of raw materials, and four new salts. The sharp endothermic peaks of VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA were 177.86 °C, 112.85 °C, 146.21 °C, and 143.15 °C, respectively. All four new salts show melting point between the free base and acid melting point, indicating the possibility of novel solid forms. The values of melting points indicate that the thermal stability was VLF:PA > VLF:4-HA ≈ VLF:SA > VLF:4-CA. At the TG pattern (Figure S1), there is no evidence of weight loss of solvent or water, which is consistent

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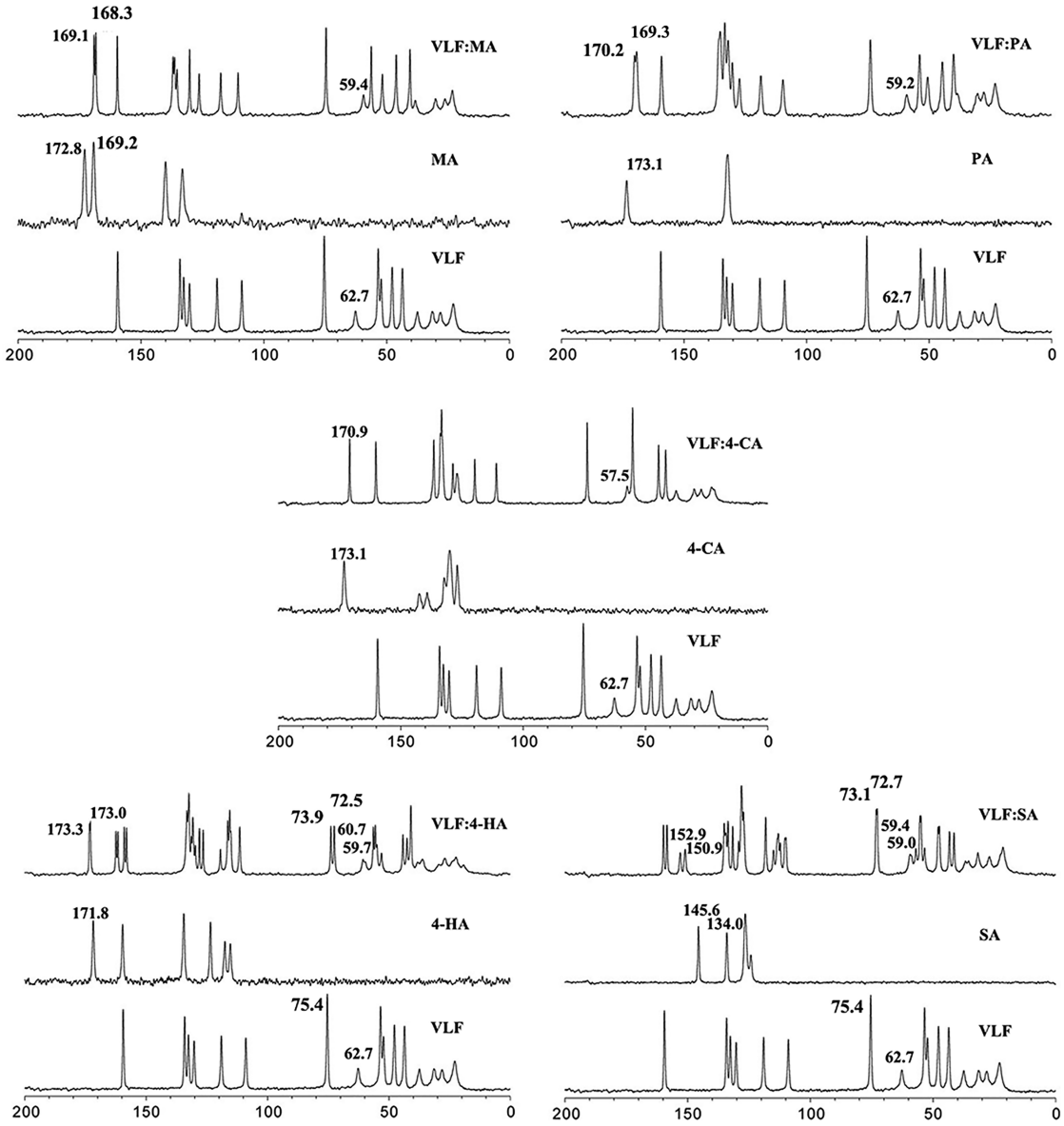

Figure 5. 13C CP/MAS TOSS NMR spectra of VLF:MA, VLF:PA, VLF:4-CA, VLF:4-HA, VLF:SA, and their starting materials.

Table 2. Chemical Shifts of C14 in Venlafaxine (VLF) and Its Salts

atom	VLF	VLF:CA	VLF:PA	VLF:4-CA	VLF:4-HA	VLF:SA
C14	62.7	59.4	59.2	57.5	60.7/59.7	59.4/59.0

with the high purity of the materials. The weight loss accompanies the decomposition process.

3.5. Stability Study. As shown in Table 3, VLF:PA, VLF:4-CA, and VLF:4-HA remained stable over 10 days under three conditions, temperature (60 ± 1 °C), humidity (90% ± 5% RH, 25 °C), and light (4500 lx ±500 lx, 25 °C), since there was no obvious change in the accelerated conditions. VLF:HCl was stable under high temperature and the light condition for 10 days, but has the polymorphic transformation trend to form 4 (hydrate) under the high humidity condition for 10 days. VLF:SA was stable under high temperature for 10 days, but metastable under the high humidity and the light condition for 10 days.

3.6. Solubility Study. The solubility of venlafaxine, VLF:MA, VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA in pure water was evaluated. The solubility of venlafaxine, VLF MA, VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA is 0.39, 288.10, 5.71, 22.61, 47.73, and 36.50 mg mL^{-1}, respectively. The retention time of venlafaxine and salt are almost the same, but different from the coformers. The HPLC retention times were VLF (3.184 min), VLF:HCl (3.158 min), VLF:MA (1.440 min, 3.168 min), VLF:PA (1.595 min, 3.162 min), VLF:4-CA (3.161 min, 4.68 2 min), VLF:4-HA (1.978 min, 3.154 min), and VLF:SA (1.292 min, 3.158 min), respectively. All the five salts have a higher solubility than the free base. A small amount of the solid phase at the end of solubility measurement was isolated and dried to a glass plate, and PXRD and DSC methods were used to analyze the samples. The results demonstrate that the samples were not completely identical with the starting polymorphic forms in each case.

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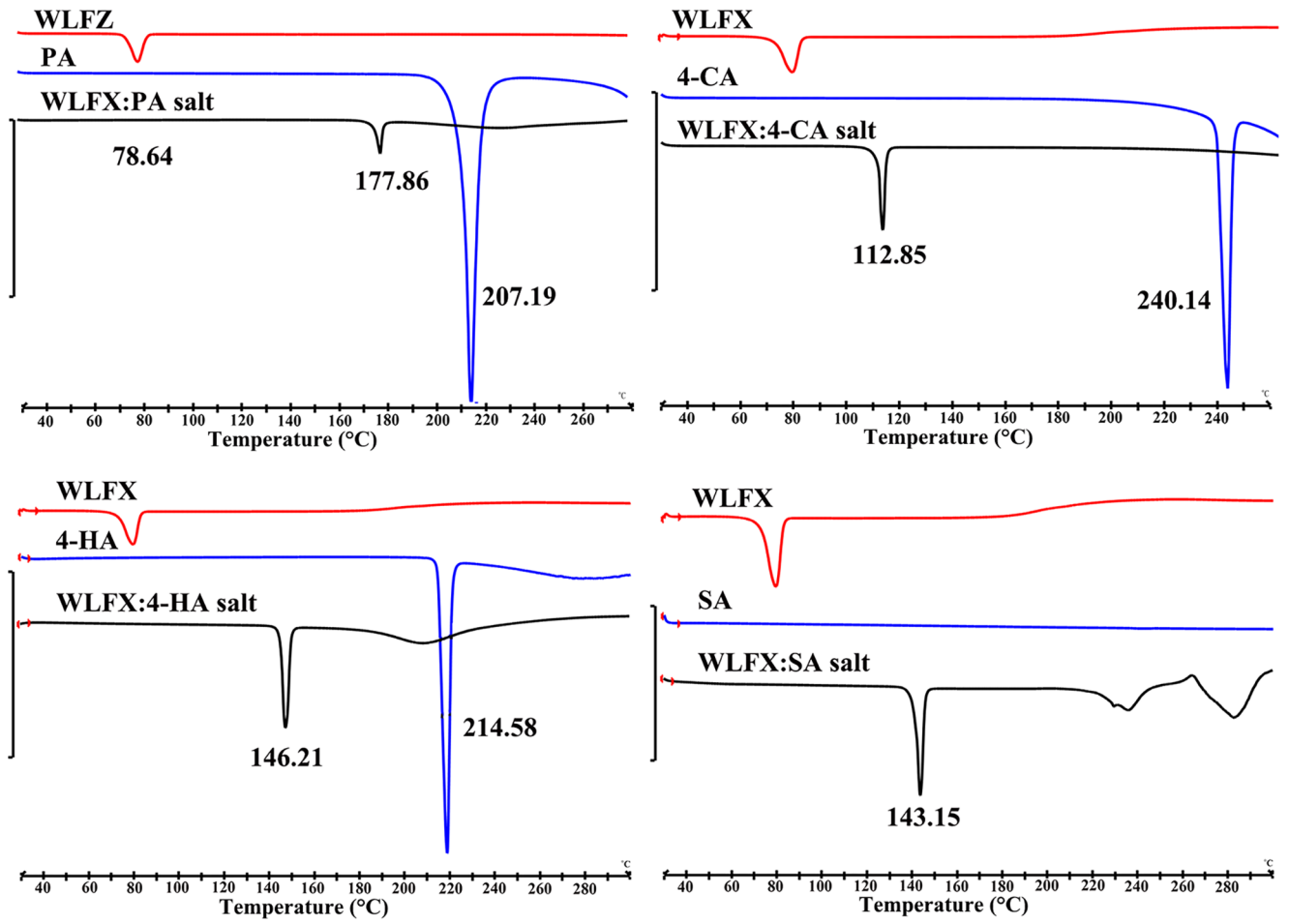

Figure 6. DSC thermograms of venlafaxine salts and the raw materials.

Table 3. Results of the Stability Study

		temperature		humidity		light	
no.	sample	5 days	10 days	5 days	10 days	5 days	10 days
1	VLF (I)[31]	I + II	I + II	I	I	I	I + III + amorphous
2	VLF:HCl(2)[5]	2	2	2 + 4	2 + 4	2	2
3	VLF:PA(A)	A	A	A	A	A	A
4	VLF:4-CA(a)	a	a	a	a	a	a
5	VLF:4-HA(i)	i	i	i	i	i	i
6	VLF:SA(α)	α	α	$\alpha + \beta$	$\alpha + \beta$	$\alpha + \gamma$	$\alpha + \gamma$

This indicate that, during the dissolution process, there were accompanied by transformation and recrystallization.

The dissolution profiles of venlafaxine, VLF:MA, VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA in pure water were also evaluated (Figure S2). The five venlafaxine salts show the same dissolution profiles in pure water (pH 7.0) as VLF:HCl, and the API was released within the first 20 min.

3.7. Glucose Consumption Assay. To evaluate the *in vitro* hypoglycemic activity of venlafaxine salts, the glucose consumption assay was done. HL-7702 cells were treated with venlafaxine salts for 12 h, and metformin was used as a positive control. As shown in Table 4, we found that 100 μM of VLF:HCl or VLF:MA caused a significant increase of glucose consumption ($p < 0.01$ or 0.05 vs DMSO) and had a higher glucose consumption index. Its efficacy was comparable to that of metformin at 5 mM. But VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA did not show any hypoglycemic activity *in vitro*.

Table 4. Results of Glucose Consumption Assay[a]

no.	sample	glucose consumption (mM)	glucose consumption index
1	DMSO	1.90 ± 0.18	1.00
2	metformin	2.58 ± 0.24***	1.36
3	VLF free base	1.97 ± 0.28	1.03
4	VLF:HCl	2.47 ± 0.21**	1.30
5	VLF:MA	2.46 ± 0.40*	1.29
6	VLF:PA	2.23 ± 0.31	1.17
7	VLF:4-CA	1.97 ± 0.06	1.03
8	VLF:4-HA	2.02 ± 0.01	1.06
9	VLF:SA	1.98 ± 0.26	1.04

[a]Values of glucose consumption are the mean ± SD of quintuplet. Values of glucose consumption index are the mean of drug treatment divided by the mean of DMSO treatment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs that of DMSO treatment.

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4. CONCLUSIONS

The aim of this study was to investigate structural changes and salt formation of venlafaxine with versatile acids using different preparation methods and characterize their physicochemical properties. In this paper, four new venlafaxine salts were prepared and characterized. The spatial structure and interaction information may help to understand the solid state properties of organic molecules. Crystal structure analysis shows that there exist reliable N−H···O ionic bonding and O−H···O hydrogen-bonding interactions between the amino group and the hydroxyl group of venlafaxine and the hydroxyl group or/and the carbonyl group of the various acids. The liquid assisted grinding method and slurry method can be used to synthesize the salts.

Although the 4-CA molecule is almost planar and did not have a chiral center in the chemical structure, the VLF:4-CA salt can crystallize in the chiral space group. This means 4-CA maybe used as a selector reagent for racemic venlafaxine or analogue organic molecules.

SXRD, PXRD, ssNMR, DSC, and TGA can be used to identify and characterize the different salts. These salts do not need to always have a high solubility to be useful. The low solubility maybe has a higher pH suitable for injections and drops. The five salts all have the lower solubility than that of VLF:HCl. Among of the salts, VLF:PA has the lowest solubility. Except for VLF:SA, other venlafaxine salts are stable under the three test conditions. Since the hydrochloride salt raw material is metastable at high humidity environment, and may cause problems during the manufacturing and storage process, the new salts may provide new API candidates for venlafaxine commercial production. On the basis of the glucose consumption assay, VLF:HCl and VLF:MA could significantly increase glucose consumption and have a higher glucose consumption index. VLF:PA, VLF:4-CA, VLF:4-HA, and VLF:SA did not show any hypoglycemic activity *in vitro*. Comprehensive consideration of the solubility, stability and safety, VLF:4-HA was the most appropriate salt for improving the safety of venlafaxine by minimizing the hypoglycemic effect.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.cgd.0c00007.

The ionic bonds and hydrogen-bond geometry, the main powder XRD peaks of salts and their raw materials, the TGA patterns of VLF salts, the dissolution profiles of VLF salts in pure water (PDF)

Accession Codes

CCDC 1960399−1960401 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via 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.

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Notes

The authors declare no competing financial interest.

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