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Articles

Anhydrates and Hydrates of Olanzapine: Crystallization, Solid-State Characterization, and Structural Relationships

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Ⓦ This paper contains enhanced objects available on the Internet at http://pubs.acs.org/crystal.

ABSTRACT: Olanzapine, a novel benzodiazepine agent used in the treatment of schizophrenia and related psychoses, crystallizes in 25+ crystal forms, seven of which are pharmaceutically relevant: three anhydrates (I−III), three dihydrates (B, D, and E), and a higher hydrate. X-ray crystal structures of the thermodynamically stable anhydrous form (I), two dihydrates (B and D), a higher hydrate, and a Rietveld-refined structure of dihydrate E have permitted a detailed analysis of the conformational, hydrogen bonding, and crystal packing preferences of olanzapine. The symmetry and hydrogen-bonding interactions in the crystal forms have also been characterized by ^{13}C and ^{15}N CP/MAS NMR spectroscopy. Using the crystallographic and spectroscopic data, significant structural relationships have been identified between the crystal forms of olanzapine. The present study demonstrates the utility of integrating crystallography, spectroscopy, and crystal modeling in detailed structural investigations of polymorphism (and solvate formation) and for rationalizing crystallization outcomes. This study also shows that polymorphism and hydrate formation can be used to optimize the physical presentation of pharmaceutical solids.

Introduction

One of the primary goals of crystal engineering[1,2] is to design and control the way molecules crystallize, producing materials with specific properties (e.g., second-harmonic generation, conductivity, thermochromism, photoactivity, etc.). Typical strategies direct molecular association through strong intermolecular interactions, such as hydrogen bonding, electrostatic and/or charge-transfer interactions, and control bulk properties by simply varying molecular structure.[3−5] If crystals of pharmaceuticals could be engineered, then properties, such as stability, bioavailability, and processibility, could be optimized.[6] Traditional approaches to crystal engineering are generally not applicable to pharmaceutical solids, however, since only limited changes to molecular structure can be tolerated to design a bulk drug material with optimal physical properties. Indeed, structural modifications to drug molecules, most commonly in the form of prodrugs, are typically driven by bioavailability considerations.[7,8]

Given the structural limitations placed on pharmaceuticals, different approaches, including salt formation,[9−11] complexation,[12−16] cocrystallization,[17,18] solvate formation,[19] and polymorphism, have been used to manipulate the supramolecular structure in pharmaceutical solids. Of these methods, salt formation, complexation, cocrystallization, and solvate formation are limited by the toxicity of the counterions, guest molecules, and solvents. Salt formation is also obviously limited to compounds with ionizable groups. Polymorphism,[20,21] considered to many to be the nemesis to crystal engineering,[22] and hydrate formation are widespread phenomena that can be viewed as opportunities to safely manipulate the physical properties of pharmaceutical solids.[23] In polymorphic solids, structure−property relationships are governed only by differences in the spatial arrangement of molecules in a crystal, and in some cases, variations in molecular conformation.[24] While true polymorphs can have significantly different

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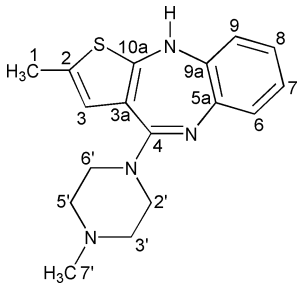
physical properties, the incorporation of water in a crystal lattice (i.e., formation of a hydrate) can have even more dramatic effects on the physical properties of a pharmaceutical solid. Hence, controlling polymorphism in pharmaceutical solids must include provisions for hydrate formation, and vice versa.

Olanzapine, 2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-*b*][1,5]benzo-diazepine, is a member of a novel benzodiazepine class of antipsychotic drugs with demonstrated efficacy in the treatment of schizophrenia and related psychoses.[25,26] Polymorphism and hydrate formation have proven to be particularly powerful means to alternate crystal forms of the drug. Olanzapine crystallizes in at least 25 solid forms, including three polymorphic anhydrates (I−III), three polymorphic dihydrates (B, D, and E), and a higher hydrate. This paper reports the preparation and structural characterization of these seven pharmaceutically relevant crystal forms by X-ray crystallography and solid-state NMR spectroscopy. The molecular recognition processes responsible for the polymorphism and hydrate formation of olanzapine have been examined, and the structural relationships between the anhydrates and the hydrates have been used to rationalize its crystallization behavior.



Experimental Procedures

Materials. Olanzapine was provided by Lilly Research Laboratories.

Form I. Olanzapine (270 g) was suspended in ethyl acetate (2.6 L). The stirred suspension was heated to 76 °C to dissolve the solids. The solution was then cooled to ambient temperature, at which time a crystal slurry formed. The solid product was isolated by vacuum filtration and dried in vacuo at 50 °C. Yield = 197 g, TGA mass loss (exptl) 0.0%.

Form II. A sample of mostly form II was prepared by desolvating olanzapine methanolate at 50 °C. TGA mass loss (exptl) 0.0%.

Form III. Olanzapine (1.5 g) was suspended in $CHCl_3$ (5 mL). The suspension was heated to reflux to dissolve the solids. The solution was then cooled to ambient temperature. Hexanes (15 mL) were added to the stirred solution, at which time a crystal slurry formed. The solid precipitate (mostly form III) was isolated by vacuum filtration and washed with hexanes (10 mL). Yield = 867 mg.

Dihydrate B. Olanzapine (5 g) was suspended in ethyl acetate (50 mL) and toluene (6 mL). The suspension was stirred and heated to 80 °C to dissolve the solids. The solution was then cooled to 60 °C, and water (30 mL) was added. The solution was further cooled to room temperature to produce a crystal slurry. Yellow, rhombohedral crystals were isolated by vacuum filtration, washed with H_2O (10 mL), and air-dried. Yield = 4.16 g, TGA mass loss (exptl) 10.1%, (theory) 10.3%.

Dihydrate D. Olanzapine form I (5 g) was suspended in water (50 mL) at ambient temperature and slurried for 5 days. The solid product was isolated by vacuum filtration, washed

Dihydrate E. Olanzapine (3 g) was suspended in ethyl acetate (60 mL) and toluene (3.6 mL). The suspension was stirred and heated to 80 °C to dissolve the solids. The solution was then cooled to 65 °C, and water (6 mL) was added. The solution was further cooled to ambient temperature to produce a crystal slurry. Yellow, rhombohedral crystals were isolated by vacuum filtration, washed with H_2O (5 mL), and air-dried. Yield = 2.47 g, TGA mass loss (exptl) 10.6%, (theory) 10.3%.

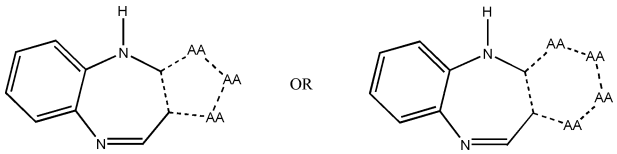
Higher Hydrate. Olanzapine (2 g) was suspended in CH_2Cl_2 (12 mL). The suspension was stirred and heated to reflux to dissolve the solids. Water (1.5 mL) was added as the solution was cooled to ambient temperature, at which time a crystal slurry formed. The crystal slurry was cooled to 0 °C, and a wetcake (2.7 g) was isolated by vacuum filtration and washed with CH_2Cl_2 (∼10 mL).

General Methods. Thermogravimetric analyses were performed on a Seiko Simultaneous Thermo-Gravimetric Analyzer Model 220. Samples (3.5 mg) were run from 25 to 350 °C at a rate of 10 °C/min.

XRD patterns were obtained on a Siemens D5000 X-ray powder diffractometer, equipped with a CuKα source (λ = 1.54056 Å) and a Kevex solid-state detector, operating at 50 kV and 40 mA. Each sample was scanned between 4 and 35° in 2θ, with a step size of 0.03° and a scan rate of 2 s/step.

Solid-state ^{13}C and ^{15}N NMR spectra were collected on a Varian Unity spectrometer operating at a ^{1}H resonance frequency of 400 MHz. All experiments were performed using cross polarization (CP), high power decoupling, and magic angle spinning (MAS = 7 kHz). Hartmann−Hahn match parameters for ^{13}C and ^{15}N were determined using hexamethylbenzene (HMB) and glycine-^{15}N, respectively. Typical ^{13}C acquisition parameters include 90° pulse width 5 μs, contact time 1.1 ms, relaxation delay 5 s, acquisition time 0.05 s, and spectral width 50 kHz. Interrupted decoupling spectra were acquired with a 40 or 50 μs delay without decoupling prior to acquisition. Chemical shifts were referenced using sample replacement to the methyl group of HMB, which resonates at 17.3 ppm. Typical ^{15}N acquisition parameters include 90° pulse width 7 μs, contact time 2.5 ms, relaxation delay 5 s, acquisition time 0.1 s, and spectral width 35 kHz. Chemical shifts were referenced using sample replacement to glycine-^{15}N, which resonates at −6.39 ppm [$^{15}NH_4Cl$ = 0.0 ppm].

Crystallographic Literature Search. A search of the Cambridge Structural Database[27] was conducted for benzodiazepines. From a connectivity search of the fragments shown below, six uncharged structures (five unique), for which coordinates are available, were retrieved (see Supporting Information).



X-ray Structure Determinations. Single crystals of form I were grown by vapor diffusion of *n*-pentane into a dry ethyl acetate solution of olanzapine. Dihydrate B crystals were obtained by diffusing water into a saturated toluene solution of olanzapine. Dihydrate D crystals were obtained by slow evaporation of a 3:1 acetonitrile/water solution of olanzapine. Single crystals of the higher hydrate were isolated from ethyl acetate/toluene/water.

Crystal data for form I were collected on an Enraf-Nonius CAD4 diffractometer. Three standard reflections were measured every 97 reflections; no crystal decay was detected. Lorentz and polarization corrections were applied to the data; no corrections were made for absorption. The structures were solved by direct methods using *Shelx86*[28], and the remaining atoms were located in succeeding difference Fourier synthesis. The structures were refined in full-matrix least-squares using

Table 1. Crystal Data, Data Collection, and/or Refinement for Olanzapine Form I and the Hydrates

	form I	dihydrate B	dihydrate D	dihydrate E[a]	higher hydrate
experimental formula	$C_{17}H_{20}N_4S$	$C_{17}H_{20}N_4S{\cdot}2\ H_2O$	$C_{17}H_{20}N_4S{\cdot}2\ H_2O$	$C_{17}H_{20}N_4S{\cdot}2\ H_2O$	$C_{17}H_{20}N_4S{\cdot}2.5\ H_2O$
formula weight	312.44	348.47	348.47	348.47	357.47
crystal dim. (mm)	0.26 × 0.24 × 0.21	0.22 × 0.13 × 0.10	0.11 × 0.24 × 0.36	NA	0.20 × 0.22 × 0.25
crystal system	monoclinic	monoclinic	triclinic	monoclinic	monoclinic
space group	$P2_1/c$ (no. 14)	$P2_1/c$ (no. 14)	P-1(no. 2)	$C2/c$ (no. 15)	$C2/c$ (no. 15)
a (Å)	10.383(1)	9.8691(12)	9.927(5)	24.5195	25.130(2)
b (Å)	14.826(3)	12.7156(15)	10.095(5)	12.3495	12.2377(11)
c (Å)	10.560(8)	14.3853(16)	10.514(6)	15.2179	14.9116(14)
α (deg)	90	90	84.710(10)	90	90
β (deg)	100.616	92.969(2)	62.665(8)	125.824	124.984(1)
γ (deg)	90	90	71.183(8)	90	90
V (Å^3)	1597.8(7)	1802.8(4)	884.1(8)	3736.3	3757.2(6)
Z	4	4	2	8	8
ρ_{calc} (g cm^{-3})	1.299	1.284	1.309	1.239	1.274
temperature (K)	293	173(2)	128(2)		198(2)
radiation	CuKα	MoKα	MoKα		MoKα
wavelength (Å)	1.54184	0.71073	0.71073		0.71073
monochromator	none	graphite	graphite		graphite
abs. coeff. (cm^{-1})	17.61	0.197	0.200		0.196
h	0−11	−13 to 13	−13 to 13		−16 to 33
k	0−16	−11 to 16	−13 to 13		−12 to 15
l	−11 to 11	−19 to 11	−13 to 13		−18 to 16
θ range (deg)	2−60	2.07−28.29	1.07−14.11		1.94−28.27
F000	664.0	744.0	372		1568
no. of unique data	2485	4193	3976		4378
data used	2139 ($I > 3\sigma(I)$)	2417 ($I > 2\sigma(I)$)	3119 ($I > 2\sigma(I)$)		2923 ($I > 3\sigma(I)$)
no. of variables	279	227	27		230
largest shift/esd	0.16	0.004	0.01		0.172
R	0.043	0.0663	0.0829		0.0730
Rw	0.057	0.1525	0.2530		0.2073
goodness of fit	2.007	0.974	1.148		1.040

[a] Rietveld refinement.

method with terms of 0.020 and 1.0.[30] Atomic scattering factors and the values for Δf′ and Δf″ were taken from *International Tables for X-ray Crystallography*.[31] Anomalous dispersion effects were included in F_c.[32] Plots of $\sum w(||F_o| - |F_c||)^2$ versus $|F_o|$, reflection order in data collection, sin θ/λ, and various classes of indices showed no unusual trends. The NH hydrogen atoms were located and their positions and isotropic thermal parameters refined; the other hydrogens were located and added to the structure factor calculations but were not refined. Using the *Cerius2* crystal modeling program,[33] the water hydrogen atoms were placed in locations consistent with hydrogen bonding (as determined by short N···O distances) for visualization purposes.

Crystal structures of dihydrates B and D and the higher hydrate were determined and refined using similar procedures. Diffraction data were collected using a Bruker SMART system P4 diffractometer using MoKα radiation and CCD detection.[34] Cell refinement and data reduction were accomplished using the *SAINT* software programs.[35] The structure was solved by direct methods using Siemens *SHELXTL-PLUS*.[36] Non-hydrogen atoms were refined anisotropically. All other hydrogen atoms were included in the structure factor calculations and placed in idealized positions ($d_{C-H} = 0.95$ Å) with assigned isotropic thermal parameters ($B = 1.2B$ of bonded atoms). Experimental details of the structure determinations are given in Table 1 (see Supporting Information).

Rietveld Refinement of Dihydrate E. A dihydrate E trial crystal structure was constructed using X-ray structure data collected for an isostructural EtOH−water mixed solvate.[37] The structure was interactively Rietveld-refined[38] using the *DBWS* program[39] until the simulated powder pattern matched the experimental X-ray powder pattern of dihydrate E. The results of the Rietveld refinement of dihydrate E are also given in Table 1. The R factor of 20% is somewhat high; however, there does seem to be reasonable agreement between the powder patterns. Given the quality of the experimental powder pattern, the structure was not further refined (see Supporting Information).

space of olanzapine and to calculate the energy difference between conformations. The commercial program *Spartan* (Version 5.0) was used.[40] The conformer search was restricted to geometry optimization (RHF/3-21G*) of conformers produced by systematically varying the N5−C4−N1′−C6′ torsion angle of the observed conformer in form I in 60° jumps. The energy difference between the two minima obtained from the conformer search was calculated using Hartree−Fock (3-21G* and 6-31G* basis sets) and SVWN (DN, DN*, and DN** basis set) density functional models.

Results and Discussion

Crystallization. Form I, the most stable nonsolvated crystal form of olanzapine, was directly crystallized from dry organic solvents, including EtOAc, THF, acetone, and toluene. Forms II and III are desolvates, having been prepared only by desolvating MeOH, CH_2Cl_2, or $CHCl_3$ solvates of olanzapine. The desolvation of these olanzapine solvates proved to be difficult to control, as mixtures of forms I, II, and/or III were routinely encountered. The comparatively harsh drying conditions required to desolvate the MeOH solvate, for example, frequently resulted in form II/III materials contaminated with form I. Forms II and III (free of form I) could be obtained by desolvating the CH_2Cl_2 or $CHCl_3$ solvates under mild conditions; however, no conditions were identified that would yield pure form II or III.

The crystalline dihydrates and the higher hydrate could be crystallized from pure water or mixtures of water and EtOAc or toluene. Dihydrate B, the kinetic form produced by slurrying olanzapine (form I) in water, could be crystallized in pure form from EtOAc−toluene−water at moderately high temperatures (e.g., 55 °C).

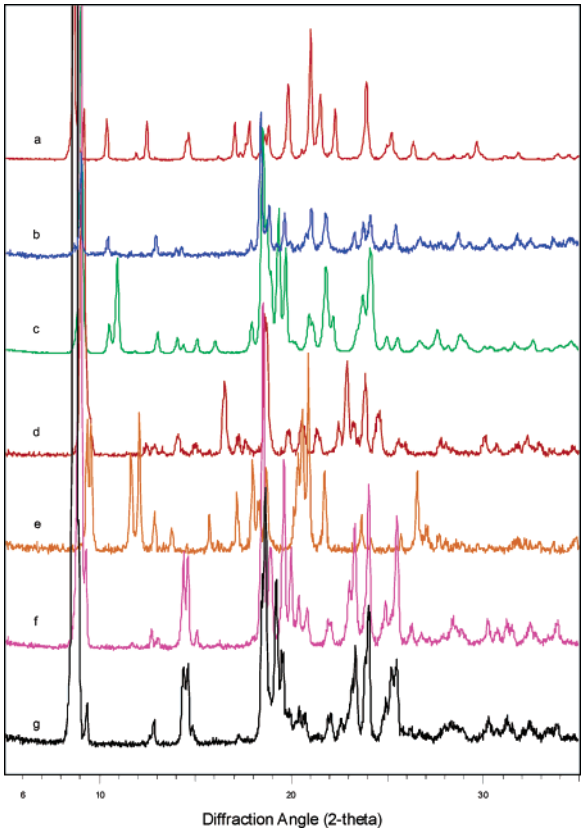


Figure 1. Powder X-ray diffraction patterns of olanzapine (a) form I, (b) form II (+ form I contaminant), (c) form III (+ form II contaminant), (d) dihydrate B, (e) dihydrate D, (f) dihydrate E, and (g) the higher hydrate.

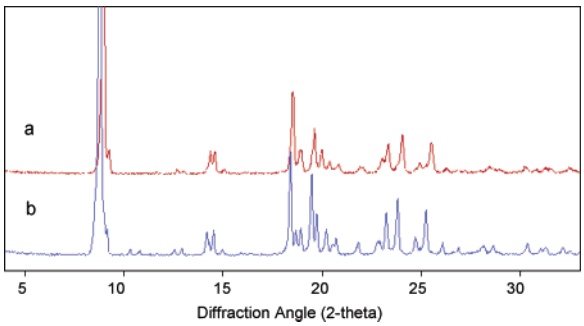


Figure 2. Similar experimental XRD patterns of (a) dihydrate E and (b) the EtOH−H_2O mixed solvate of olanzapine reveal that these crystal forms are isostructural.

crystal form that contains 2−2.5 mol of water and has only been observed in wetcakes of olanzapine. As the higher hydrate wetcake was air-dried to a flowable powder, the material rapidly lost the first of three waters of crystallization and converted to dihydrate E. Thus, while mixtures of dihydrate B and the higher hydrate crystallized from EtOAc−toluene−water between 25 and 55 °C, dihydrates B and E were obtained as the solid products. Dihydrate E was isolated in pure form by mildly drying the higher hydrate, which was exclusively crystallized at or below ambient temperature. Dihydrate D, the thermodynamically stable hydrated crystal form of olanzapine, could be isolated by slurrying any of the crystal forms in EtOAc−H_2O or pure water at ambient temperature for several days.

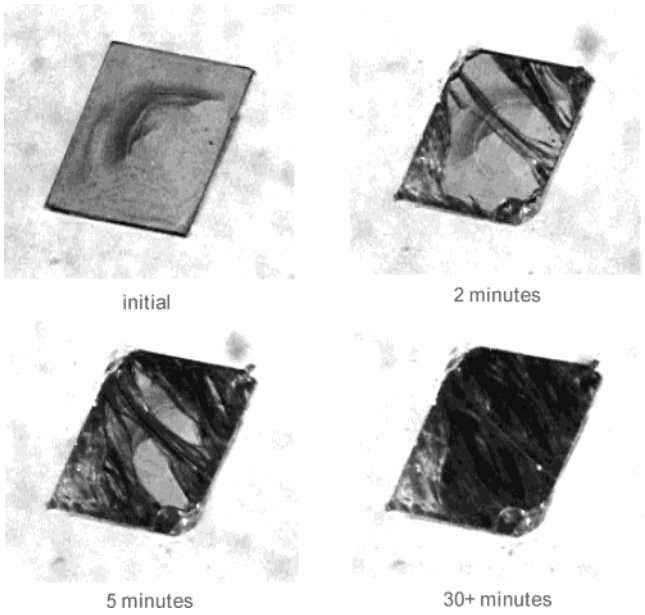


Figure 3. Crystals of the higher hydrate fracture as they desolvate to dihydrate E within minutes of isolating them from the crystallization solution.

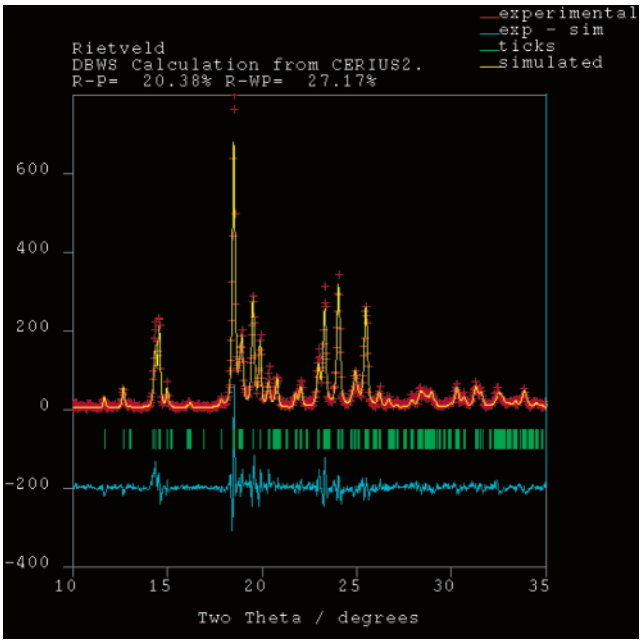


Figure 4. Simulated, experimental, and difference XRD patterns from the Rietveld refinement of olanzapine dihydrate E.

terns, Figure 1. The powder patterns of the higher hydrate and its desolvation product, dihydrate E, were strikingly similar, suggesting no gross structural changes accompanying the dehydration process. The high purity of form I and the hydrated crystal forms could be confirmed by comparing the experimental powder patterns to those calculated from single-crystal X-ray diffraction data; however, in practice, solid-state NMR spectroscopy proved much more useful for establishing the phase purity of the olanzapine samples (vide infra).

Isostructurality and the Rietveld Refinement of Dihydrate E. Powder X-ray diffraction was particularly

Table 2. Selected Torsion Angles (deg) for Olanzapine Crystal Forms[a]

	form I	dihydrate B	dihydrate D	dihydrate E	higher hydrate
N5−C4−N1′−C2′	12.5	7.9	9.7	4.2	6.2
C10a−C3a−C4−N5	35.2	37.3	35.8	37.7	35.1
C3a−C4−N5−C5a	5.4	4.5	5.8	4.0	5.1
C4−N5−C5a−C9a	−43.4	−44.8	−44.8	−44.0	−42.7
C5a−C9a−N10−C10a	55.9	58.2	52.6	58.5	58.2
C9a−N10−C10a−C3a	−56.2	−59.6	−52.6	−59.1	−58.2
N4′−C5′−C6′−N1′	−57.4	−56.6	−58.6	−57.4	−56.8
N1′−C2′−C3′−N4′	59.0	58.8	56.3	57.9	58.4

[a] Angles are reported for the same conformational enantiomer.

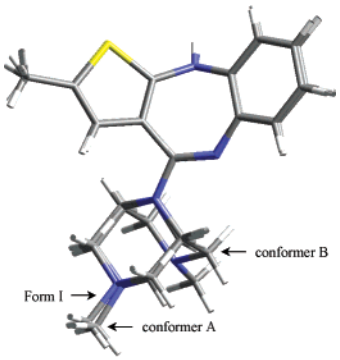


Figure 5. Molecular structure and conformation of olanzapine observed in form I. This conformer and its enantiomer are also present in dihydrates B, D, and E and in the higher hydrate. Conformers A and B are the two energy minima produced in a search for conformational minima.

Ⓦ A 3D rotatable image in PDB format is available.

observed between the powder patterns of dihydrate B and the MeOH and EtOH solvates (not shown), for example, revealed that these crystal forms are isostructural, or nearly so. A particularly useful observation was the isostructurality of dihydrate E and several mixed solvates, including the EtOH−water solvate, as shown in Figure 2.

Efforts to obtain single crystals of dihydrate E suitable for an X-ray structure determination were unsuccessful because this crystal form could not be crystallized directly from solution, and single crystals of the higher hydrate, from which dihydrate E was obtained, fractured as the first of three waters of crystallization was lost from the crystal lattice, Figure 3. Since the X-ray structure of the isostructural EtOH−water solvate was available, a crystal model of dihydrate E was generated by simply replacing the EtOH ethyl group in the mixed solvate with a hydrogen to create the second water of crystallization; the dihydrate structure was then Rietveld-refined. The energy minimization proceeded to yield a structure with a simulated powder pattern that closely matched that of the experimental pattern of dihydrate E ($R = 20.4$), Figure 4.

Conformational Analysis. Olanzapine adopts mirror-related conformations, which rapidly interconvert in solution by inversion of the diazepine ring.[41] This molecular motion is sufficiently frozen in each crystal form, however, such that pairs of opposite enantiomers are observed. Importantly, the same conformers of olanzapine are observed in each crystal structure (for which X-ray data is available). One of the two mirror-related enantiomers present in form I is depicted in

Table 3. Relative Conformational Energies (kJ/mol) of Olanzapine Conformers

basis set	conformer A	conformer B
ab initio		
3-21G*	1.42	0
6-31G*	0	4.53
density functional (SVWN)		
DN	0	1.70
DN*	0	4.62
DN**	0	5.38

benzodiazepine substituents occupying equatorial positions. The diazepine ring is puckered, as evidenced by the 127° dihedral angle between the planes of the thiophene and benzene rings. The piperazine and puckered benzodiazepine rings are nearly coplanar (N5−C4−N1′−C6′ torsion angle = 12°). This relatively coplanar orientation, which has also been observed in dibenzodiazepines, may be attributed to the partial double bond between the piperazine and the diazepine rings. Selected torsion angles describing the molecular conformation of olanzapine in form I, dihydrates B, D, and E, and the higher hydrate are given in Table 2.

Because all of the known crystal structures of olanzapine feature the same pair of conformational enantiomers, a conformational energy minimum has likely been realized.[42] The Cambridge Crystallographic Database was searched for benzodiazepines fused to five- and six-membered rings to determine whether similar molecular conformations are present in structurally similar molecules. Five unique, uncharged structures were retrieved, all of which featured a fused 6−7−6 tricyclic ring system and a 1-piperazinyl side chain.[43] No structures of benzodiazepines fused to five-membered rings were found. Like olanzapine, the five dibenzodiazepine analogues adopted puckered conformations, with dihedral angles between the six-membered rings ranging from 118 to 129°. Additionally, in each of the analogues, the 1-piperazinyl side chain adopts a chair conformation that is in a relatively coplanar orientation with respect to the tricyclic ring system. Like olanzapine, which adopts both conformational enantiomers in all of its crystal structures, all but one of the five structural analogues feature two enantiomeric puckered conformations.

A search for conformational minima was also conducted to assess whether the conformations of olanzapine selected by crystal forces are geometrically close to the global minimum. The conformational search produced two energetic minima, A and B (Figure 5).[44] Conformer A is characterized by a N5−C4−N1′−C6′ torsion angle of 8.9° and a thiophene/benzene ring dihedral angle of 131° and is remarkably similar to that

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