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RESEARCH PAPER

Solubilization and Solid-State Characterization of a Poorly Soluble 5-α Reductase Inhibitor

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ABSTRACT

GI197111X is a 5-alpha reductase inhibitor for the treatment of androgenetic alopecia. Equilibrium solubilities of GI197111X were determined in multiple solvents or cosolvents. A polymorph screen was conducted using suspension equilibration and solution recrystallization methods. Single crystals were grown from pyridine/water and crystal structure was determined using a Bruker SMART diffractometer. Crystal structure data were imported into Cerius2 to provide visualization of the crystal structure and calculation of the simulated X-ray powder diffraction (XRPD) pattern. The solubility of GI197111X was low at 25°C in all vehicles suitable for animal and human dosing. The solubility of 6.4 mg/mL in Capmul MCM made it the only choice for a soft gel dosage form for phase I/II. Solution recrystallization and suspension equilibration of GI197111X have produced only one crystal form. Crystal structure data: orthorhombic $P2_1\ 2_1\ 2_1$; a = 10.8960(6) Å, b = 11.5683(6) Å, c = 20.9019(11) Å; unit cell volume 2634.65(24) $Å^3$; Z = 4; calculated density = 1.248 g/cc. The molecule has seven chiral centers, and single-crystal analysis eliminated all possible stereoisomers except the expected conformation or its enantiomer. Hydrogen bonds occur from both carbonyl oxygens to an H–N group. Simulated vacuum-based crystal morphology (habit) calculated using the Bravais–Friedel–Donnay–Harker, Growth Morphology, and Hartman-Perdok modules in Cerius2 was a close match to the morphology observed by light microscopy.

Key Words: GI197111X; Polymorph; Crystallization; Solubility; Thermal analysis; Powder X-ray diffraction; Single crystal X-ray; Crystal structure.

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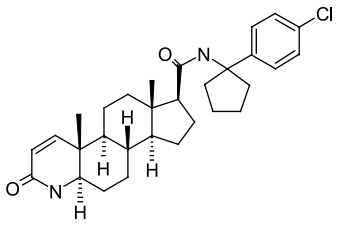
INTRODUCTION

GI197111X (4a,6a-Dimethyl-2-oxo-2,4a,4b,5,6,6a,7,8,9,9a,9b,10,11,11a-tetradecahydro-1H-indeno[5,4-f]quinoline-7-carboxylic acid [1-(4-chloro-phenyl)-cyclopentyl]-amide) is a 5-alpha reductase inhibitor for the treatment of androgenetic alopecia.[1] The compound molecular structure, chemical formula, and molecular weight are shown in Fig. 1. GI197111X is practically insoluble in water. Solubility studies were conducted to search for acceptable toxicology and clinical trial formulation. Screening for and characterizing polymorphs are essential for the successful development of a drug candidate, because different polymorphs have different physicochemical properties.[2,3] The report also details the results of polymorph screen studies, which included suspension equilibration and solution recrystallization. Single crystals were grown from pyridine/water, and crystal structure was determined using a Bruker SMART diffractometer. Crystal structure data were imported into Cerius2 to provide visualization of the crystal structure and calculation of the simulated X-ray powder diffraction (XRPD) pattern. The preliminary results of the study were previously presented in a poster presentation.[1]

EXPERIMENTAL

Materials

GI197111X was manufactured at GlaxoSmithKline Inc., Research Triangle Park, North Carolina. Captex 200 (medium chain esters), Capmul MCM (mono-diglycerides of medium chain fatty acids), and Capmul PG-8 (propylene glycol monocaprylate) were purchased from Abitec (Columbus, OH). Labrafil (oleoyl macrogol-6-glycerides linoleoyl macrogol-6-glycerides) and Gelucire 44/14 (glycerol esters of fatty acids) were purchased from Gattefosse (Paramus, NJ). Vitamin E-TPGS (vitamin E–tocopheryl polyethylene glycol succinate) was purchased from Eastman Kodak (Kingsport, TN). Hydroxypropyl-beta-cyclodextrin (HP-beta-cyclodextrin) was purchased from Spectrum Chemicals (New Brunswick, NJ). Sulfobutylether-beta-cyclodextrin (SBE-beta cyclodextrin) was purchased from Research Diagnostics Inc. (Flanders, NJ). All other materials were reagent grade or better and used without further purification.



Formula: $C_{30}H_{39}ClN_2O_2$; MW: 495.1

Figure 1. Molecular structure of GI197111X.

Table 1. Summary of GI197111X solubility data.

Vehicle	Solubility (mg/mL)
Water	<0.003
Propylene glycol (PG)	3.0
PEG-400	0.69
40% HP-beta-CD	0.56
40% SBE-beta-CD	0.91
Captex 200	1.0
Labrafil	2.1
Capmul PG-8	7.5
Capmul MCM	6.4
25% TPGS/75% PG	2.7
50% TPGS/50% PG	3.2
Gelucire 44/14	2.3

Equilibrium Solubilities

Equilibrium solubilities at 25°C were conducted by weighing excess solid of GI197111X into glass vials, adding approximately 2–3 mL of vehicle (see Table 1 for list of vehicles), sonicating, and vortexing for several minutes. The vials were equilibrated in shaking water baths at 25°C, with samples periodically withdrawn for assay. When the final value was in agreement with the previous one, equilibrium was assumed to be reached. X-ray powder diffraction analysis of the filtered solid showed no evidence of polymorphic form conversion.

The solubility of GI197111X was quantitated by high-performance liquid chromatograph (HPLC) method (see method below). The specificity, sensitivity, linearity, accuracy, precision, and ruggedness of this method are sufficient for the determination. Solubility results listed as ''≤'' imply visual estimates and were not done as equilibrium experiments. Typically, the samples were periodically sonicated and vortexed over the course of 1 week. If solid remained, the samples were heated to completely dissolve and allowed to cool to room temperature. In all cases precipitation was observed upon cooling and assured that the solubility was below the selected value.

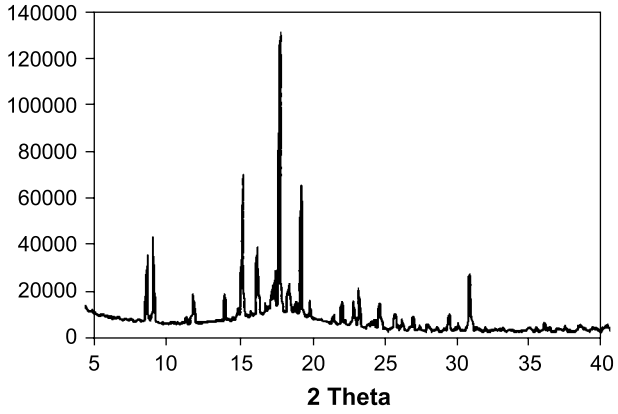


Figure 2. X-ray powder diffraction pattern of GI197111X.

HPLC Method

Instrument: Waters™ 717 Plus Autosampler;
Column: Inertsil ODS-2, 4.6 × 150 mm, 5μm;
A = 0.05% aqueous TFA;
B = Acetonitrile (0.05% TFA);
Ramp from 50% B to 90% B over 15 min, hold at 90% B for 5 min and immediate return to 50% B for 10 min—a total run time of 30 min;
Flow rate = 1 mL/min;
UV detection at 225 nm;
Injection volume = 10 μL.

Suspension Equilibration

Suspensions were prepared with 10 mg/mL of GI197111X in 50% organic/water and equilibrated at 25°C for 6 days and 80°C for 3 days. The organic solvents examined were pyridine, methanol, isopropanol, acetonitrile, and Capmul MCM. The suspensions were filtered, air-dried, and analyzed by XRPD.

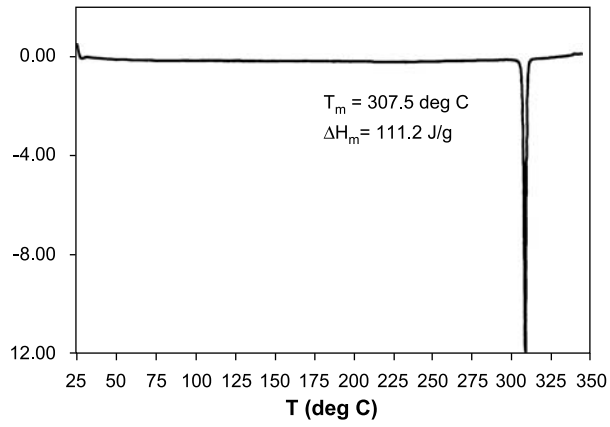


Figure 3. DSC scan of GI197111X.

Table 2. GI197111X crystal structure data

Space group: orthorhombic $P2_1\ 2_1\ 2_1$
Cell dimension: a = 10.8960(6) Å, b = 11.5683(6) Å, c = 20.9019(11) Å
Unit cell volume: 2634.65(24) $Å^3$
FW = 495.10 g/mol
Z (# molecules in unit cell) = 4
Calculated density = 1.248 g/cc

Solution Recrystallization

Suspensions were prepared at 2.5 mg/mL and heated to 80°C to dissolve material, followed by warm filtration. The clear solutions were then allowed to evaporate slowly at room temperature. The crystals were collected by vacuum filtration, air-dried, and analyzed by XRD.

Differential Scanning Calorimetry (DSC)

A model 910S differential scanning calorimeter (TA Instruments, New Castle, DE) equipped with a data station (Thermal Analyst 2200, TA Instruments, New Castle, DE) was used to determine the DSC curves. GI197111X samples were each placed in an open aluminum pan and heated at 10°C/min under nitrogen purge at 40 mL/min.

X-Ray Powder Diffraction (XRPD)

X-ray powder diffraction patterns were obtained with a Scintag, Inc. XDS2000 diffractometer equipped with a copper source and a peltier-cooled solid state detector.

Single X-Ray Crystallography

Single crystals were grown from pyridine/water by slow evaporation and submitted to the X-ray Crystallographic Facility located in the Chemistry Department, University of North Carolina, Chapel Hill, NC (UNC report code c98001). The 2θ and intensity data were collected at −100°C using a Bruker SMART diffractometer. Unit cell dimensions were obtained from 5953 reflections. Structure solution and refinement was based on 4630 unique reflections (R_f = 0.062 and R_w = 0.071). The program used for data reduction and analysis has been described.[4]

Table 3. Single crystal X-ray diffraction unit cell and fractional atomic coordinates.[a]

	x	y	z	Biso[b]
C11	0.25510 (24)	0.19541(15)	0.08814(6)	10.99(13)
C2	0.2487 (6)	0.1451 (6)	0.00935(21)	5.9 (3)
C3	0.1936 (6)	0.2137 (4)	−0.0374 (3)	6.7 (3)
C4	0.1888 (5)	0.1712 (4)	−0.10015(21)	4.57 (22)
C5	0.2334 (3)	0.0633 (4)	−0.11532(17)	3.19 (18)
C6	0.2861 (4)	−0.0011 (5)	−0.06621(20)	4.60 (23)
C7	0.2938 (5)	0.0385 (7)	−0.00456(23)	5.9 (3)
C8	0.2266 (3)	0.0182 (3)	−0.18330(17)	2.51 (15)
C9	0.2142 (3)	−0.1136 (3)	−0.18888(20)	3.39 (17)
C10	0.1588 (5)	−0.1360 (4)	−0.25378(22)	4.84 (23)
C11	0.1045 (4)	−0.0224 (4)	−0.27755(19)	3.36 (18)
C12	0.1119 (3)	0.0589 (3)	−0.22047(18)	2.62 (16)
N13	0.33458 (24)	0.0590 (3)	−0.22004(13)	2.21 (12)
C14	0.4514 (3)	0.0237 (3)	−0.20958(16)	2.03 (15)
O15	0.47776 (21)	−0.05004(22)	−0.16944(11)	2.70 (11)
C16	0.5460 (3)	0.0786 (3)	−0.25354(16)	2.03 (14)
C17	0.6808 (3)	0.0654 (3)	−0.22915(17)	2.52 (15)
C18	0.7598 (3)	0.0437 (3)	−0.28868(15)	2.19 (14)
C19	0.6769 (3)	0.0749 (3)	−0.34430(16)	1.71 (13)
C20	0.7096 (3)	0.0454 (3)	−0.41344(15)	1.71 (13)
C21	0.8403 (3)	0.0814 (3)	−0.43026(17)	2.15 (15)
C22	0.8688 (3)	0.0741 (3)	−0.50145(17)	2.13 (15)
C23	0.7745 (3)	0.1427 (3)	−0.53817(15)	1.77 (13)
N24	0.80420 (24)	0.1538 (3)	−0.60630(13)	1.96 (11)
C25	0.7263 (3)	0.2063 (3)	−0.64653(16)	2.01 (14)
O26	0.75276 (23)	0.22929(20)	−0.70275(10)	2.48 (11)
C27	0.6031 (3)	0.2301 (3)	−0.61970(17)	2.27 (15)
C28	0.5648 (3)	0.1795 (3)	−0.56749(17)	2.23 (15)
C29	0.6428 (3)	0.0983 (3)	−0.52871(16)	1.93 (14)
C30	0.6253 (3)	−0.0250 (3)	−0.55503(16)	2.28 (15)
C31	0.6130 (3)	0.1051 (3)	−0.45612(15)	1.72 (13)
C32	0.4845 (3)	0.0599 (3)	−0.43855(16)	2.21 (14)
C33	0.4533 (3)	0.0802 (3)	−0.36781(16)	2.15 (14)
C34	0.5488 (3)	0.0304 (3)	−0.32301(16)	1.92 (14)
C35	0.5425 (3)	−0.1020 (3)	−0.32096(17)	2.32 (15)
H3	0.158	0.286	−0.026	7.5
H4	0.140	0.216	−0.129	5.4
H6	0.309	−0.077	−0.080	5.4
H7	0.336	−0.010	0.025	6.7
H9[a]	0.292	−0.149	−0.180	4.2
H9[b]	0.166	−0.136	−0.153	4.2
H10[a]	0.095	−0.193	−0.252	5.6
H10[b]	0.215	−0.167	−0.285	5.6
H11[a]	0.154	0.004	−0.312	4.1
H11[b]	0.026	−0.021	−0.299	4.1
H12[a]	0.115	0.141	−0.228	3.4
H12[b]	0.042	0.055	−0.192	3.4
H13	0.323	0.124	−0.248	3.0
H16	0.528	0.160	−0.257	2.8
H17[a]	0.683	−0.004	−0.204	3.3
H17[b]	0.706	0.134	−0.207	3.3
H18[a]	0.784	−0.036	−0.288	3.0

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Table 3. Continued.

	x	y	z	Biso[b]
H18[b]	0.828	0.096	−0.291	3.0
H19	0.669	0.158	−0.343	2.5
H20	0.701	−0.037	−0.419	2.5
H21[a]	0.851	0.161	−0.420	2.9
H21[b]	0.894	0.032	−0.406	2.9
H22[a]	0.948	0.105	−0.511	2.9
H22[b]	0.865	−0.004	−0.517	2.9
H23	0.775	0.217	−0.518	2.6
H24	0.885	0.121	−0.609	2.8
H27	0.550	0.282	−0.642	3.1
H28	0.481	0.190	−0.555	3.0
H30[a]	0.665	−0.031	−0.596	3.1
H30[b]	0.539	−0.041	−0.560	3.1
H30[c]	0.661	−0.080	−0.526	3.1
H31	0.614	0.187	−0.447	2.5
H32[a]	0.431	0.103	−0.466	3.0
H32[b]	0.486	−0.021	−0.448	3.0
H33[a]	0.449	0.162	−0.360	2.9
H33[b]	0.374	0.048	−0.359	2.9
H35[a]	0.463	−0.125	−0.306	3.1
H35[b]	0.605	−0.131	−0.293	3.1
H35[c]	0.555	−0.133	−0.363	3.1

[a]UNC report code c98001.
[b]Biso is the mean of the principal axes of the thermal ellipsoid.

Molecular Modeling

Single-crystal structure data were imported into Cerius2 (Accelrys Inc., San Diego, CA), v. 4.2 and Materials Studio (Accelrys Inc., San Diego, CA), v. 2.0 to provide visualization of the crystal structure and calculation of the simulated XRPD pattern. Simulated vacuum-based crystal morphology (habit) was calculated using the Bravais–Friedel–Donnay–Harker, Growth Morphology, and Hartman–Perdok modules in Cerius2.

RESULTS AND DISCUSSION

GI197111X is practically insoluble in water, with water solubility less than 0.003 mg/mL (Table 1). The formulation strategy for GI197111X was to develop a solution or self-emulsifying dosage form. The solvents listed in Table 1 are those that could be used for human dosing as a solution-in-bottle for early clinical studies as well as those that are compatible with gelatin capsule shells. In addition to solutions using good solvents or cosolvent mixtures, other approaches that can be used for poorly soluble, nonionizable drugs include: solid state manipulation (e.g., amorphous form), particle engineering (e.g., nanomilling), solubilization by lipids (e.g., in emulsions, microemulsions, micelles, liposomes), and solubilization with complexing agents (e.g., cyclodextrins).[5–7] However, the solution dosage form is the

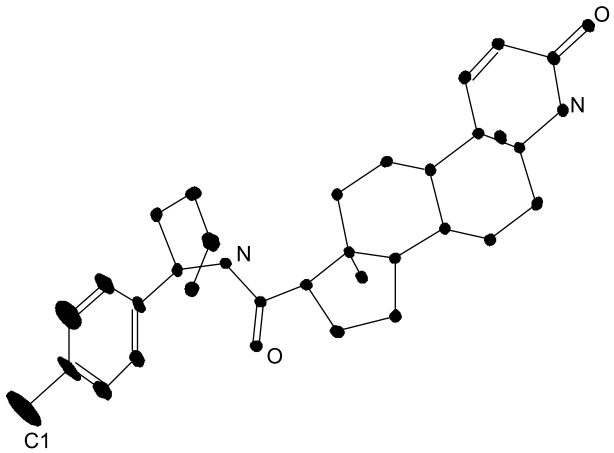

Figure 4. Thermal ellipsoid plot of asymmetric unit with hydrogen atoms not shown. Atoms shown at 90% probability being within ellipsoid.

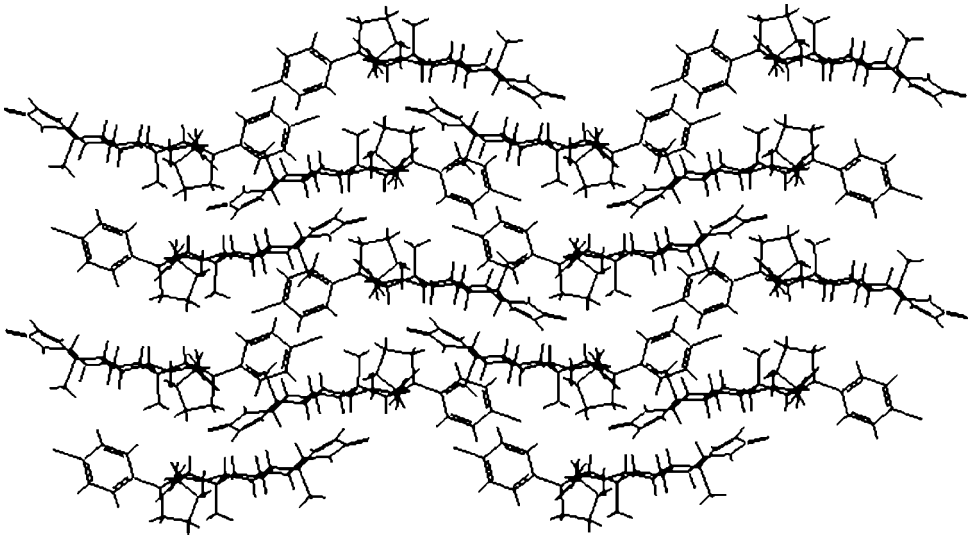

Figure 5. Crystal packing diagram of $1 \times 2 \times 2$ ($a \times b \times c$) unit cells viewed down the a-axis.

simplest in terms of development, scale-up, and manufacture, which is the reason this approach was undertaken in preference to the other ones, especially to minimize effort in early clinical evaluation before safety and efficacy are established.

All of the solubility results are compiled in Table 1. The low solubility in this broad range of vehicles is not too surprising, considering that GI197111X's melting point is quite high, 308°C. The solubility of such high melting drugs is generally limited by the high lattice energy of the crystal structure, which is implied by the high heat of fusion, 111 J/g in this case. Unfortunately, the solubility in PEG-400 (0.69 mg/mL) was too low to be practical value for toxicology and clinical studies, as the concentration for tox and clinical formulation is 2 mg/mL. Proplene glycol (PG) could be used in animal and human studies, but it was found that a surfactant was needed to prevent immediate precipitation of GI197111X from this vehicle upon mixing with 0.1 N HCl. Thus, the 50% TPGS/50% PG formulation was used in toxicology experiments.

Capmul MCM had relatively ''high'' solubility (6.4 mg/mL with water) and was selected for the phase I/II clinical program, due to its compatibility with soft gel capsules. Gelucire 44/14 was also evaluated, but crystallization was observed at room temperature in the wax form. Capmul PG-8 did not progress due to its

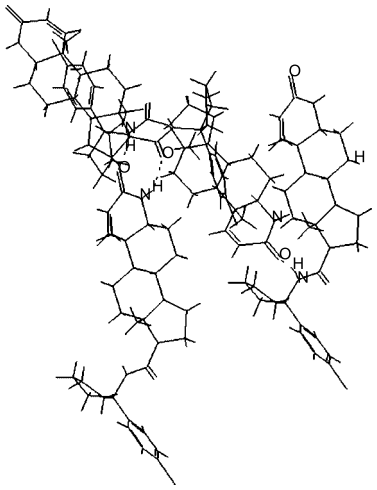

Figure 6. Partial packing diagram showing hydrogen bonding between carbonyl oxygens and H–N groups.

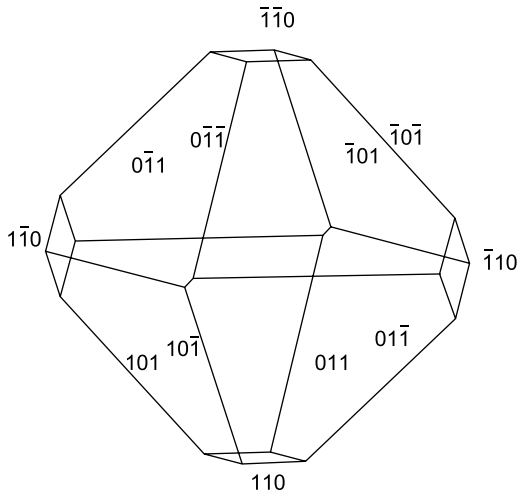

Figure 7. Hartman–Perdok (calculated) morphology.

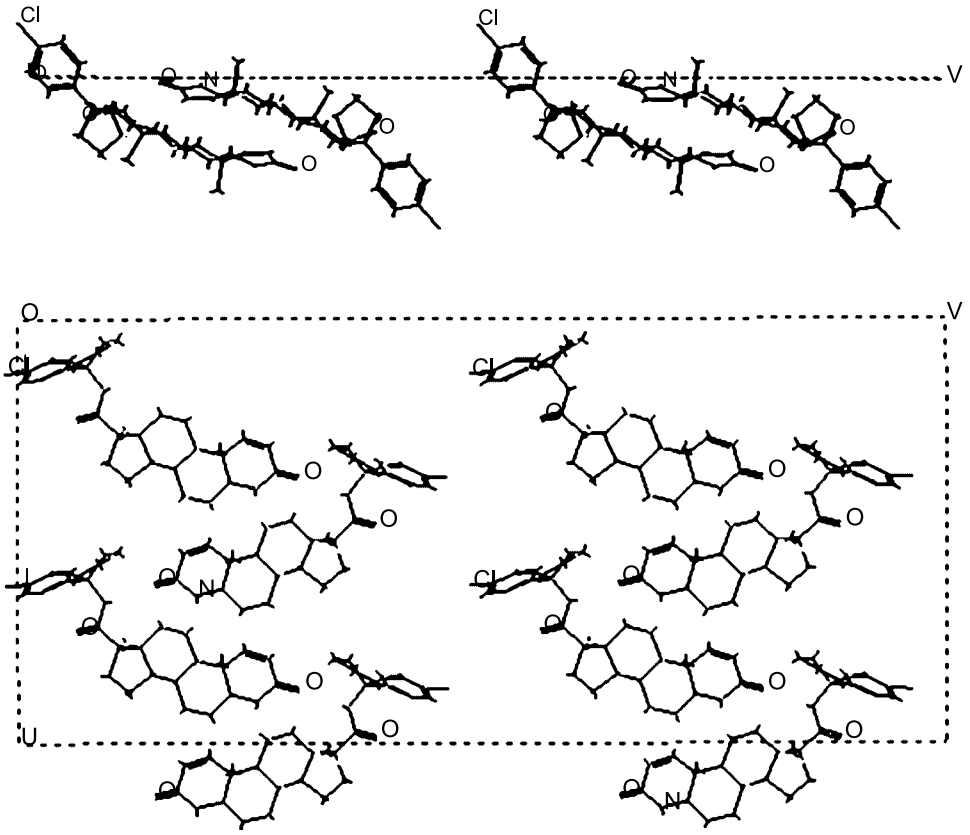

Figure 8. Surface of (0 1 1) face shown on edge (top figure) and viewed from above (bottom figure).

propensity to absorb water and potential regulatory hurdles in establishing generally regarded as safe (GRAS) status. For simplicity in manufacturing as solution-in-bottle, 25% TPGS/75% PG was chosen as the first-time-in-human formulation.

There are no major differences in the powder XRD patterns for all samples studies in the polymorph screen (Fig. 2). Likewise, all samples exhibited similar thermal behavior with melting points about 308°C (Fig. 3). Solution recrystallization and suspension equilibration of GI197111X in pyridine, methanol, isopropanol, acetonitrile, and their 50% organic/water mixtures have produced only one crystal form. Recrystallization from the formulation vehicle Capmul MCM with and without 10% water produces the same crystal form found with the synthetic process solvents. To date, these experiments have revealed the existence of only one polymorphic form of GI197111X.

The crystal structure data are listed in Table 2 and the Unit Cell and Fractional Atomic Coordinate information is provided in Table 3. The crystal structure is composed of one molecular conformation (one molecule in the asymmetric unit). The molecule has seven chiral centers, and single-crystal analysis eliminated all possible stereoisomers except the expected conformation or its enantiomer. The crystal structure asymmetric unit is presented in Fig. 4. The C-C-N bond angle between the chlorophenyl ring, connecting carbon atom of the five-membered ring, and the nearest nitrogen atom is 109.7°. A crystal packing diagram viewed down the a-axis is presented in Fig. 5.

Hydrogen bonds occur from both carbonyl oxygens to an H–N group. Each carbonyl and H–N group is involved in only one hydrogen bond, and the hydrogen bonds connect different molecules (intermolecular bonding) as shown in Fig. 6. No solvent of crystallization was detected. A Connolly Surface

Table 4. Low-temperature single crystal (−100°C) and room-temperature Rietveld refined unit cell parameters.

	Original single-crystal result	Rietveld refined result	% Change
a	10.8960	10.9852	+0.9
b	11.5683	11.7056	+1.2
c	20.9019	20.8775	−0.1
Density	1.248	1.227	−1.7

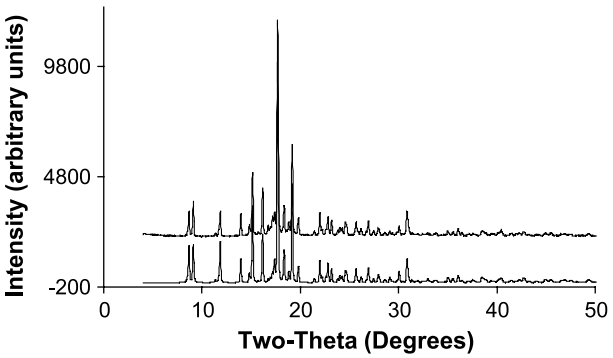

Figure 9. Overlay of experimental (top) and Rietveld refined simulated XRD patterns.

calculation revealed pockets that could accommodate solvents no larger than water and no channels that could act as solvent tunnels.

Simulated vacuum-based crystal morphology (habit) was calculated using the Bravais–Friedel–Donnay–Harker, Growth Morphology, and Hartman–Perdok modules in Cerius2. The Hartman–Perdok (calculated) morphology was a close match to the morphology observed by light microscopy. The calculated morphology with labeled faces is shown in Fig. 7. The dominant faces are the (0 1 1, 0-1 1, 0-1 1, 0-1-1) and (1 0 1,-1 0 1, 1 0-1,-1 0-1) families. The surfaces of each crystal face were viewed using the Surface Builder tool within Cerius2. The surface of the (0 1 1) face is shown in Fig. 8. The surface chemistry is nonhydrophillic, which is consistent with the low aqueous solubility.

Using a room-temperature experimental X-ray powder diffraction pattern, the unit cell low temperature single crystal structure was Rietveld refined. Without refining for preferred orientation in the powder pattern, the refinement of the unit cell, powder XRD baseline, peak profile, and XRPD sample displacement error gave a R_{wp} of 15.5%. Including preferred orientation in the refinement dropped the R_{wp} to 12.8%. Less than 2% change in unit cell parameters were observed after refinement, as shown in Table 4. An overlay of the refined simulated powder pattern and the experimental pattern is provided in Fig. 9.

CONCLUSIONS

The solubilities of GI197111X were evaluated in many vehicles suitable for animal and human dosing. The solubility of 6.4 mg/mL in Capmul MCM made it the only choice for a soft gel dosage form for phase I/II. The TPGS/PG mixtures provided the simplest formulations for toxicology and first-time-in-human evaluation. Only one crystal form was identified for GI197111X. The molecule has seven chiral centers, and single-crystal analysis eliminated all possible stereoisomers except the expected conformation or its enantiomer. Simulated vacuum-based crystal morphology (habit) using the Bravais–Friedel–Donnay–Harker, Growth Morphology, and Hartman–Perdok modules in Cerius2 Cerius2 was a close match to the morphology observed by light microscopy. Structural analysis and molecular modeling are complementary techniques in studying the solid state behavior of the GI197111X.

ACKNOWLEDGMENT

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REFERENCES

1. Zhu, H.; Varlashikin, P.; Sacchetti, M. Solubilization and Solid State Characterization of a Poorly Soluble Compound, A Poster Presentation in 16th AAPS Annual Meeting, Toronto, Canada, Nov. 10–14, 2002.
2. Haleblian, J.K. Characterization of habits and crystalline modification of solids and their pharmaceutical applications. J. Pharm. Sci. **1975**, *64*, 1269–1288.
3. Byrn, S.R. *Solid-State Chemistry of Drugs*; Academic Press: New York, NY, 1982.
4. Gabe, E.J.; LePage, Y.; Charland, J.-P.; White, P.S. NRCVAX—an interactive program system for structure analysis. J. Appl. Crystallogr. **1989**, *22*, 384–387.
5. Yalkowski, S.H. *Solubility and Solubilization in Aqueous Media*; American Chemical Society: Washington, D.C., 1999.
6. Jinno, J.; Oh, D.; Crison, J.R.; Gordon, A. Dissolution of ionizable water-insoluble drugs: the combined effect of pH and surfactant. J. Pharm. Sci. **2000**, *89*, 268–274.
7. Redenti, E.; Szente, L.; Szejtli, J. Cyclodextrin complexes of salts of acidic drugs. Thermodynamic properties, structural features, and pharmaceutical applications. J. Pharm. Sci. **2001**, *90*, 979–986.