Structure Determination and Characterization of Carbendazim Hydrochloride Dihydrate

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ABSTRACT

The objective of this study was to synthesize and characterize the hydrochloride salt of carbendazim with the aim of improving the intrinsic solubility of the parent compound. Carbendazim hydrochloride dihydrate was synthesized for the purpose of increasing the aqueous solubility of the parent drug, carbendazim. This was done with the commonly used saturation and cooling method. The structure was determined by single crystal radiograph crystallography, and the hydrochloride salt was found to be a dihydrate. The salt crystallized in a P 2_1 2_1 2_1 (#19) space group, which is typical for nonplanar, achiral, and noncentrosymmetric molecules. The asymmetric unit is comprised of 1 molecule each of carbendazim and chloride and 2 water molecules. The carbendazim molecules arrange themselves in a helical structure, with the waters and the chloride molecules in the channel linking the helix. The crystal lattice is held together by numerous hydrogen bonds, as well as van der Waals interactions. The melting point of the salt is 125.6°C. The solubility of the salt is 6.08 mg/mL, which is a thousand-fold increase from the intrinsic solubility (6.11 μg/mL) of the free base.

KEYWORDS: carbendazim dihydrate hydrochloride, radiograph crystallography, helical structure, enantiotropic

INTRODUCTION

Carbendazim (methyl, 2-benzimidazolecarbamate), shown in Figure 1, is a well-known antifungal agent that has been discovered recently to have anticancer properties. The compound is an ampholyte with a basic pKa of 4.48 and acidic pKa of 10.8. Ni et al[1] reported a melting point of 320°C for carbendazim and an intrinsic solubility of 6.11 μg/mL. The poor water solubility, in turn, leads to poor dissolution.

The goal is to deliver carbendazim orally. As stated by Hendriksen et al,[2] solubility is essential if an orally administered drug is to be absorbed. Lipinsky et al[3] also showed that drugs with poor aqueous solubility normally have poor bioavailability, and, therefore, salt synthesis may be helpful in resolving this problem. Salt formation is a mode of changing the pharmacokinetic properties of a drug by modifying its physical and chemical properties. There are different criteria to consider when selecting a conjugate acid or base for salt formation. The type of salt to be prepared is dependent on the chemical properties (pKa, logP) of the drug and the counter ion, the mode of preparation, the safety of the counter ion, and the route of administration. The yield, rate, and quality of crystallization, as well as cost and availability of conjugate acid/base, also need to be considered.[4]

The most common criteria for counter ion selection was proposed by Tong and Whitesell,[5] where they claimed that for the preparation of salts of a basic drug, the pKa should be at least 2 pH units lower than the pKa of the drug. For weakly basic drugs, a salt of an inorganic acid (eg, hydrochloride, sulfate, or phosphate) or a sulfonic acid (mesylate or isothionate) could be considered.[6]

Hydrochlorides are the most commercially marketed salts (43%) as earlier reported by Berge et al[7] and Bighley et al[8] compiled data from the 1993 edition of Martindale, the Extra Pharmacopeia, and showed that 44% of the marketed salts are hydrochloride. Hydrochloride salts are common because of the low molecular weight and low toxicity. The use of hydrochloric acid as the conjugate acid for salt formation in this case was based on the criteria described above. In this article we focus on the synthesis and characterization of the hydrochloride salt of carbendazim.

MATERIALS AND METHODS

Materials

Carbendazim was provided by Procter & Gamble Company (Cincinnati, OH) and used as received. The hydrochloric acid (37% American Chemical Society reagent) was purchased from Sigma (St Louis, MO). Millipore water was used throughout the experiment.

Preparation of Carbendazim-Hydrochloride Dihydrate Salt

Ten milliliters of 0.1 mol/L HCl was saturated with carbendazim at 80°C and stirred at 250 rpm for 30 minutes. The solution was filtered with a 0.45-m filter and allowed to cool to the ambient room temperature (approximately

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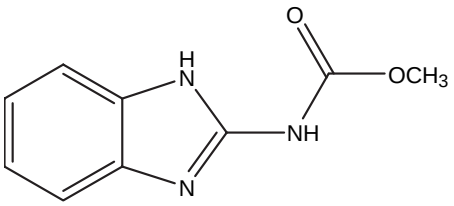


Mol Wt: 191.19
pKa: 4.48, 10.80
MP: 593 K
Aq sol: 6.11 mcg/ml

Mol Wt: 36.46
pKa: <0
MP: 159 K
BP: 188 K

Figure 1. Carbendazim and hydrochloric acid structure and physical parameters.

25°C). Crystals were observed within 24 hours. The crystal was examined under polarized light using a Leica DMLP polarizing microscope (E Licht Co, Denver, CO). The photomicrographs were taken using a Nikon camera attached to the microscope.

Single Crystal Radiograph Crystallography

A colorless single-needle crystal of the salt, with dimensions of 0.08 × 0.12 × 0.55 mm, was mounted on a glass fiber and immersed in a stream of nitrogen gas. The temperature was set at 170 K at a power setting of 50 kV and 40 mA. Radiograph data was collected on a Bruker SMART 1000 CCD detector, and refinement was performed with a SMART 1000 system using graphite monochromated Mo Kα radiation (0.71073Å). The solution was achieved using direct methods followed by Fourier synthesis as described by Massa.[9] SHELXS-90 and SHELXL-97 were used to solve and refine the structure, respectively. The molecular graphics were performed using Bruker SHELXTL, Version 5.0 (Bruker, Madison, WI).

Thermal Analysis

A differential calorimeter (DSC), DSC Q1000 by TA Instruments (New Castle, DE), was used to determine the melting point. Indium was used to calibrate the DSC. A sample of 1 to 2 mg was run using crimped aluminum pans and heated at a rate of 5°C/min. Thermogravimetric analysis (TGA) was performed using a TGA Q-50 thermogravimetric analyzer (TA Instruments, New Castle, DE) where samples of 1 to 2 mg were placed in an open aluminum pan and heated at a rate of 2 to 15°C/min.

High-Performance Liquid Chromatography Method

Samples were analyzed by a high-performance liquid chromatography instrument (Beckman, Fullerton, CA) consisting of a Beckman 125 solvent delivery system, a Beckman 507e autosampler, and a Beckman 168 diode array detector. The data were analyzed using System Gold Chromatographic Acquisition Software (Beckman, Fullertown, CA). The chromatographic system was adopted from Ni et al[1] with the following slight modifications: column, Pinnacle ODS amine column (250 × 4.6 mm); mobile phase, 40:60 mixture of 20 mmol/L phosphate buffer (pH 3) /Acetonitrile; flow rate, 1 mL/min; ultraviolet detection, 280 nm; injection volume, 20 μL; and retention time, approximately 3.5 minutes.

Solubility Determination

Multiple solutions of carbendazim hydrochloride salt were rotated end to end for 24 to 48 hours in 4-mL glass vials containing 1 to 2 mL of Millipore water at room temperature (25°C). The saturated solutions were filtered using 0.45-μm Millipore filter and assayed by high-performance liquid chromatography.

Moisture Absorption Study

The moisture sorption of the hydrochloride salt was determined by exposing weighed amounts (approximately 2 to 3 mg) of salts in 4-mL glass vials placed in sealed desiccators containing saturated salt solutions.

The study was performed at relative humidity values of 43% (saturated solution of potassium carbonate) and 81% (saturated solution of potassium bromide) at 25°C. The humidity was measured using a Humidity/Temp Monitor 800016 by Sper Scientific (Scottsdale, AZ). The samples were stored in the desiccators for 8 days, after which they were weighed to calculate the percentage of weight change.

RESULTS AND DISCUSSION

Single Crystal Structure Determination

The crystals were synthesized as described and observed under the microscope. They were found to be biofringent under polarized light and are rod-like with rough-wedged edges as shown in Figure 2.

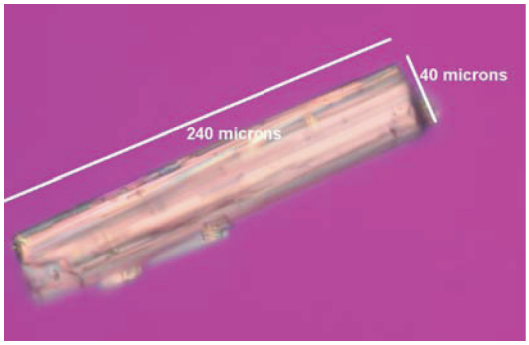


Figure 2. Photomicrograph of a crystal of carbendazim-hydrochloride dihydrate.

Table 1. Crystal and Structure Refinement Data for Carbendazim Hydrochloride

Empirical formula	$C_9 H_{14} Cl N_3 O_4$	
Formula weight	263.68	
Temperature	170(2) K	
Wavelength	0.1707 Å	
Crystal color	opaque	
Crystal size/mm	0.55 × 0.12 × 0.08	
Crystal system	Orthorhombic	
Space group	P 2_1 2_1 2_1 (#19)	
Unit cell dimensions	a = 5.6916(7) Å	α = 90°
	b = 13.3375(17) Å	β = 90°
	c = 15.4889(19) Å	γ = 90°
Volume	1175.8(3) $Å^3$	
Z	4	
Density (calculated)	1.490 Mg/m^3	
Absorption coefficient	0.333 mm^{-1}	
F(000)	552	
Limiting indices	$-7 \leq h \leq 7$, $-16 \leq k \leq 16$, $-18 \leq l \leq 19$	
Reflections used	12994	
Independent reflections	2321	
θ range for data collection	2.01−26.11°	
Absorption coefficient	none	
Max. and min transmission	0.9732 and 0.98380	
Refinement method	Full-matrix least squares on F^2	
Data/restraints/parameters	2321/0/170	
Goodness-of-fit on F^2	1.073	
Final R indices [I>2sigma(I)]	R1 = 0.0397, wR2 = 0.0714	
R indices (all data)	R1 = 0.0523, wR2 = 0.0754	
Largest difference peak and hole	0.301 and -0.188 e.$Å^{-3}$	
RMS difference density	0.057 e.$Å^{-3}$	
Flack Parameter	−0.03(7)	

After data collection and refinement, the systematic absences and intensity statistics indicated the space group to be P 2_1 2_1 2_1 (#19), which was consistent with the refinement. The absolute configuration was determined by refinement of the Flack parameter. This was found to be -0.03,[1] where the expected values are 0 (within 3 estimated standard deviations) for correct and +1 for inverted absolute structure. The crystal and structure refinement data are summarized in Table 1.

Hydrogen Bonding and Crystal Packing

The hydrochloride salt crystallized in the orthorhombic space group P 2_1 2_1 2_1 (#19), which is noncentrosymmetric,

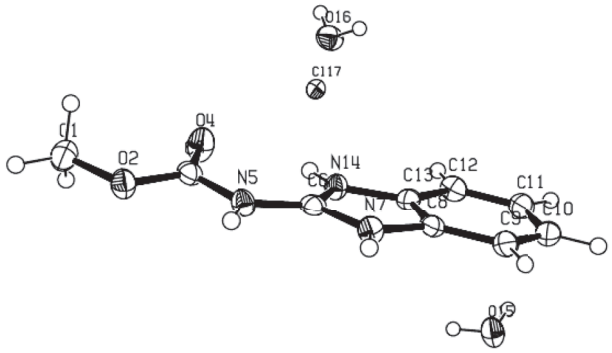


Figure 3. Carbendazim hydrochloride assymetric unit.

polar, chiral, and belongs to the laue class mmm. The space group P 2_1 2_1 2_1 depicts a system that consists of 3 orthogonal axes, a, b, and c, with a 2-fold screw axis running along each direction. The application of these symmetry operations generates 4 equipoint transformations, which, in turn, leads to a general position multiplicity of 4 in the unit cell.

The asymmetric unit is the minimum group of atoms of which the positions, together with those formulated by the symmetry operations of the space group, generate the complete contents of the unit cell. Based on the multiplicity, there are 4 asymmetric units (Z = 4) in the unit cell, and each consists of 1 drug molecule, 1 hydrochloride, and 2 water molecules, as shown in Figure 3.

It is apparent from Figure 3 that the imidazole nitrogen N14 is protonated. This forms a positive charge on the drug molecule, which is stabilized through charge distribution

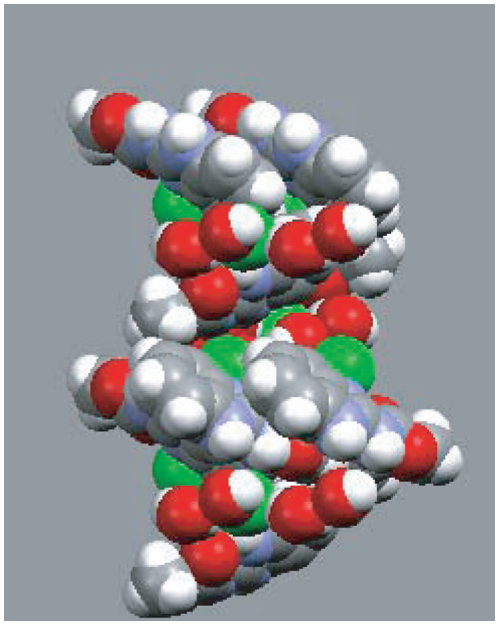
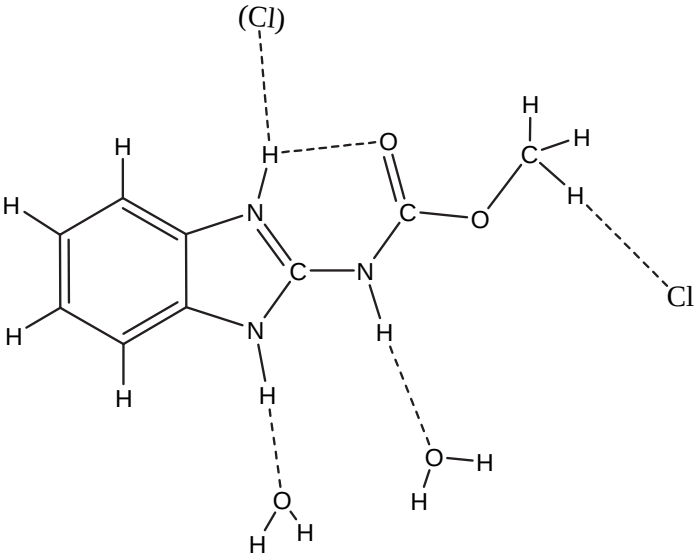


Figure 4. Helical packing of carbendazim hydrochloride.



Where (Cl) is not part of the asymmetric unit

Figure 5. Schematic of the hydrogen bonding of carbendazim hydrochloride.

among N5, N7, and N14. The charged chloride species acts as the counter ion.

The carbendazim molecules arrange themselves in a helical structure, as shown in Figure 4, with the waters and the chloride molecules linking the carbendazim molecules. The crystal is held together by hydrogen bonding and through π-π interactions involving the benzimidazole rings. These interactions are significant in maintaining the crystallinity of the system.

There are weak hydrogen bonds with the chloride ion (N14-H14A-Cl17), which are attributable to the high electronegativity of chloride, its large size, and its proximity to hydrogen. The hydrogens on the terminal methyl group are partially positive because of the electronegativity of the neighboring oxygen atoms, hence the presence of a weak hydrogen bond C(1)-H(1A)-Cl(17) with chlorine. The donor/acceptor distance is 3.693 Å, which is less than the van der Waals distance (4.08 Å), and the angle is almost linear (157.82). The hydrogen bonding is depicted in the schematic in Figure 5 and the bonding angles and distance are shown in Table 2.

There is a weak intramolecular hydrogen bond between the oxygen (O4) of the carbamate group and the proton (H14) on the imidazole nitrogen (N14). This forms a 6-membered ring with a bond angle of 116.12°. The proton, H14, also forms a hydrogen bond with the chloride ion with an angle of 147.6°, which is less than ideal, probably because of competition with the other hydrogen bond. Additionally, the size and electronegativity of the atoms (chlorine and oxygen) repel each other.

Thermal Analysis

The DSC and TGA plots for the salt are shown in Figure 6. The first endotherm at 69.5°C corresponds to a weight loss of 13.1%. This is in agreement with the loss of 2 waters of hydration, which would produce a 13.6% weight loss. The major endotherm occurs at 125°C and corresponds to a 17% weight loss, which is approximately the loss of HCl. This is confirmed by a positive chloride test on the gas released at this temperature and negative chloride test of the residue. The remaining endotherms are likely unstable forms of the anhydrous carbendazim.

Solubility of Hydrochloride Salt

The solubility of the salt is determined on its ability to either reinforce or destroy the structure of water in their vicinity. Most organic salts are chaotropic as is observed in

Table 2. Hydrogen Bonding Table for Hydrochloride Salt*

D-H	d(D-H)	d(H..A)	<DHA	d(D..A)	A
N5-H5A	0.88	1.885	170.74	2.758	O16
N7-H7A	0.88	1.786	174.79	2.664	O15
N14-H14A	0.88	2.198	116.12	2.703	O4
N14-H14A	0.88	2.364	147.66	3.143	Cl17
O15-H15B	0.804	2.299	160.82	3.07	Cl17
O16-H16B	0.771	2.395	149.07	3.082	O16
O16-H16B	0.771	2.493	127.06	3.021	O15
O16-H16A	0.818	2.391	173.74	3.205	Cl17
O15-H15A	0.88	2.452	151.04	3.249	Cl17
O15-H15A	0.88	2.622	125.17	3.211	O4
C(1)-H(1A)	0.88	2.767	157.82	3.693	Cl17

*D and A refer to donor and acceptor atoms respectively.
Distances in Å and angles in °C.

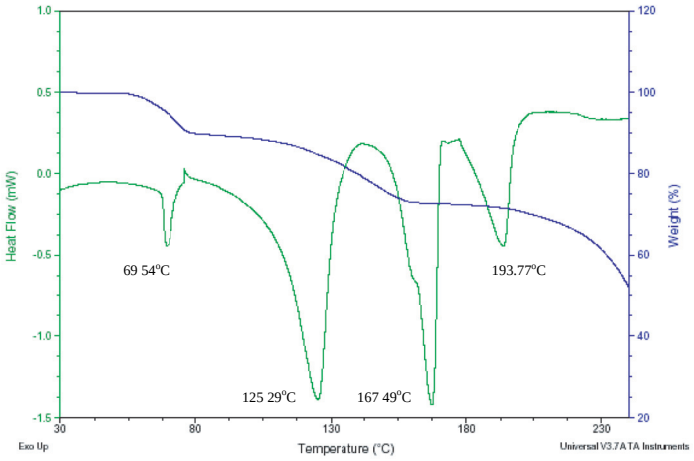


Figure 6. DSC and TGA of the hydrochloride salt.

the case of carbendazim dihydrate hydrochloride, where the salt ionizes and the basic and acidic moieties disrupt the ordered structure of water. The solubility of the dihydrate salt was determined to be 6.08 mg/mL (approximate pH 3.0), which is 1,000 times that of the parent drug.

Moisture Absorption Study

Tong and Zograf[10] showed that exposure of crystals to elevated relative humidities and temperatures could affect crystallinity. Assessment of the ability of a compound to adsorb moisture is an important developability criterion. This is used as a guide to the pharmaceutical industry as to what ambient conditions are necessary when handling a specific compound. At 43% and 81%, the percentage change in weight was minimal, at 0.36% and 0.59%, respectively. Parenthetically, this salt was synthesized as a hydrate, thus, its capability of moisture absorption is limited.

CONCLUSION

The synthesis of the hydrochloride salt of carbendazim was successful. The salt showed a marked increase in solubility from the free base. This will, in turn, yield a higher dissolution rate. The increase in solubility will help in achieving the targeted oral dose.

ACKNOWLEDGMENTS

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