COMMUNICATION

Discovery of a Stable Molecular Complex of an API With HCl: A Long Journey to a Conventional Salt

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Received 3 July 2007; revised 8 October 2007; accepted 17 October 2007

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21264

ABSTRACT: We report formation and characterization of the first pharmaceutically acceptable and stable molecular complex of a mono-HCl salt of Compound 1 with HCl. The novelty of this discovery is due to the fact that there is only one major basic site in the molecule. Thus this complex is reminiscent of other noncovalent crystalline forms including solvates, hydrates, cocrystals and others. To the best of our knowledge, the observed bis-HCl salt appears to be the first example of an active pharmaceutical ingredient in a form of a stable HCl complex. The paucity of stable complexes of APIs with HCl is likely due to the fact that HCl is a gas at ambient conditions and can easily evaporate compromising physical (and chemical) stability of a drug. The bis-HCl salt was chemically/physically stable at low humidity and the molecular HCl stays in the lattice until heated above 140°C under nitrogen flow. Structure solution from powder diffraction using the Monte Carlo simulated annealing method as well as variable temperature ATR-FTIR suggest the possibility of weak hydrogen bonding between the molecular HCl and the nitrogen atom of the amide group. Two years later after the search for a suitable pharmaceutical salt began, the elusive conventional mono-HCl salt was obtained serendipitously concluding the lengthy quest for a regular salt. This work emphasizes the necessity to be open-minded during the salt selection process. It also highlights the difficult, lengthy and often serendipitous path of finding the most appropriate form of an API for pharmaceutical development. J Pharm Sci 97:3721–3726, 2008

Keywords: salts; cocrystal; HCl cocrystal; HCl complex

This article contains supplementary material, available at www.interscience.wiley.com/jpages/0022-3549/suppmat.

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Journal of Pharmaceutical Sciences, Vol. 97, 3721–3726 (2008)

In memory of Jacqueline Smitrovich.

Finding a suitable crystalline form of an active pharmaceutical ingredient (API) for pharmaceutical product development is of utmost importance. Some critical attributes of the API include crystallinity, chemical and physical stability, solubility in the physiological pH range (2–8) and

processability. Of these, crystallinity is an extremely important parameter as it allows: (a) process chemists and chemical engineers to isolate, purify and control the morphology of the material; and (b) pharmaceutical scientists to develop a suitable dosage form.[1] Active pharmaceutical ingredients have been reported to exist in various forms including salts, solvates, hydrates, and recently cocrystals.[2,3] In recent years, a high-throughput approach has become standard when a salt screen is designed according to the structure of the compound and its pK_a.[4] Conventional wisdom dictates that in order to form a stable salt, at least a two-unit difference in pK_a is required.[5] Recently, this conventional system has been somewhat challenged by crystal engineering of cocrystals, which rely on hydrogen bonding and do not require existence of a pK_a difference between the acid and base.[6,7] Here we report the formation and characterization of the first pharmaceutically acceptable and stable complex of the mono-HCl salt of Compound 1 with HCl. This discovery conflicts with "conventional wisdom" due to the presence of only one pK_a in the molecule.

Compound 1 was a development candidate for oral administration.[8] A pharmaceutically acceptable crystalline form of *N*-{(1*S*)-1-[2-(1-{[(3*S*,4*R*)-1-*tert*-butyl-4-(2,4-difluorophenyl)pyrrolidin-3-yl]carbonyl}piperidin-4-yl)-5-chlorophenyl]ethyl}acetamide (Compound I; Fig. 1), had been elusive for nearly a year. Numerous crystallization attempts involving multiple high-throughput screens as well as conventional bench-top crystallization attempts yielded three crystalline forms of Compound I—a tetrafluoroborate salt, a perchlorate salt, and a hexafluorophosphate salt, none of which were acceptable for pharmaceutical development due to toxicity reasons.[9] After prolonged experimentation a crystalline solid precipitated out of a titration experiment of Compound I in THF against one equivalent HCl in diethyl ether. Analysis of the precipitate revealed the existence of two molecules of HCl per molecule of Compound 1 (even though only one equivalent of acid was added). As evident from the structure in Figure 1, compound 1 has only one major basic site (N3, the pyrrolidine group) having a pK_a of 7.8 (determined by potentiometric titration). Even though a search in the Cambridge Structural Database (CSD) revealed four examples of a chloride salt of an organic molecule which is a HCl complex,[10] the observed bis-HCl salt appears to be the first reported example of an API in the form of a stable molecular complex of the HCl salt and a molecule of HCl. It should be noted here that while there is an ongoing debate regarding the nomenclature of cocrystals and related multicomponent systems, the observed bis-HCl salt of Compound I should generally be better classified as a molecular complex of the mono-HCl salt with a molecule of HCl, thus being a subset of multicomponent crystals which include solvates, hydrates, salts, clathrates, and other inclusion crystals.[11–13] The paucity of pharmaceutically acceptable and stable complexes with HCl is likely due to the fact that HCl is a gas at ambient conditions and can easily evaporate compromising physical (and chemical) stability of a drug.

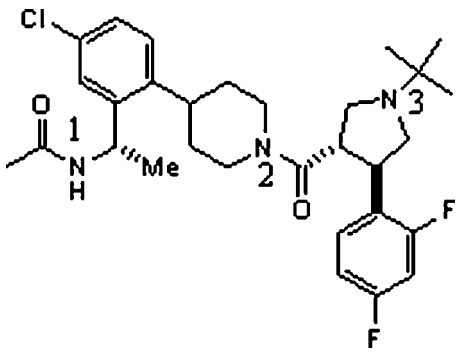

Figure 1. Structure of Compound 1.

Discovery of the crystalline bis-HCl salt was quite a relief for the development teams as the only other options for development were amorphous HCl salt and the amorphous free base both of which were plagued by significant chemical stability concerns. On a large scale, the bis-HCl salt can be reproducibly crystallized from ethanol with >98.5% purity. Detailed synthetic procedures for the synthesis of Compound 1 as well as its various salts can be found elsewhere.[8] Elemental analysis, $AgNO_3$ titration, and TG-MS unambiguously indicated the presence of two molecules of HCl per one molecule of Compound I. Surprisingly, based on the TGA data the second HCl molecule remains in the lattice until heated above 140°C under nitrogen flow at which point the material turns into amorphous mono-HCl salt. The bis-HCl salt has four known phases, an isopropyl acetate solvate, a hemihydrate, and two anhydrous polymorphs known as Form I and Form II which have a monotropic relationship where Form I is the thermodynamically most stable form.[14] The hemihydrate form can be obtained from Form II at greater than 50% relative humidity (RH). The bis-HCl salt is highly

soluble in water (>500 mg/mL) and slightly hygroscopic, gaining 0.2% of water at 60% relative humidity (RH) and deliquescing above 80% RH. It should be noted that the hygroscopicity of the bis-HCl salt is highly dependent upon the degree of crystallinity. If the bis-HCl salt is subjected to compression or high relative humidity (>70% RH), amorphous material may be generated, which is quite hygroscopic. The bis-HCl salt was found to be chemically and physically stable by itself and in mixtures with common excipients such as starch, HPMS, microcrystalline cellulose and lactose when stored for 4 weeks at 40°C and low relative humidity (20% RH). Exposure of the bis-HCl to humidities >70% resulted in a gradual loss of crystallinity accompanying a gradual loss of HCl, culminating in the formation of an amorphous solid. This gradual loss of crystallinity at high humidity made the bis-HCl salt less attractive (but not impossible) for further pharmaceutical development, as care should be taken in controlling humidity during processing and on storage. Therefore, the development team did not abandon hopes for finding a conventional crystalline mono-HCl salt, which still remained amorphous at the time.

The fact that there is only one major basic site suggests that the second HCl is somehow associated with the crystal lattice through non-covalent interactions. The crystals of the bis-HCl salt were too small for single-crystal structure solution. Hence a Monte Carlo simulated annealing method was utilized to determine the structure from the powder pattern (Fig. 2).

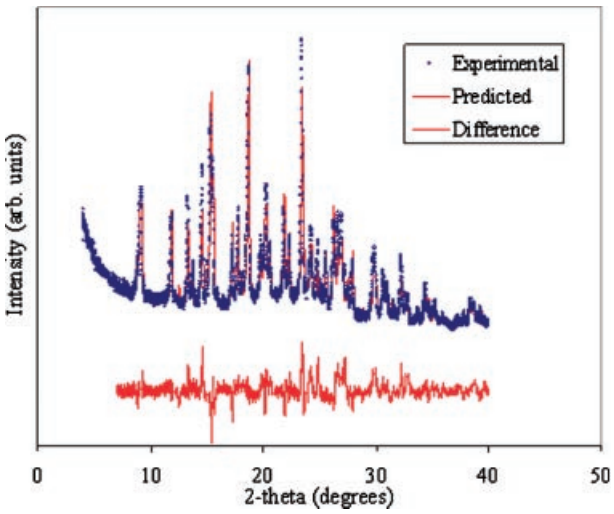

Figure 2. Comparison of experimental and predicted powder patterns of the bis-HCl salt.

Limitations imposed by the low resolution of the pattern are reflected in the Rwp of 0.11 for the final solved structure. The agreement of the predicted diffraction pattern with experimental data is heartening enough to draw some general conclusions about the structure of the bis-HCl salt (Fig. 3). Close examination of the structure shows one chloride in close proximity to N3. The interatomic distance of 2.425 Å is consistent with proton transfer at this site. On the other hand, the second HCl appears to occupy the space between the difluorophenyl moiety and the pyridinyl ring. Close examination of the environment around this HCl molecule reveals the possibility of weak hydrogen bonding between the proton on HCl and the nitrogen atom of the secondary amide group (N1 in Fig. 1). Assuming a H–Cl bond length of 1.36 Å, the interatomic distance between this proton and the amide nitrogen (N73 in the image) is 2.515 Å, which is within the limit of a weak hydrogen bonding distance. The same distance analysis performed on Cl–HN71 and Cl–HN72 (indicated hydrogen bonding) reveals interatomic distances of 2.878 Å for Cl–HN71 and 3.36 Å for Cl–HN72. These are higher than that for Cl–HN73, indicating a higher probability of hydrogen bonding between the amide nitrogen, N73 (N1 in Fig. 1), and HCl. These data are consistent with the ^{15}N solid state NMR data (see discussion below), which indicated that N72 (N2 in Fig. 1) does not possess a close approaching proton. This molecular HCl is released from the lattice upon heating above 140°C as shown by TG-MS data (see supplementary information). Additionally variable temperature ATR-FTIR was performed on samples of the bis-HCl salt. Changes as a function of temperature were noted above 140°C (see supplementary information) particularly in the regions 1449–1461 cm^{-1} and 1529–1537 cm^{-1}. These are associated with N–H deformation and bending modes and N–H stretching vibrational modes of amides, respectively. These changes are hypothesized to arise due to the evolution of HCl adjacent to the amide moiety, from the lattice upon heating.

^{15}N Solid State NMR variable contact time data are in agreement with the simulations (Fig. 4). The spectrum for the bis-HCl salt displays significantly increased relative intensity for N1 and N3. This suggests stronger proton coupling for N1 and N3 compared to N2. Nitrogen sites with strong proton coupling will yield an extremely broad NMR peak, virtually undetectable compared to baseline in a "low" decoupler strength

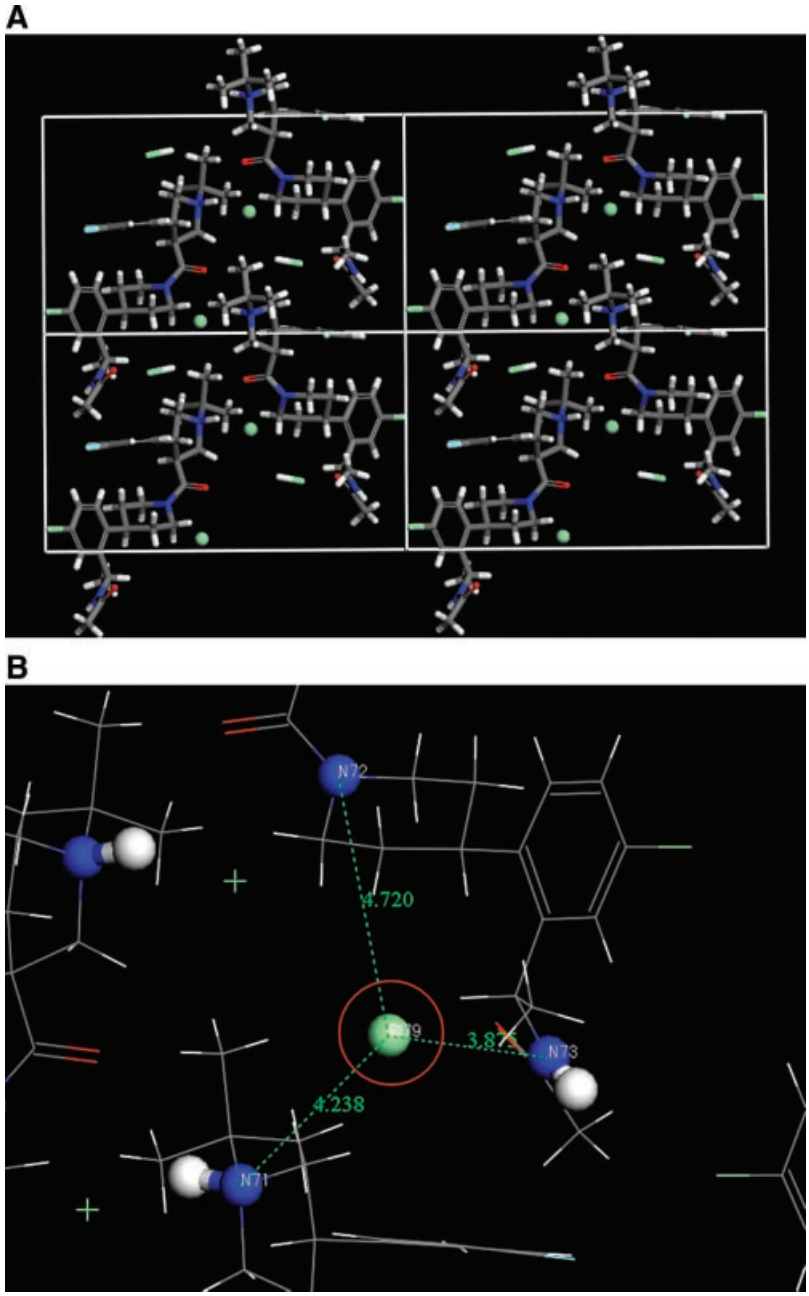

Figure 3. (A, top) Predicted crystal structure of the bis-HCl salt from powder pattern. (B, bottom) Environment around the 2nd chloride species (encircled by a red circle). The proton is removed for clarity.

experiment. The spectrum from this experiment performed on the bis-HCl salt clearly indicates that N2 does not possess a strongly coupled (close approaching) proton. The combined nitrogen solid state NMR data indicate the presence of close approaching protons to N1 and N3 and not to N2 (see Fig. 1 for numbering scheme).

A year after the bis-HCl salt was discovered (being almost 2 years after the search for an acceptable pharmaceutical form began) the elusive conventional mono-HCl salt crystallized serendipitously from a concentrated aqueous solution of the bis-HCl salt. The crystalline mono-HCl salt showed approximately 10 mg/mL

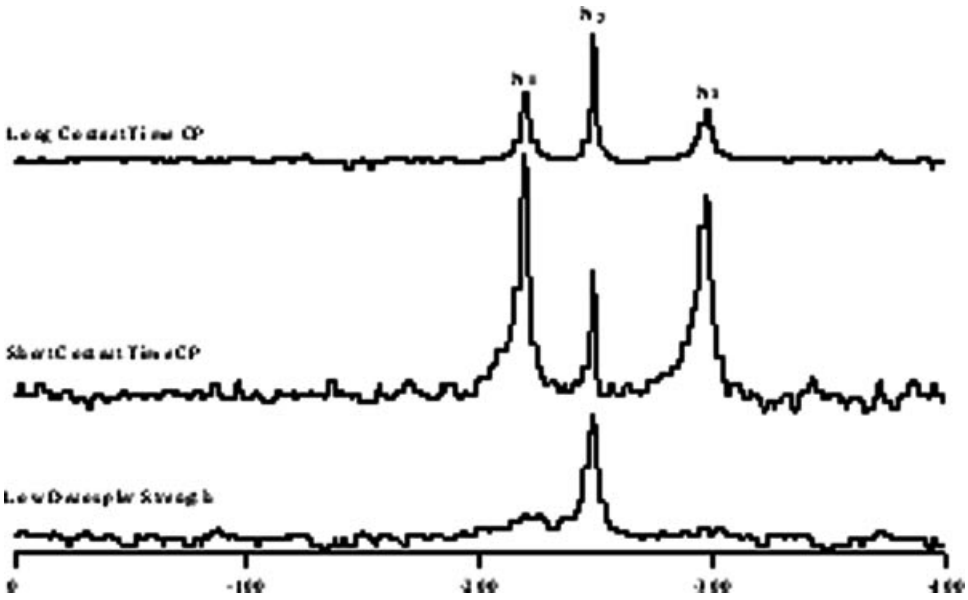

Figure 4. ^{15}N Solid state NMR data for the bis-HCl salt of Compound I, indicating that N1 and N3 have close approaching protons while N2 does not. N1, primary amide nitrogen; N2, central amide nitrogen; N3, pyrrolidine nitrogen.

solubility in water (vs. >500 mg/mL for the bis-HCl salt). Such decrease in solubility did not affect *in vivo* performance. Discovery of the crystalline mono-HCl salt was quite a relief as there was always a danger of disproportionation of the bis-HCl salt to the mono-HCl salt in solution in the worse possible moment. Moreover, the bis-HCl salt was not an ideal development candidate due to the gradual loss of HCl at high humidity leading to chemical/physical instability and corrosivity. The search for the crystalline mono-HCl salt lasted for more than 2 years involving numerous high-throughput screens as well as multiple bench crystallization experiments.

In conclusion, a stable HCl complex of the mono-HCl salt (bis-HCl) of Compound I has been discovered and characterized. The observed bis-HCl salt appears to be the first example of an active pharmaceutical ingredient in the form of a stable HCl complex. This discovery demonstrates the necessity to be open-minded and flexible during the salt selection process, which used to be dominated by the requirement of having a difference of at least two pK_a units in order to form a stable salt. The subsequent discovery of the mono-HCl salt, which has been elusive for more than 2 years, not only concludes the long quest for a conventional crystalline form of Compound I but also highlights the difficult, lengthy and often serendipitous path of finding the most appropriate form of an API for pharmaceutical development.

ACKNOWLEDGMENTS

The authors would like to thank the following colleagues for their contribution to this work: Ralph Calabria, Alex Chen, Yu Cheng, Jennifer Chilenski, Danielle Euler, Mike Hobbs, Ashkan Kamali, Arlene McKeown, Drazen Ostovic, Ivan Santos, Cecile Savarin, Feroze Ujjainwalla.

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