Crystal Structures of Tiotropium Bromide and Its Monohydrate in View of Combined Solid-state Nuclear Magnetic Resonance and Gauge-Including Projector-Augmented Wave Studies

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ABSTRACT: Tiotropium bromide is an anticholinergic bronchodilator used in the management of chronic obstructive pulmonary disease. The crystal structures of this compound and its monohydrate have been previously solved and published. However, in this paper, we showed that those structures contain some major errors. Our methodology based on combination of the solid-state nuclear magnetic resonance (NMR) spectroscopy and quantum mechanical gauge-including projector-augmented wave (GIPAW) calculations of NMR shielding constants enabled us to correct those errors and obtain reliable structures of the studied compounds. It has been proved that such approach can be used not only to perform the structural analysis of a drug substance and to identify its polymorphs, but also to verify and optimize already existing crystal structures. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2285–2292, 2015

Keywords: solid state NMR; *ab initio* calculations; crystal structure; polymorphism; tiotropium bromide; GIPAW calculations; solid state characterization

INTRODUCTION

There is an increasing interest in the pulmonary route of administration for both local and systemically acting drugs or vaccines.[1] Inhaled aerosol therapy is capable of transferring the drug directly to the target organ, hence systemic drug levels can be reduced, whereas systemic exposure and adverse drug effects lowered.[2]

A very important decision in the development of inhaled medications is to select the best solid form of an active pharmaceutical ingredient (API) for particular pharmaceutical formulation. Each API polymorph has its specific physicochemical properties. Therefore, it is not easy to find the most appropriate solid form of API, taking into consideration possible polymorphism of pure API and of its solid derivatives (salts, solvates, and cocrystals). Various API polymorphs can crystallize in the different ways, forming crystals of different shapes and sizes. In some cases, aerosolization of a given polymorph can be very difficult because of its strong cohesive and adhesive properties.[3] Besides, it may happen that over specific time metastable polymorphs undergo transformations into other forms during drug formulation or storage, spontaneously or as a result of interaction with excipients. Such transformations can greatly affect drug bioavailability.[4]

It should also be emphasized that for each polymorphic form of API, a separate patent protection can be obtained. This may be of a great importance for the fast-growing market of generic drugs. Furthermore, good quality crystal structures have to be included in input files of some computational procedures used to determine solubility and other physicochemical properties of API.[5]

Therefore, accurate and reliable methods and procedures have to be developed to characterize API polymorphs and determine precisely their crystal structure. The characterization of API polymorphs can be accomplished by the powder X-ray diffraction (PXRD), or single-crystal X-ray diffraction, Fourier transform infrared spectroscopy, Raman spectroscopy, solid-state nuclear magnetic resonance (ssNMR), thermal analysis, and scanning electron microscopy; each of those analytical methods provides different information and has its own advantages and disadvantages. In many cases, ssNMR is the method of choice because of following important practical aspects.[6–8] It can be used to analyze a final drug form without any need of special sample preparation, then ssNMR spectra do not usually pose problems with interpretation and the method can also be used for quantitative analysis. Any changes in the API structure (phase transitions) caused by molecular interactions or chemical bonding between API and associated excipients can easily be identified using the ssNMR spectra.

Tiotropium bromide (TIO), (1α,2β,4β,5α,7β)-7-[(2-hydroxy-2,2-di-2-thienylacetyl)oxy]-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.$0^{2,4}$]nonane bromide (Fig. 1) is an anticholinergic bronchodilator used in the management of chronic obstructive pulmonary disease. Unlike ipratropium and atropine that nonselectively block all three muscarinic receptors, TIO is more selective for the M_1 and M_3 receptors, from which it dissociates much more slowly. As a consequence, TIO is more potent bronchodilator than ipratropium, and has a much longer duration of action. A single dose of inhaled TIO produces bronchodilation that is sustained for 24 h or more.[9] In a retrospective analysis of two studies performed in the United States,[10,11] it was found that treatment with

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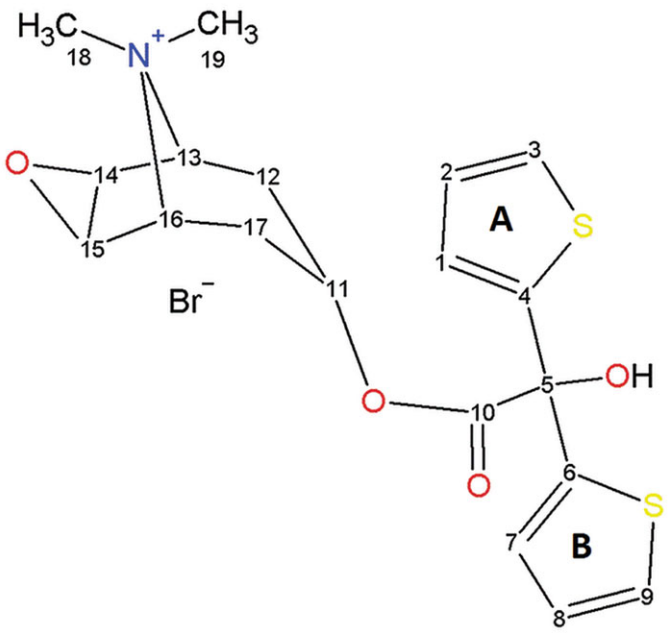

Figure 1. The structural formula and atom numbering of tiotropium bromide (TIO).

tiotropium reduced average total annual healthcare costs by approximately thousand US dollars per patient. This financial saving was entirely because of reduction in the hospitalization costs.[12] D'Souza et al.[13] in their review article stated that treatment with tiotropium is undoubtedly more cost-effective than with ipratropium.

Tiotropium was synthesized and crystallized in various polymorphic forms,[14,15] including hydrates and cocrystals, but only one of them, that is, tiotropium bromide monohydrate, is present in the commercially available drugs (Spiriva®, Tiova®). In this study, we wish to focus on the anhydrous tiotropium bromide (TIOA) and its monohydrate (TIOH), for which four and three crystallographic information files, respectively, have been deposited in Cambridge Structural Database (CSD).[16] However, only two of them contain complete structural data, the other five provide limited information without 3D atoms coordinations. Therefore, we can only make use of the two available structures with full crystallographic information: GUYGOX03 (structure of TIOA) and GUYGUD01 (structure of TIOH). It is worth mentioning that the other five structures have been assigned with the following comment from the CSD administration: "No reply to request for data." The information about all of the deposited structures can be found in Table 1.

The main aims of our study were the following: (1) to assess the quality of the existing CSD crystal structures of TIOA and TIOH, (2) to develop a practical and nondestructive analytical procedure to distinguish TIO polymorphs, (3) to determine correct structures of TIO that can be used in the further analysis.

In this work, experimental and theoretical methods were applied in tandem: high-resolution solid-state ^{13}C NMR spectroscopy and quantum mechanical calculations of NMR shielding constants. For NMR, cross-polarization (CP) under magic angle spinning (MAS) was employed.

Our principal intention was to verify the hypothesis that the quality of the published API crystal structure can be improved by using the density functional theory (DFT) method of geometry optimization for periodic systems,[19] together with two experimental methods of structure quality verification: ssNMR and PXRD.

MATERIALS AND METHODS

Sample Preparation

Tiotropium bromide was obtained using a three-step synthesis according to known methods described previously.[20–22] First, dimethyl glioxalate was reacted with 2-thienylmagnesium bromide. Then, the obtained methyl di(2-thienyl)glycolate was subjected to transesterification with scopine hydrobromide in the presence of potassium carbonate to give scopine di(2-thienyl)glycolate. The final anhydrous tiotropium bromide was obtained by quaternisation of scopine di(2-thienyl)glycolate with methyl bromide. Monohydrate tiotropium bromide was prepared using previously published procedures.[18]

NMR Spectroscopy

The high-resolution ^{13}C NMR spectra were collected at 298 K on a Bruker Avance 400 WB spectrometer using 100 MHz resonance frequency (B_0 = 9.4 T). The CP experiments[23] were performed with high-power proton decoupling using Bruker 7-mm MAS probe with zirconia rotors driven by dry air. The MAS rate was set at 7 kHz and the Hartmann-Hahn condition was matched using adamantane. We used a $\pi/2$ pulse of 4 μs, and a recycle delay of 50 and 30 s for TIOA and TIOH, respectively (both optimized). Chemical shifts were referenced to TMS using glycine as an external secondary standard (δ_{CO} = 176.5 ppm from TMS). The dipolar dephased experiments were carried out with dipolar filters to suppress the CP/MAS NMR signals from ^{13}C nuclei strongly coupled to protons (CH and CH_2 groups). A 50-μs delay before the FID acquisition results in the selective dephasing of magnetizations from methine and methylene groups was inserted. Conventional 1D and 2D NMR solution spectra in deuterated acetone were recorded on a Varian Unity Plus 300 MHz spectrometer (7.0 T, 298 K). The NMR spectra were processed with the ACD/SpecMenager NMR program.[24]

Gauge-Including Projector-Augmented Wave CASTEP Calculations

The quantum chemical calculations of geometry, energy, and NMR shielding constants were carried out with the CASTEP program[25,26] implemented in the Materials Studio 6.1 software.[27] Geometry optimizations and calculations of NMR chemical shielding were performed using the plane wave pseudopotential formalism and the Perdew–Burke–Ernzerhof exchange–correlation functional, defined within the generalized gradient approximation and the dispersion–interaction contributions were considered using the Tkatchenko–Scheffler method[28] for density functional theory dispersion correction. All the calculations were performed with ultrasoft pseudopotentials calculated on the fly; the quality of calculations was set to fine as implemented in the CASTEP standards. CASTEP default values for the geometry convergence criteria were used. The kinetic energy cutoff for the plane waves was set to 550 eV. Brillouin zone integration was performed using a discrete 1 × 1 × 1 Monkhorst-Pack k-point sampling for a primitive cell. The computation of shielding tensors was performed using the gauge-including projector-augmented wave (GIPAW) method of Pickard et al.[29] Additional computational data can be found

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