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Molecular and crystal structure of praziquantel. Spectroscopic properties and crystal polymorphism

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ABSTRACT

Schistosomiasis is a parasitic disease widely extended worldwide, mainly in the tropics and subtropics. Its pharmacological treatment is approached with praziquantel (PZQ), chemically named (*RS*)-2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazin-[2,1a]-isoquinolin-4-one. The PZQ commercial preparation is actually a racemic mixture, in which only the levo-enantiomer possesses anthelmintic activity. The knowledge of its spectroscopic and other chemical-physical properties is important to improve its applications. Therefore, the molecular and crystal structure of praziquantel have been investigated by means of calculations with classical mechanics and quantum mechanics methods based on Density Functional Theory (DFT), reproducing the experimental crystallographic and spectroscopic properties. Several crystal lattices of PZQ have been studied. Most of the intramolecular and intermolecular interactions of PZQ molecules in the crystal structure have been discussed. The vibration frequencies of the PZQ molecule were calculated with different molecular simulations and the assignments of some bands have been confirmed, such as, those of carbonyl groups.

1. Introduction

Praziquantel is the drug of choice for schistosomiasis. It is highly effective against all species of Schistosomas and against cestodes. It is usually administered in a single oral dose, which not only facilitates compliance but also has few side effects at short and long term. For its effectiveness, safety and cost-effectiveness features, the World Health Organization considers the praziquantel to be an essential drug (Passerini et al., 2006; WHO, 2015).

Schistosomiasis remains one of the most prevalent parasitic infections in the world caused by trematodes of the genus *Schistosoma*, which currently affects 250 million people worldwide out of a total of 783 million at risk (in 74 developing countries) (Trastullo et al., 2015) with about a half million deaths per year, mainly in the tropic and subtropical zones (Chitsulo et al., 2000; Rodrigues et al., 2010; Steinmann et al., 2006). There are two clinical forms of schistosomiasis, intestinal (caused by *Schistosoma mansoni* and *Schistosoma japonicum*) and urogenital (caused by *Schistosoma haematobium*). The adult worms of *S. mansoni* and *S. japonicum* reside in the mesenteric veins and *S. haematobium* in the host veins bladder plexus, place where fertilization occurs after male and female coupling, and subsequent eggs deposition. In particular, the *S. mansoni* extends almost all of Africa and South America; *S. haematobium* in Africa and the Middle East and *S. japonicum* in China, the Philippines and some islands of Southeast Asia. Schistosomiasis is acquired by contact with fresh water contaminated with larvae of the parasite, called cercariae, released by different species of molluscs (intermediate hosts), which can penetrate into the individual skin (Hackey and Stirewalt, 1956). Pharmacology treatment mainly deals with the use of praziquantel for both intestinal and urinary way. Schistosomiasis prevention is problematic, since there is no vaccine, although its research is currently underway. The only possibility is chemotherapy treatment, for which there are three drugs, oxamniquine, albendazole and praziquantel. PZQ is active against all species of the parasite (Cioli et al., 1995), it has a high efficiency, low toxicity and is easy oral administration. This coupled with its versatility and low cost production makes it the chosen, as the drug of choice (Doenhoff et al., 2000). It is also effective against other parasitic infections, such as, flukes and tapeworms that infect pets. Hence it is considered an anthelmintic of broad spectrum. Its main disadvantage is its ineffectiveness against juvenile forms of the parasite (Cioli and Pica-Mattoccia, 2003). It is considered that the result of using a single drug for treating a widespread disease, might lead to such a dangerous situation as drug resistance.

Praziquantel was discovered at the beginning of the 1970s, when Bayer A.G. and E. Merck found that the group pyrazinoisoquinoline were effective anthelmintics (Groll, 1984; Andrews et al., 1983; Davis, 1982). In the PZQ molecule, chemically named (*RS*)-2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-

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pyrazin[2,1*a*]-isoquinolin-4-one (EP, 2011; USP, 2013), the oxo group in position 4 is essential for drug activity and that any modification of the skeleton ring suppresses activity. Furthermore, PZQ has a chiral center at the position 11b. The commercial preparation is actually a racemic mixture composed of equal parts "levo" isomer R (−), and "dextro" S (+), in which only the levo-enantiomer possesses molluscicide activity (Pax et al., 1978). Therefore, the PZQ anthelmintic activity is associated mainly with *R*-(−)-enantiomer (Andrews, 1985).

The PZQ action mechanism is not exactly known, although most of the evidence points to an alteration of calcium homeostasis (Greenberg, 2005; Doenhoff et al., 2008). PZQ can act on various stages of development Schistosomas and can affect adult parasites, miracidia and cercariae, but has little or no effect on the eggs, sporocysts or schistosomula.

PZQ is hydrophobic and has low aqueous solubility. However, it is well absorbed through the gastrointestinal tract (Andrews, 1985). Oral doses are required to overcome high first pass liver metabolism and get appropriate target tissues concentrations (Leopold et al., 1978). With regard to their biopharmaceutical properties, PZQ is classified in Class II in Biopharmaceutics Classification System (BCS) (Lindenberg et al., 2004; González-Esquivel et al., 2005). This means that it has very low water solubility and high permeability. Furthermore, PZQ shows drug resistance. At present there are numerous reports of *Schistosoma* strains with decreased drug sensibility. Due to its problematic, the PZQ continues being an object of study. However, surprisingly only a few works (Ahamed et al., 2012; Rodrigues et al., 2010) have been found related with the study of the molecular structure and intermolecular interactions in the crystal lattice, and spectroscopic properties of this drug. On the other hand, the racemate (*RS*)-PZQ crystallizes in an anhydrous crystal form that is different to that of the enantiomer (R) or (*S*)-PZQ, which are hemihydrates. Then, the racemate is a different crystal polymorph to the pure enantiomer PZQ. Previous works have tried to find new polymorphs of PZQ but always they obtained the same crystal forms (Toro et al., 2014). Therefore, one of the aims of this work is to find a computational methodology, which describes properly the molecular structure, and crystal lattices of PZQ and to identify normal vibration modes of the IR spectrum of PZQ.

2. Computational methodology

Calculations based on the Density Functional Theory (DFT) were performed by means of the DMOL3 program (Delley, 2000) using the generalized gradient approximation (GGA), and the revised Perdew–Burke–Ernzerhof functional (RPBE) for the exchange–correlation potential (Hammer et al., 1999). Tridimensional periodical boundary conditions can be applied in this code, which uses molecular orbitals as a linear combination of atomic orbitals (LCAO), employing double zeta extended base functions including polarization functions (DNP) (Delley, 1990). Pseudopotentials with semi-core correction (DSPP) were used (Delley, 2002) in order to describe the interactions between electrons of the inner shells of atoms and valence electrons. The convergence threshold in the self-consistent cycle (SCF) of the energy calculation was 10^{-6} Ha. The calculation of frequency was based on the harmonic approximation using finite displacements of every atom. The harmonic vibrational frequencies were calculated by diagonalization of the Hessian matrix of the second derivative of the energy with respect to geometric changes generated by finite atomic displacements. This approach was previously used in other crystalline systems with good results (Escamilla-Roa and Sainz-Díaz, 2014).

On the other hand, since quantum mechanical methods described above may be difficult to explain the weak dispersion interactions in some cases, modeling using empirical interatomic potentials was also used for comparison. This approach is based on classical mechanics considering atoms as spheres with charges and interatomic potentials dependent of the distance between atoms. These potentials are characterized by a number of parameters that have been preset with empirical observables constituting force fields. Several force fields (FF) were used, such as Compass (Sun, 1998), Universal (Rappe and Goddard, 1991) and the recently optimized CVFFH, based on the consistent valence force field (CVFF) (Heinz et al., 2006), that has provided good results in previous studies (Martos-Villa et al., 2013). To carry out these FF calculations, the Discover and Forcite programs were used within Materials Studio package (Accelrys, 2012). Different conditions were tested for calculating Van der Waals and Coulomb interactions. Atom based interactions with a cut-off of 18.5 Å was used for Universal and Compass FFs. With the CVFFH FF, the Ewald method with a cut-off of 15.5 Å yielded better convergence in the calculations according to previous works (Sainz-Díaz et al., 2011). Therefore, these conditions have been defined to undertake this work. On the other hand, the vibration frequencies were calculated following the same harmonic approximation used in the DFT calculations by means of finite displacements of every atom, where the energies are calculated with the FF.

In preliminary calculations various methods of atomic charges assignments were explored within the COMPASS FF: the Compass charges included in its own FF, and those calculated by the QEQ Method (charge equilibration) (Rappe and Goddard, 1991; Rick et al., 1995). The use of atomic charges of the own Compass FF yielded the closest values of lattice parameters to the experimental data. On the other hand, for CVFFH two water models were explored: SPC one (charges values of 0.41 and −0.82 for H and O atoms, respectively), and TIP4P model (charges of 0.417 and −0.834 for H and O atoms, respectively) (Rick et al., 1995; Berendsen et al., 1987). Net atomic charges associated to the electrostatic potential (ESP charges) (Besler et al., 1990) were calculated at quantum mechanical level using DMOL3 and they were applied to the COMPASS and CVFFH calculations.

The work was carried out using a modeling and simulation of materials software, Materials Studio 6.0 (Accelrys Inc., 2012) as well as graphical interface to calculate, analyze and study the crystal and molecular structures.

3. Results and discussion

3.1. Praziquantel molecular structure

The crystal structure of (*RS*)-PZQ showed a certain disorder in the atomic positions, then the PZQ molecule was extracted from the experimental crystallographic data of the hemihydrate of (*R*)-PZQ crystal (Meyer et al., 2009) where no disorder was found in atomic positions. This molecule was fully optimized alone, in gas phase, with different theoretical methods (Fig. 1) comparing the calculated geometry with the only available experimental data that is in solid state (Table 1). Assuming that this calculated molecular structure is for an isolated molecule as in gas phase, the comparison with experimental values from a solid state can be considered valid for comparing the different calculation methods used in this work.

In general, the calculated C═O bond distances are close to those from experimental data. In the hemihydrate crystal structure, the C═O bond attached to the cyclohexyl group is much longer than those calculated. In this crystal structure, this carbonyl group interacts with a water molecule by hydrogen bonds enlarging the C═O bond. In the OC—N bond, in both cases, *d*(OC(Cy)—N) and *d*(OC—N), the calculated distances are larger than the experimental, being the closest those calculated with Compass. The same occurs with the bond H_2C—N of the CH_2 joined to the carbonyl and with the HC—N bond, the experimental values are close to those calculated with Compass. The OC—N and OC(Cy)—N bonds are shorter than the HC—N and H_2C(CO)—N bonds, due to the resonant delocalization of π electrons between N atom and carbonyl group.

Net atomic charges based on the Mulliken approximation and also atomic charges fitted to the electrostatic potential (ESP charges) were calculated (Besler et al., 1990) (see Tables S1 and S2 in Supplementary