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Research paper

A new soluble and bioactive polymorph of praziquantel

Debora Zanolla[a], Beatrice Perissutti[a,*], Nadia Passerini[b], Michele R. Chierotti[c], Dritan Hasa[d], Dario Voinovich[a], Lara Gigli[e], Nicola Demitri[e], Silvano Geremia[a], Jennifer Keiser[f], Paolo Cerreia Vioglio[g], Beatrice Albertini[b]

[a] Department of Chemical and Pharmaceutical Sciences, University of Trieste, P.le Europa 1/via L. Giorgieri 1, 34127 Trieste, Italy
[b] Department of Pharmacy and BioTechnology, University of Bologna, Via S. Donato 19/2, 40127 Bologna, Italy
[c] Department of Chemistry and NIS Centre, University of Torino, V. Giuria 7, 10125 Torino, Italy
[d] Leicester School of Pharmacy, De Montfort University, The Gateway, LE1 9BH Leicester, United Kingdom
[e] Elettra – Sincrotrone Trieste, S.S. 14 Km 163.5 in Area Science Park, 34149 Basovizza, Trieste, Italy
[f] Helminth Drug Development Unit, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Socinstr. 57, CH-4051 Basel, Switzerland
[g] Aix-Marseille Université, CNRS, ICR (UMR 7273), 13397 Marseille cedex 20, France

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ABSTRACT

Praziquantel is the only available drug to treat Schistosomiasis. However, its utilization is limited by many drawbacks, including the high therapeutic dose needed, resulting in large tablets and capsules difficult to be swallowed, especially from pediatric patients. In this study, an alternative option to overcome these disadvantages is proposed: to switch to a novel crystalline polymorph of racemic compound praziquantel. The preparation of the crystalline polymorph was realized via a neat grinding process in a vibrational mill. The new phase (Form B) was chemically identical to the starting material (as proved by HPLC, ^{1}H NMR, and polarimetry), but showed different physical properties (as evaluated by SEM, differential scanning calorimetry, thermogravimetry, ATR-FTIR spectroscopy, X-ray powder diffraction, and solid-state NMR). Furthermore, the crystal structure of the new phase was solved from the powder synchrotron X-ray diffraction pattern, resulting in a monoclinic *C2/c* cell and validated by DFT-D calculation. Moreover the simulated solid-state NMR ^{13}C chemical shifts were in excellent agreement with the experimental data. The conversion of original praziquantel into Form B showed to affect positively the water solubility and the intrinsic dissolution rate of praziquantel. Both the in vitro and in vivo activity against *Schistosoma mansoni* were maintained. Our findings suggest that the new phase, that proved to be physically stable for at least one year, is a promising product for designing a new praziquantel formulation.

1. Introduction

Praziquantel (PZQ) is an antihelmintic drug largely used for the treatment of Schistosomiasis. It is estimated that at least 230 million people worldwide are infected by the genus Schistosoma [1], mainly with *Schistosoma haematobium*, *S. japonicum* and *S. mansoni*. Praziquantel is included in the WHO Model List of Essential Drug for the treatment of adults and children [2]. The drug is well tolerated and safe, however it is classified as a BCS class II drug, hence characterized by high permeability, low solubility [3] and extensive first-pass metabolism [4]. The recommended dosage for the treatment of schistosomiasis is 20 mg/kg three times a day which has to be repeated after 4 to –6 weeks. For at-risk populations a 40 mg/kg single dose is used as preventive chemotherapy. Since children are the main target of treatment, research is needed to enhance the solubility and the bioavailability of PZQ, in order to reduce the high therapeutic doses and therefore the dimension of tablets, which are difficult to swallow particularly for pediatric patients [5]. Several studies aimed to enhance PZQ properties such as the preparation of solid dispersion of the drug with Povidone [6–8] , or with Sodium Starch Glycolate [9], Praziquantel-Beta-Cyclodextrins systems [10,11], solid lipid nanoparticles [12], coground systems [13], fast dispersible granules [14] and melt

Abbreviations: ASU, Asymmetric Unit; ATR-FTIR, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy; CPMAS, Cross Polarization Magic Angle Spinning; DFT-D, Dispersion-corrected Density Functional Theory; DSC, Differential Scanning Calorimetry; HPLC-UV, High Performance Liquid Chromatography Ultraviolet Detection; IC50, Half Maximal Inhibitory Concentration; IDR, Intrinsic Dissolution Rate; NMR, Nuclear Magnetic Resonance; PZQ, Starting Praziquantel; SD, Standard Deviation; RSD, Relative Standard Deviation; SEM, Scanning Electron Microscopy; SSNMR, Solid State Nuclear Magnetic Resonance; TGA, Thermogravimetric Analysis; XRPD, X-Ray Powder Diffraction

* Corresponding author.
E-mail address: bperissutti@units.it (B. Perissutti).

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granulation combined with ultrasonic spray congealing [15].

In this study, an alternative way to improve solubility and hence to possibly reduce the therapeutic dose is proposed, namely to switch to a crystalline polymorph of PZQ racemic compound.

Polymorphism, a property inherent to the solid state, is the ability of a compound to exist in more than one crystalline form that have different arrangements in the solid state. Polymorphs have different relative intermolecular and/or interatomic distances, or unit cells. Sometimes structural differences are significant, resulting in very different physical (e.g. mechanical, thermal, etc.) and chemical properties (e.g. chemical reactivity). In other cases differences are very subtle making it difficult to distinguish different crystal forms [16–18]. The search for these polymorphic varieties recently became a topic of major interest.

Several authors have already testified the existence of crystal modifications of PZQ. The commercial racemic compound crystallizes in an anhydrous crystal form. Its structure (TELCEU in CSD) belongs to the triclinic space group P-1 with four crystallographically independent molecules in the asymmetric unit with a *syn* conformation (concerning the two C = O groups present in the molecules), where two of them are disordered [19].

Differently from the racemic PZQ, the pure enantiomers (R) or (S)-PZQ crystallize in hemihydrates having different intermolecular interactions than those of the racemic crystal [20,21]. Racemic praziquantel is well known also as an excellent candidate to form co-crystals with several aliphatic dicarboxylic acids, such as oxalic, malonic, succinic, maleic, fumaric, glutaric, adipic, and pimelic acid [19].

Recently, the formation of a diastereoisomeric cocrystal pair with L-malic acid in presence of acetone and the subsequent isolation of R-enantiomer has been also documented [22].

As regards to anhydrous polymorphic varieties of racemic praziquantel, previous works have tried to obtain new polymorphs of racemic PZQ, by slow evaporation using several solvents, but always they obtained the same triclinic crystal form [23].

By exploiting the full potential of a multitechnique approach here we report a detailed investigation of a new polymorphic anhydrous crystalline form of PZQ (Form B) obtained by a neat grinding method in absence of solvent. The identification of the polymorph was carried out by differential scanning calorimetry, thermogravimetry, SEM analysis, solid-state NMR (SSNMR) combined with DFT-D calculations, ATR-FTIR. The crystalline structure was solved from the powder X-ray diffraction pattern and deposited in the Cambridge Crystallographic Data Center with deposition number: 1,557,658. The new phase have been also fully characterized for its biopharmaceutical properties (determining saturation solubility and intrinsic dissolution rate), and performing in vitro and in vivo antihelmintic assays.

2. Materials and methods

2.1. Materials

Praziquantel (PZQ) Ph. Eur. grade ((11bRS)-2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4-H-pyrazino[2,1-a]isoquinolin-4-one) was kindly donated by Fatro S.p.A. (Bologna, Italy). PZQ impurity A (2-Benzoyl-1,2,3,6,7,11b-hexahydro-4-H-pyrazino[2,1-a]isoquinolin-4-one) and impurity B (2-Cyclohexanecarbonyl-2,3,6,7-tetrahydro-pyrazino[2,1-a]isoquinolin-4-one) were Ph. Eur. grade and purchased from Endotherm Gmbh (Saarbruecken, Germany). HiPersolv Chromanorm Methanol (Ph. Eur. for HPLC Gradient Grade) and Ethanol (Ph. Eur.) were purchased from VWR Chemicals BHD PROLABO®.

2.1.1. Preparation of Form B

Praziquantel was milled on its own, by neat grinding, in a vibrational mill-Retsch MM400 (Retsch GmbH) which was equipped by 2 screw-type zirconium oxide jars, each with a capacity of 35 ml. A ceramic material like zirconium oxide was selected due to its high density (5.9 g/cm^3), allowing for a high energy input. The amount of powder to be introduced in the milling jar was determined in 0.800 g per jar, and three zirconium oxide spheres of 15 mm (weighing 10.72 g) were used as milling media. The vibrational frequency of 20 Hz was applied for a time of 4 h without interruption.

After the treatment, the solid product were stored at 25 °C in desiccators over anhydrous calcium chloride.

2.2. Methods

In all evaluations the ground sample was analyzed in comparison to starting PZQ racemic compound.

2.2.1. Determination of drug content

Reverse phase HPLC-UV method was used for the quantification of PZQ, using a method adapted from literature [24] and validated according to a slight modification in column length. The HPLC system used consisted of two mobile phase delivery pumps (LC-10 ADVP, Shimadzu, Japan), a UV–Vis detector (SPD-10Avp, Shimadzu, Japan), an autosampler (SIL-20A, Shimadzu, Japan), an interface (SCL-10Avp, Simadzu, Japan) for the acquisition of data through a software Ez-Star and a column Kinetex 5 μm C18 100 Å (150 × 4.60 mm, Phenomenex, Bologna, Italy). The mobile phase comprised of methanol and water (65:35 V/V), the flow rate was 1 ml/min and absorbance readings were conducted at fixed wavelength of 220 nm. The retention time of PZQ was about 5.5 min and the run time was set at 12 min. Quantification was carried out by integration of the peak areas using the external standardization method. Under these conditions, the linear calibration curve of PZQ was obtained in the range of 0.3–10 mg/l (r^2 = 0.99996). As reference, a fresh stock solution was prepared each time before starting the analysis. The standard solution of Praziquantel was prepared by dissolving 10 mg of PZQ in 20 ml of methanol HPLC-grade. The solution was stirred for several minutes and then diluted with the mobile phase in ratios 1:10 and 1:20 to obtain a drug concentration of 2.5 mg/l (ppm). According to the PZQ monograph in the Eur. Ph. (Ed. 8.0), specified impurities, named impurity A and impurity B, have to be detected. The linear calibration curve of each impurity was obtained in the range of 0.05–1 mg/l (r^2 = 0.9993 and 0.9994, for impurity A and B, respectively). The retention times of the impurities were at 3.45 min and 11.2 min. Both impurities were absent in the reference solution. The same procedure was used to characterize starting PZQ solid form and the milled sample Form B.

The determination of the PZQ content into the milled samples was determined by dissolving about 10 mg, exactly weighed, of sample in 20 ml of methanol HPLC-grade. The obtained solution was then diluted 1:200 with the mobile phase, corresponding to about 2.5 mg/l of PZQ, in order to ensure the linearity of the analytical response. In the sample solution, the retention time of PZQ was at about 5.4–5.5 min. Each sample solution was analyzed in triplicate and the mean of the sum of the peak responses of Praziquantel was then calculated. The results were expressed as the percentage of PZQ recovery with respect to the sum of all peaks present in the chromatogram (PZQ and eventual related impurities and/or other detectable related products).

2.2.2. Polarimetric analyses

Optical rotations of the samples were measured on a Polarimeter Jasco P-2000 (Lecco, Italy), with a λ = 589 nm and a concentration of 1 g/100 ml in ethanol, according to the method reported in literature [25,26]. Ethanol was preferred in place of $CHCl_3$, in order to prevent formation of air bubbles in the cell.

2.2.3. X-Ray powder diffraction (XRPD)

Starting PZQ material was tested on a conventional diffractometer equipped with an RTMS X'celerator detector, using a Ni-filtered Cu Kα radiation (wavelength 1.5418 Å). A small amount of powder (15–25 mg) was gently pressed on a glass slide to give a flat surface. The