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Design and synthesis of molecular probes for the determination of the target of the anthelmintic drug praziquantel

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ABSTRACT

Schistosomiasis is a highly prevalent neglected tropical disease caused by blood-dwelling helminths of the genus *Schistosoma*. Praziquantel (PZQ) is the only drug available widely for the treatment of this disease and is administered in racemic form, even though only the (*R*)-isomer has significant anthelmintic activity. Progress towards the development of a second generation of anthelmintics is hampered by a lack of understanding of the mechanism of action of PZQ. In this Letter, we report an efficient protocol for the small-scale separation of enantiomers of **2** (hydrolyzed PZQ) using supercritical fluid chromatography (SFC). The enantiopure **2** was then used to develop several molecular probes, which can potentially be used to help identify the protein target of PZQ and study its mode of action.

Schistosomiasis is an intravascular parasitic infection caused by trematode worms of the genus *Schistosoma*. The disease affects approximately 249 million people including 114 million school-age children, 90% of whom live on the African continent where *Schistosoma mansoni* and *Schistosoma haematobium* are the predominant causative agents.[1] Praziquantel (PZQ, **1**) is the least expensive, easiest to use and most readily available of all current anti-schistosomal drugs.[2,3] It is highly effective against all schistosome species that are known to infect humans and is well-tolerated, making it suitable for mass treatment campaigns. Although PZQ efficacy is high, reported cure rates range from 60% to 95%.[4,5] There are at least two explanations for this phenomenon. First, PZQ does not kill juvenile schistosomes readily between 2 and 5 weeks after infection of the definitive host.[6–9] Without rigorous follow-up treatment, this can leave a reservoir of surviving worms to continue the cycle of infection. A second potential problem is the presence of drug resistance traits in laboratory and natural populations of worms.[10–14]

Currently, PZQ (**1**) is synthesized and employed as a racemic mixture; however, the anthelmintic activity of PZQ is associated mainly with the (*R*)-stereo isomer.[15–19] The inactive (*S*)-isomer is responsible for its side effects,[18] extremely bitter taste[20] and large pill size.[21] The classical resolution of the PZQ intermediate **2** has recently been reported and has the potential to lend itself to the preparation of more optically pure versions of the drug on a commercial scale.[22] Although the molecular target of PZQ remains unknown, a number of lines of evidence suggests that it disrupts cellular Ca^{2+} homeostasis by affecting voltage gated Ca^{2+} channel function.[23–26] The lack of an efficient general small-scale method for developing molecular probes of exceptional high optical purity limits our ability to readily prepare specific molecular probes based on the active and inactive soluble configuration of the PZQ pharmacophore. Molecular probes of this type may lead to an improved understanding of the mode of action of PZQ on schistosomes. Here, we report an efficient method to obtain highly purified samples of each of the two enantiomers of hydrolyzed PZQ (**2**, see Scheme 1) on gram scales with supercritical fluid chromatography (SFC). Enantiopure (**R**)-**2** and (**S**)-**2** were used to synthesize (*R*)-PZQ, (*S*)-PZQ and other PZQ-based molecular probes. These molecular tools can potentially be used to identify the molecular target and site of localization of this drug.

Our synthesis of new molecular probes starts with racemic **2**, which is generated from (*rac*)-PZQ (**1**) using the precedented hydrolysis of **1** on a 20 g scale under acidic conditions (Scheme 1).[22] The reaction provided **2** in 77% isolated yield. Recently, SFC has gained acceptance as a valuable chromatographic technique because of improved resolution and a shorter analysis time compared to high performance liquid chromatography (HPLC).[27]

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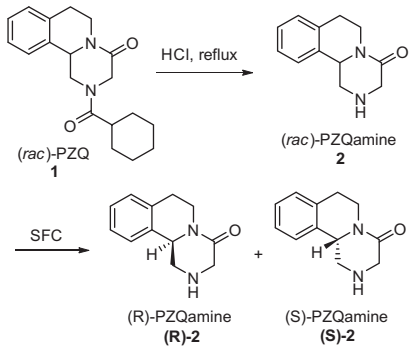

Scheme 1. Synthesis of racemic **2** and separation of the two enantiomers (**R**)-**2** and (**S**)-**2** using supercritical fluid chromatography (SFC).

Additionally, SFC generally uses supercritical CO_2 in combination with a polar organic solvent (e.g., alcohols) as the mobile phase; making it a greener and cost-effective alternative to HPLC and reverse phase liquid chromatography (RPLC). Given the advantages of SFC, we investigated its ability to separate the enantiomers of **2**. The preliminary conditions for separation were determined using the Berger SFC analytical system, ProNTo. Interestingly, a recent study on HPLC analysis of (*rac*)-**2** showed the inefficiency of both Chiralcel AD-H and OD-H columns to separate the enantiomers of **2**.[22] Our initial study using SFC indicated that the Chiralcel AD-H column worked more efficiently compared to the Chiralcel OD-H column in separating the enantiomers of **2**. Figure 1A shows the chromatogram for the separation of (*rac*)-**2** on the chiral AD-H column using 35% methanol as the mobile phase. The retention time was 3.7 ± 0.1 min for (**R**)-**2** and 4.3 ± 0.1 min for (**S**)-**2**.

Based on the above data, the following conditions were selected for subsequent work using Berger SFC preparative-scale system: Chiralcel AD-H column, methanol/supercritical CO_2 solvent system (35:65), amount per injection 7 mg, total flow 55, cycle time 3 min, injection value duration 45 s and total elution time 7 min. The SFC preparative-scale system worked efficiently to separate the enantiomers of **2** (on a gram scale) as shown by the SFC analysis of (**R**)-**2** and (**S**)-**2** in Figure 1B and C, respectively. The identity of (**R**)-**2** and (**S**)-**2** was determined by comparing their optical rotation values with the literature.[22] In addition to optical rotation, the absolute stereochemistry of (**R**)-**2** was further confirmed by X-ray analysis (CCDC 994262) as shown in Figure 2.

Once the protocol for obtaining the enantiopure (**R**)-**2** and (**S**)-**2** was established, they were then used for the synthesis of several molecular probes (Fig. 3). First, (**R**)-**2** and (**S**)-**2** were coupled with cyclohexanoyl chloride under basic conditions that provided enantiopure (*R*)-PZQ and (*S*)-PZQ, respectively.[22] Using a procedure we previously developed for the racemic **2**,[7] (**R**)-**2** and (**S**)-**2** were used to obtain the optically pure fluorescently labeled probes: (*R*)- and (*S*)-PZQ-Bodipy (compounds (**R**)-**3** and (**S**)-**3**). Additionally, we designed and synthesized a 'short-arm' PZQ molecular probe (compound **4**) containing the diazirine functionality for photoaffinity labeling studies, as well as an alkyne moiety making **4** suitable for click chemistry. Using a similar approach, we also prepared another (*R*)-PZQ-click probe having a longer photoreactive arm (compound **5**). Details of the experimental procedures for the synthesis of compounds shown in Figure 3 are in the supplementary information section. While a different method of preparation of PZQ enantiomers has been reported previously,[22] to the best of our knowledge the synthesis of molecular probes (**R**)-**3**, (**S**)-**3**, **4** and **5** in an enantiomerically pure form has not.

Next, we evaluated the activity of the synthesized compounds against sexually mature male *S. mansoni* PR-1 worms in vitro. Worms were incubated overnight in the presence of different concentrations of each compound after which they were washed and maintained for 7 days in drug-free media. The EC_{50} for each PZQ molecule at the end of this 7 day period is shown in Table 1. The EC_{50} of commercially available racemic PZQ (2.5 μM) was similar to that reported by Pica-Mattoccia and Cioli (1.6 μM).[9] The EC_{50} of (**R**)-**1** was significantly less than that of the racemic PZQ (**1**)

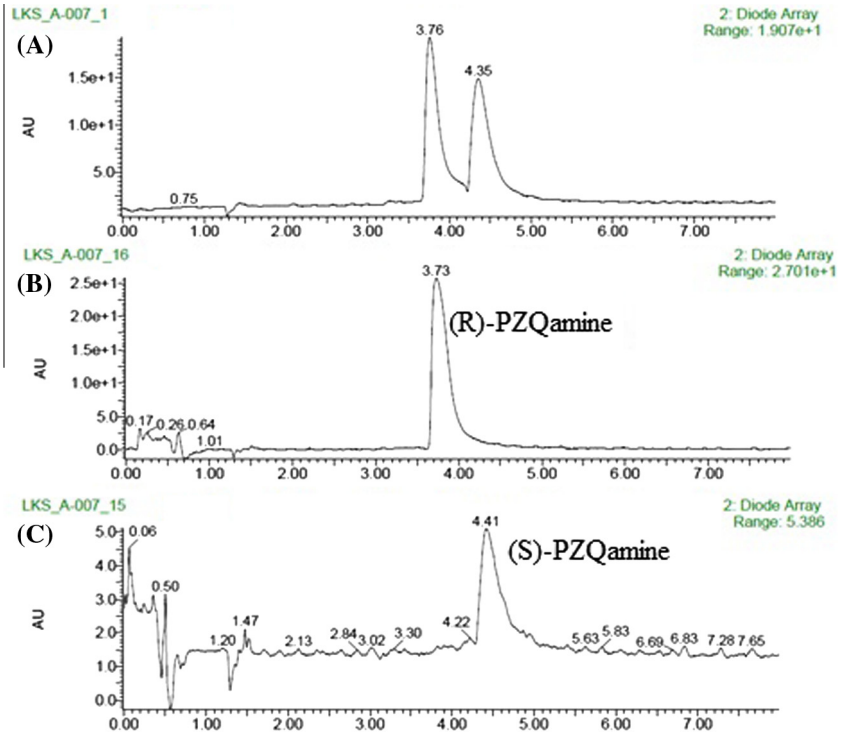

Figure 1. SFC analysis of (A) (*rac*)-**2**; (B) (**R**)-**2** and (C) (**S**)-**2**. Chiralcel AD-H column; solvent system: methanol/supercritical CO_2 (35:65).