Supplemental Data for

Metabolic profiling of *S*-praziquantel: Structure elucidation using the crystalline sponge method in combination with mass spectrometry and nuclear magnetic resonance

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Preparation of crystalline sponges

The porous crystalline sponges $[(ZnI_2)_3 \cdot (tpt)_2]_n \cdot x(cyclohexane)_m$ (**1a**) and $[(ZnI_2)_3 \cdot (tpt)_2]_n \cdot x(n\text{-}hexane)_m$ (**1b**), were prepared following the reported procedures (Biradha and Fujita, 2002; Ramadhar et al., 2015).

Single-crystal X-ray diffraction experiments

Single crystal X-ray diffraction measurements were conducted on a Rigaku Oxford Diffraction XtaLAB Synergy-R diffractometer using Cu-K_α X-ray radiation (λ = 1.54184 Å), equipped with a HyPix-Arc 150° Hybrid Photon Counting (HPC) detector (Rigaku, Tokyo, Japan) at a temperature of 100 K using a Cryostream 800 nitrogen stream (Oxford Cryostreams, UK). The software CrysAlisPro ver. 171.41.68a (*S*-/*R*-M6 praziquantel), ver. 171.41.99 (M1 praziquantel, *R*-M6 praziquantel) and ver. 171.41.113a (*S*-praziquantel, M2 praziquantel, M3 praziquantel and M4 praziquantel) was used for calculation of measurement strategy, data reduction (data integration, empirical and numerical absorption corrections and scaling) and the generation of the *h*0*l* layers. The software ShelXle was used to generate the electron density maps F_o to verify the position of metabolism (Hübschle et al., 2011). Isocontour levels are described as σ (square root of the average variance of the density).

Crystal structure analysis

All crystal structures were modeled using OLEX2 (Dolomanov et al., 2009), solved with SHELXT ver. 2014/5 and refined using SHELXL ver. 2018/1 (Sheldrick, 2015). Figures of framework and analyte were created using OLEX2. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed using the riding model. Populations of the guests in the crystal were modelled by least-square refinement of a guest/solvent disorder model under the constraint that the sum of them should equal to 100%. The number of used

constraints and restraints was tried to minimize and applied without changing the standard deviation. Solvent cyclohexane molecules in the pores were found in the difference electron density map and refined using the restraints (DFIX, DANG, SIMU, RIGU and SADI). These molecules are expected to be severely disordered due their high thermal motion. Due to their averaged structure of various geometry and orientation, some cyclohexane molecules are distorted to energetically-unfavorable (boat-shaped or twisted) structures. One “Alert A” notification was found by the validation program CheckCIF. This alert is derived from undefined solvent molecules is unavoidable due to severe disorder. The comments for the alerts are described in the CIFs using the validation response form (vrf).

Solvent masking algorithm was applied during structure refinement. The Flack parameters before and after solvent masking including their estimated standard deviations are described (Classic Flack method).

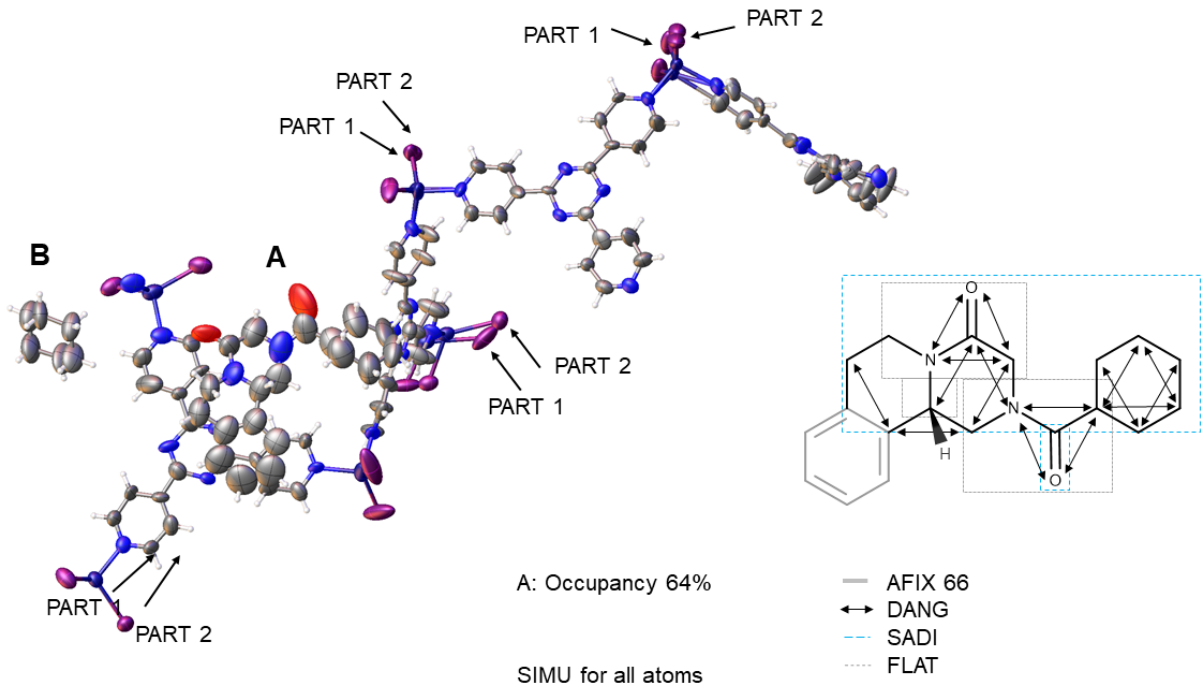
The present crystal structures are not used for exact structure analysis. They are used to confirm structure proposals derived from information obtained from mass spectrometry and knowledge of the parent structure. Therefore, details of the crystals structure (bond lengths, angles, etc.) will not be discussed.

Crystallographic data for 1a•*S*-praziquantel (after solvent masking)

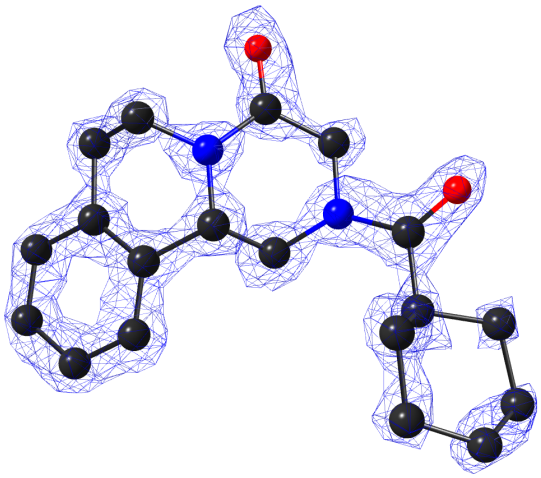
Crystal size: 342 × 180 × 97 μm^3, refined formula: $C_{87.57}H_{69.78}I_{12}N_{25.28}O_{1.28}Zn_6$, formula weight ($M_r$) = 3411.76 g/mol, light yellow needle, crystal system: monoclinic, space group *C*2, Z = 4, 27315 unique reflections merged from recorded 145718 ones (2.579° < θ < 67.078°) were used for structural analysis (R_{int} = 0.0308). Lattice parameters, R-factor on $F^2 > 2\sigma(F^2)$, weighted R-factor, and goodness-of-fit are as follows: a = 35.2195(4) Å, b = 14.7904(2) Å, c = 32.5945(4) Å, β = 103.339(1)°, V = 16520.8(4) Å^3, R = 0.0823, wR_2 = 0.2695, S = 1.123. Calculated density is 1.372 gcm^{-3}. Linear absorption coefficient (μ) is 18.857 mm^{-1}. Residual electron density (max/min) is 1.95/-1.24 eÅ^{-3}. The Flack parameters for *S*-praziquantel before and after using solvent masking were χ = 0.146(9) and χ = 0.234(8), respectively. CCDC number 2104973. The ORTEP diagram of the asymmetric unit of the framework and *S*-praziquantel is shown in Supplemental Figure 1.

The framework is refined using the constraint EADP for some disordered ZnI_2 moieties, as well as the commands SADI and DANG for some tpt rings. Three ZnI_2 moieties are disordered and refined using disorder model. One guest molecule (A) with an occupancy of 64% and one solvent molecule (B) were found in the asymmetric unit. The benzene ring was fixed using AFIX 66. The C=O double bonds, C-C and C-N single bonds were restrained using SADI command. DANG was used for some angles of the guest. In addition, FLAT was applied for the two cyclic amide functional groups. SIMU was applied for all analyte molecules. Applied restraints can be taken from Supplemental Fig. 1. Additional electron density was found that could not be assigned due to disorder. Contribution of this electron density was removed using solvent masking algorithm implemented in OLEX2. A solvent mask was calculated and 428 electrons were found in a volume of 1622 A^3 in one void per asymmetric unit. This is consistent with the presence of 9 cyclohexane molecules per asymmetric unit. Supplemental Fig. 2 shows the electron density map of the *S*-praziquantel molecule taken from the crystal structure. The parent compound interacts with the CS framework by C-H···O interactions (distance 2.49 Å) between the aromatic ring of the tpt ligand and the carbonyl group of the analyte.

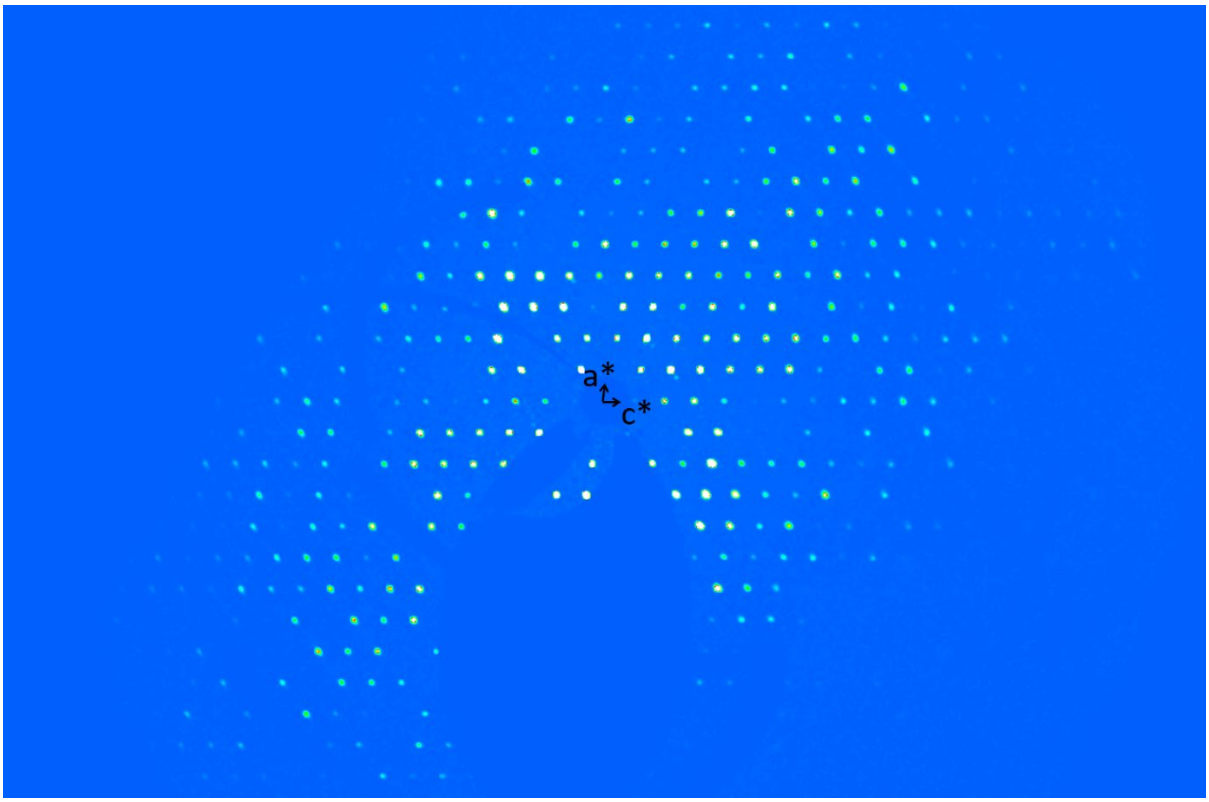
S-praziquantel was used as enantiomerically pure substance. Several examples have shown that the crystalline sponge method can be used to determine the absolute structure of soaked analytes because soaking of chiral molecules lead to lowering of the symmetry from *C*2/*c* to *C*2 or $P2_1$ (Hoshino et al., 2016; Zigon et al., 2015). To assure the correct space group assignment during data processing, extinction rules for C centering (*hkl*, *h*+*k*=odd) and the existence of the c glide plane (*h*0*l*, *l*=odd) were evaluated. The reciprocal space of **1a•*S*-praziquantel** was inspected for the *h*0*l* layer, clearly revealing Bragg spots *h*=2*n* and *l*=*n*. Therefore, the data reduction and structure solution were executed for space group *C*2. For typical *C*2/c example for comparison see Supplemental Fig. 3, after soaking see Supplemental Fig. 4.



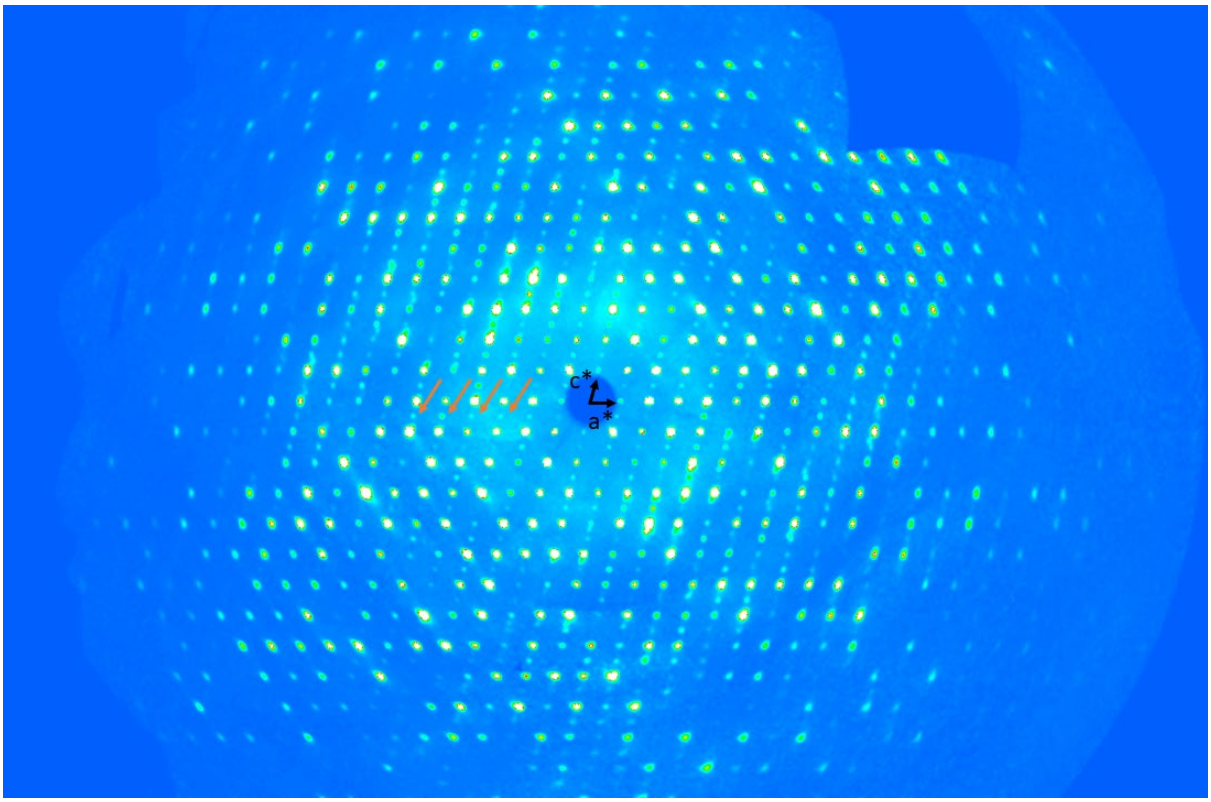
Supplemental Fig. 1. ORTEP diagram with 50% probability of the asymmetric unit of **1a•*S*-praziquantel**; restraints and constraints applied in the refinement of ***S*-praziquantel**



Supplemental Fig. 2. Electron density map F_o of ***S*-praziquantel** (contoured at the 2.02σ level)



Supplemental Fig. 3. Reciprocal *h*0*l* layer of a typical crystalline sponge crystal before soaking in *C*2/*c*. Absence of *h*=odd and *l*=odd reflexes is clearly visible.



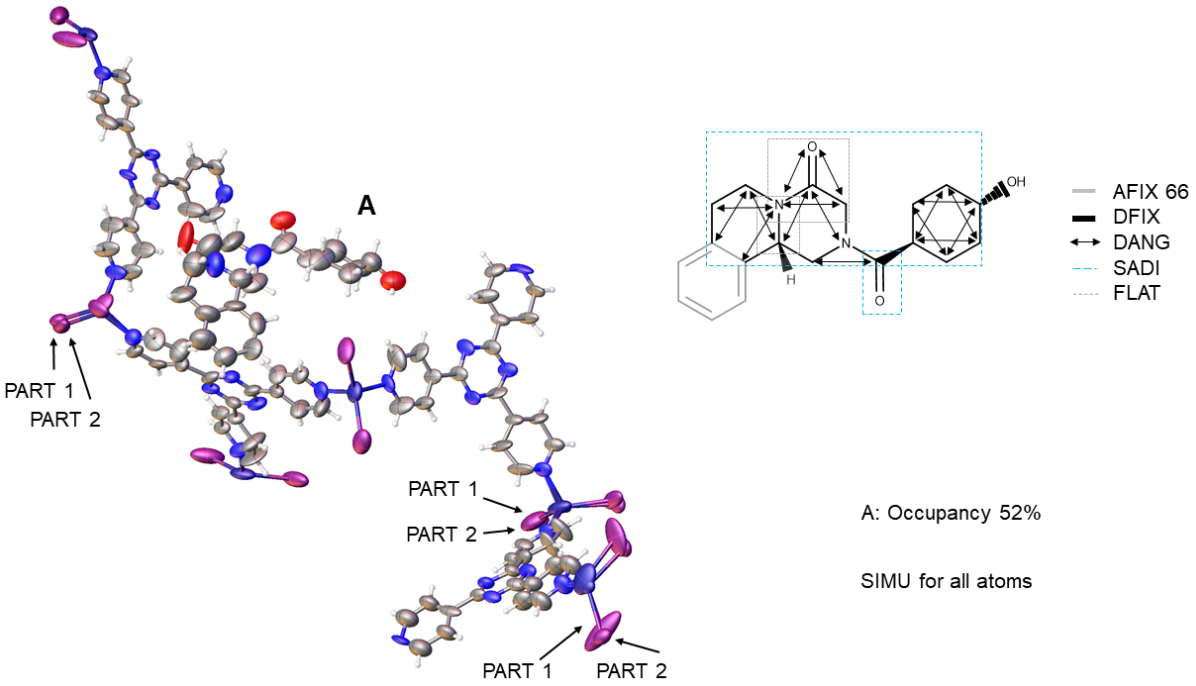
Supplemental Fig. 4. Reciprocal *h*0*l* layer after soaking ***S*-praziquantel** into the crystalline sponge. Reflexes at *h*=2*n* and *l*=*n* (highlighted with orange arrows) clearly visible.

Crystallographic data for 1b•*S-trans*-4'-hydroxy praziquantel (M1) (after solvent masking)

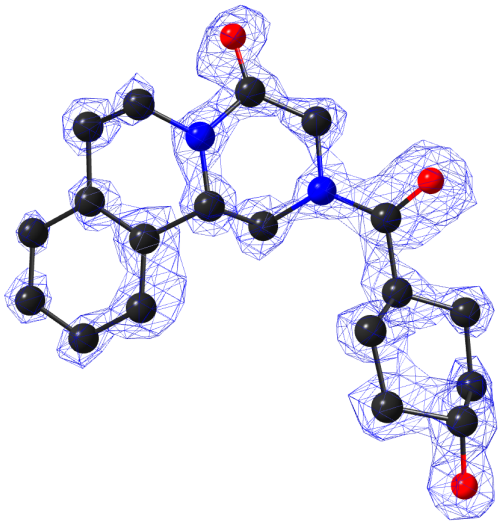
Crystal size: 253 × 213 × 97 μm^3, refined formula: $C_{81.95}H_{60.57}I_{12}N_{25.05}O_{1.57}Zn_6$, formula weight ($M_r$) = 3336.32 $^g/_{mol}$, yellow needle, crystal system: Monoclinic, space group *C*2, Z = 4, 27092 unique reflections merged from recorded 134281 ones (2.631° < θ < 67.074°) were used for structural analysis (R_{int} = 0.0386). Lattice parameters, R-factor on $F^2 > 2\sigma$ (F^2), weighted R-factor and goodness-of-fit are as follows: a = 34.1212(5) Å, b = 14.9488(2) Å, c = 30.3783(6) Å, β = 100.163(2)°, V = 15252.0(4) Å^3, R = 0.0658, wR_2 = 0.2218, S = 1.083. Calculated density is 1.453 gcm^{-3}. Linear absorption coefficient (μ) is 20.413. Residual electron density (max/min) is 1.32/-1.08 eÅ^{-3}. The Flack parameters for *S-trans*-4'-hydroxy praziquantel before and after using solvent masking were χ = 0.179(10) and χ = 0.229(8), respectively. CCDC number 2104977. The ORTEP diagram of the asymmetric unit of the framework and M1 praziquantel is shown in Supplemental Fig. 5.

The framework is refined using the restraint SIMU and constraint EADP for some carbon and nitrogen atoms of the tpt rings. Three ZnI_2 moieties are disordered and refined using disorder model. One guest molecule was found in the asymmetric unit with an occupancy of 52%. The benzene ring was fixed using AFIX 66 and DFIX, DANG, SADI and FLAT were used for some bonds and angles of the guest (see Supplemental Fig. 5). In addition, the guest molecule was refined by applying SIMU (for the complete molecule). A solvent mask was calculated and 255 electrons were found in a volume of 1024 A^3 in one void per asymmetric unit. This is consistent with the presence of 5 n-hexane molecules per asymmetric unit. The oxidation of the cyclohexane ring in position 4' in *trans* configuration is clearly visible from electron density maps (Supplemental Fig. 6).

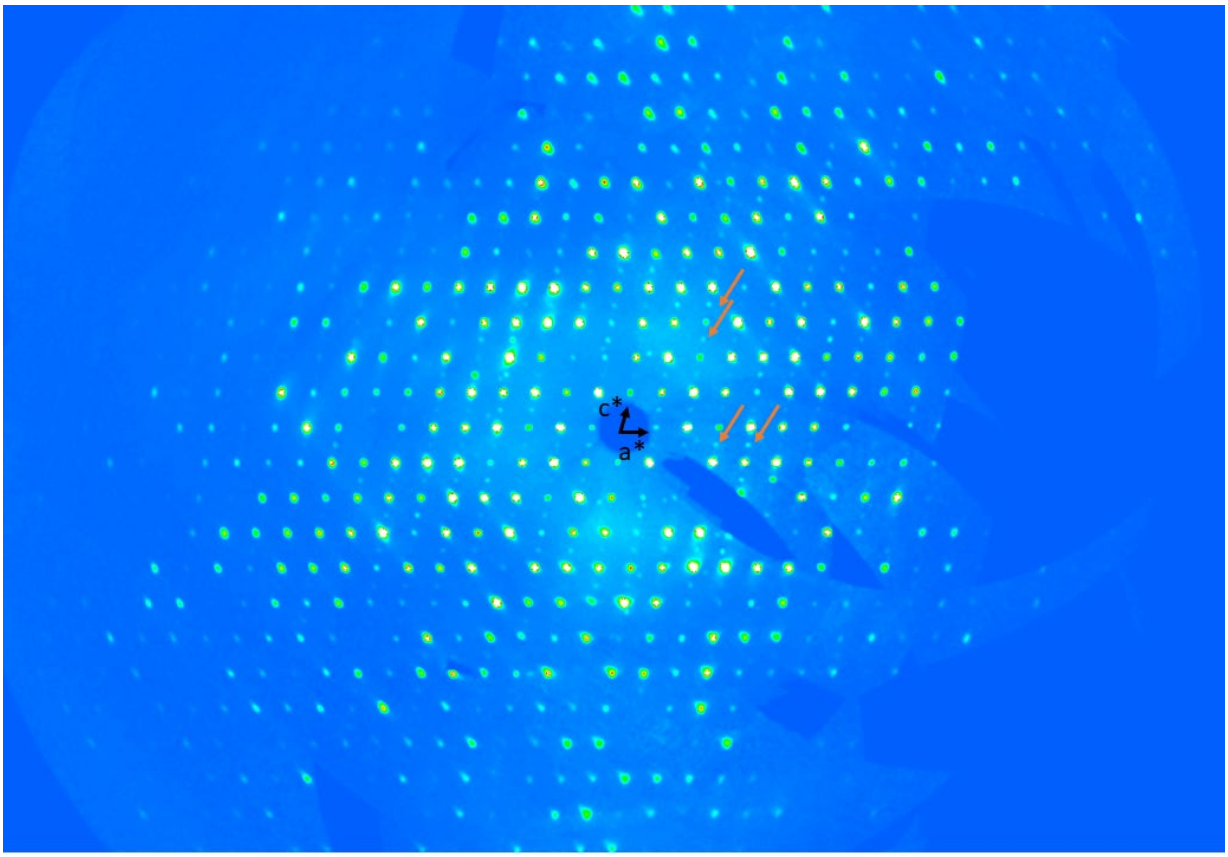
S-trans-4'-hydroxy praziquantel was used as enantiomerically pure substance. The reciprocal space of **1b•M1** was inspected for the *h*0*l* layer, revealing Bragg spots *h*=2*n* and *l*=*n* (Supplemental Fig.7).



Supplemental Fig. 5. ORTEP diagram with 50% probability of the asymmetric unit of **1b•M1 praziquantel**; restraints and constraints applied in the refinement of **M1 praziquantel**



Supplemental Fig. 6. Electron density map F_o of **M1 praziquantel** (contoured at the 2.00σ level)



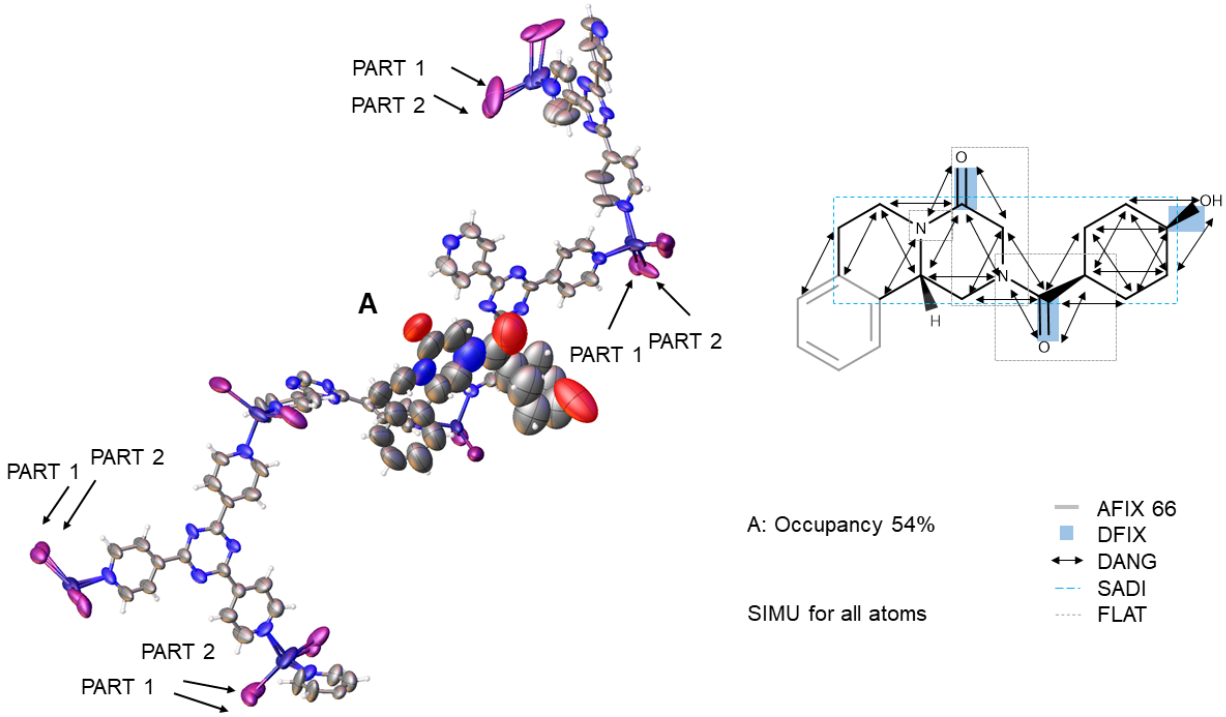
Supplemental Fig. 7. Reciprocal *h*0*l* layer after soaking ***S*-trans-4'-hydroxy praziquantel** into the crystalline sponge. Reflexes at *h*=2*n* and *l*=*n* (highlighted with orange arrows) visible.

Crystallographic data for 1b•*S-cis*-4'-hydroxy praziquantel (M2) (after solvent masking)

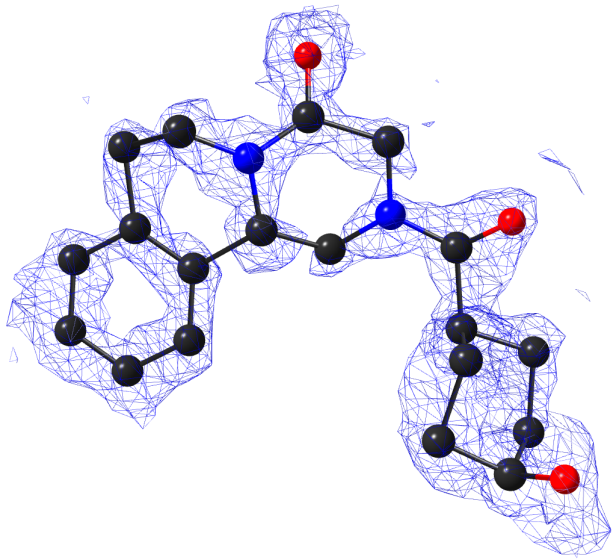
Crystal size: 264 × 136 × 112 µm^3, refined formula: $C_{81.87}H_{60.87}I_{12}N_{25.07}O_{1.61}Zn_6$, formula weight ($M_r$) = 3336.89 $^g/_{mol}$, yellow needle, crystal system: Monoclinic, space group *C*2, Z = 4, 27270 unique reflections merged from recorded 189002 ones (2.711° < θ < 67.078°) were used for structural analysis (R_{int} = 0.0286). Lattice parameters, R-factor on $F^2 > 2\sigma(F^2)$, weighted R-factor, and goodness-of-fit are as follows: a = 34.7979(5) Å, b = 14.9056(2) Å, c = 30.9525(3) Å, β = 101.311(1)°, V = 15742.7(4) Å^3, R = 0.0585, *w*R_2 = 0.2003, S = 1.101. Calculated density is 1.408 gcm^{-3}. Linear absorption coefficient (µ) is 19.777 mm^{-1}. Residual electron density (max/min) is 0.87/-0.58 eÅ^{-3}. The Flack parameters for *S-cis*-4'-hydroxy praziquantel before and after using solvent masking were χ = 0.180(9) and χ = 0.328(8), respectively. CCDC number 2104978. The ORTEP diagram of the asymmetric unit of the framework and M2 praziquantel is shown in Supplemental Fig. 8.

The framework is refined using the constraint EADP for four carbon atoms. The framework exhibits disorder of four ZnI_2 parts. One guest molecule (A) can be found in the asymmetric unit with an occupancy of 54%. AFIX 66 was applied to the benzene ring, and DFIX, DANG, SADI and FLAT were used for most bonds and angles of the guest (see Supplemental Fig. 8). SIMU was applied for the analyte molecule. A solvent mask was calculated and 440 electrons were found in a volume of 1544 A^3 in one void per asymmetric unit. This is consistent with the presence of 8.5 n-hexane molecules per asymmetric unit. The oxidation of the cyclohexane ring in position 4' in *cis* configuration is clearly visible from electron density maps (Supplemental Fig. 9).

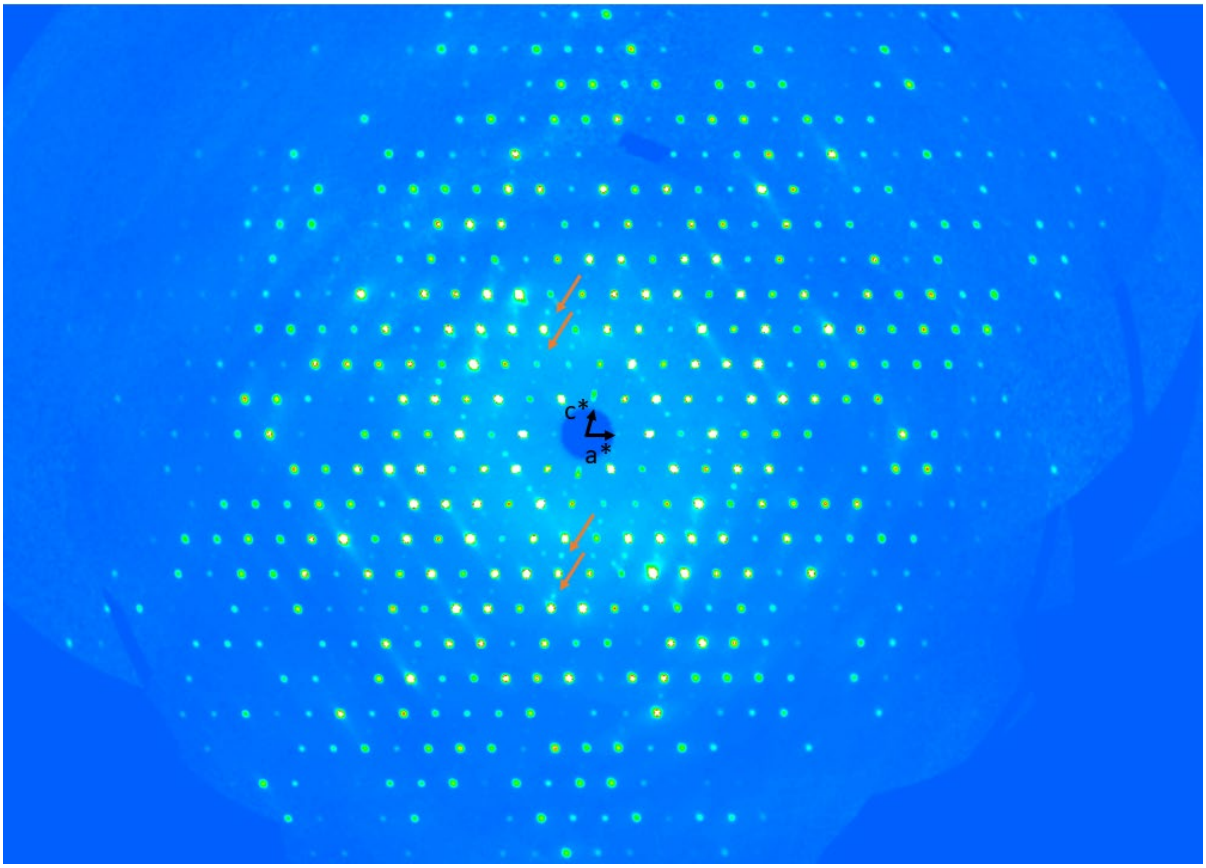
S-cis-4'-hydroxy praziquantel was used as enantiomerically pure substance. The reciprocal space of **1b•M2** was inspected for the *h*0*l* layer, revealing Bragg spots *h*=2*n* and *l*=*n* (Supplemental Fig.10).



Supplemental Fig. 8. ORTEP diagram with 50% probability of the asymmetric unit of **1b•M2 praziquantel**; restraints and constraints applied in the refinement of **M2 praziquantel**



Supplemental Fig. 9. Electron density map F_o of **M2 praziquantel** (contoured at the 3.70σ level)



Supplemental Fig. 10. Reciprocal h0*l* layer after soaking ***S-cis*-4'-hydroxy praziquantel** into the crystalline sponge. Reflexes at $h=2n$ and $l=n$ (highlighted with orange arrows) visible.

Crystallographic data for 1a•*S*-9-hydroxy praziquantel (M3) (after solvent masking)

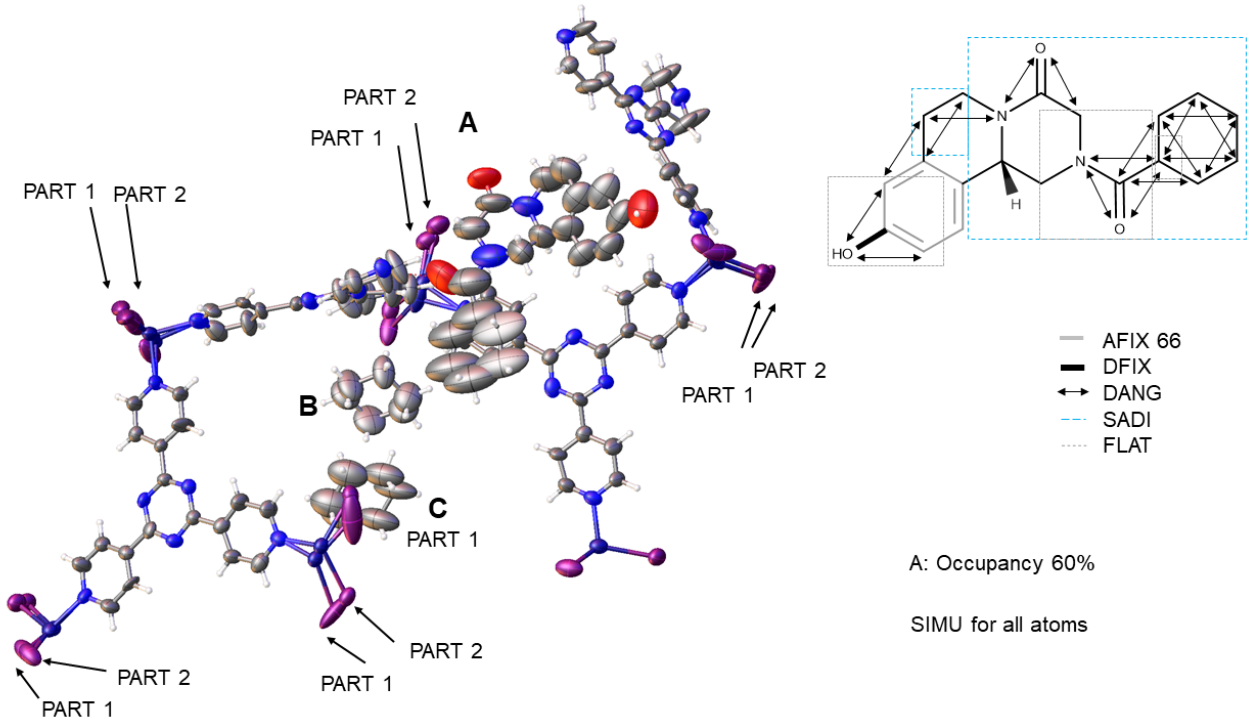
Crystal size: 178 × 120 × 71 μm^3, refined formula: $C_{91.82}H_{79.01}I_{12}N_{25.2}O_{1.8}Zn_6$, formula weight ($M_r$) = 3478.97 $^g/_{mol}$, colorless needle, crystal system: Monoclinic, space group *C*2, Z = 4, 23994 unique reflections merged from recorded 68432 ones (2.583° < θ < 67.081°) were used for structural analysis (R_{int} = 0.0152). Lattice parameters, R-factor on $F^2 > 2\sigma(F^2)$, weighted R-factor, and goodness-of-fit are as follows: a = 35.1163(6) Å, b = 14.8449(2) Å, c = 32.1504(6) Å, β = 103.042(2)°, V = 16327.6(5) Å^3, R = 0.0553, *w*R_2 = 0.1716, S = 1.113. Calculated density is 1.415 gcm^{-3}. Linear absorption coefficient (μ) is 19.093 mm^{-1}. Residual electron density (max/min) is 1.02/-1.05 eÅ^{-3}. The Flack parameters for *S*-9-hydroxy praziquantel before and after using solvent masking were χ = 0.111(9) and χ = 0.204(7), respectively. CCDC number 2107215. The ORTEP diagram of the asymmetric unit of the framework and M3 praziquantel is shown in Supplemental Fig. 11.

The framework is refined using the constraint EADP for some disordered ZnI_2 moieties and the restraint SIMU for some carbon atoms. Five ZnI_2 moieties are disordered and refined using the disorder model. One guest molecule (A) and two cyclohexane molecules (B and C) were found in the asymmetric unit. The analyte occupancy is 60%. Solvent molecule C is disordered with one ZnI_2 moiety and refined using PART 1 command. Some restraints were applied for refinement of the analyte and solvent molecules (DFIX, SADI, FLAT and DANG). The guest molecule was refined by applying SIMU (for complete molecule). The restraints used for the refinement are summarized in Supplemental Fig. 11. The benzene part of the molecule could be refined with minor constraints (AFIX 66) and the presence of oxidation at position 9 of the aromatic ring is clearly visible from the electron density map (Supplemental Fig. 12). The position of hydroxylation had to be restrained to its theoretical values by applying DFIX and DANG commands due to high mobility and not resolved disorder with solvent molecules at this part. Intermolecular hydrogen bonds could be observed by the C-H···O distance (2.17 Å) between the framework and the carbonyl of the analyte. A solvent mask was calculated and

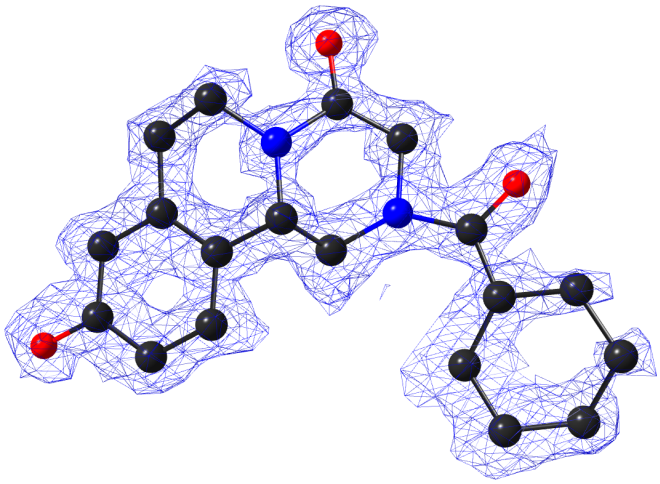
418 electrons were found in a volume of 1342 A^3 in one void per asymmetric unit. This is consistent with the presence of 8.5 cyclohexane molecules per asymmetric unit.

In the present study, the task of the crystalline sponge method was the determination of the hydroxy positions after *in vitro* incubation of the parent compound. The position of metabolism could be determined unambiguously. The combination of known parent structure, mass spectrometry and crystalline sponge analysis leads therefore to a successful structure elucidation. Furthermore, NMR analysis of the metabolite confirms the structure.

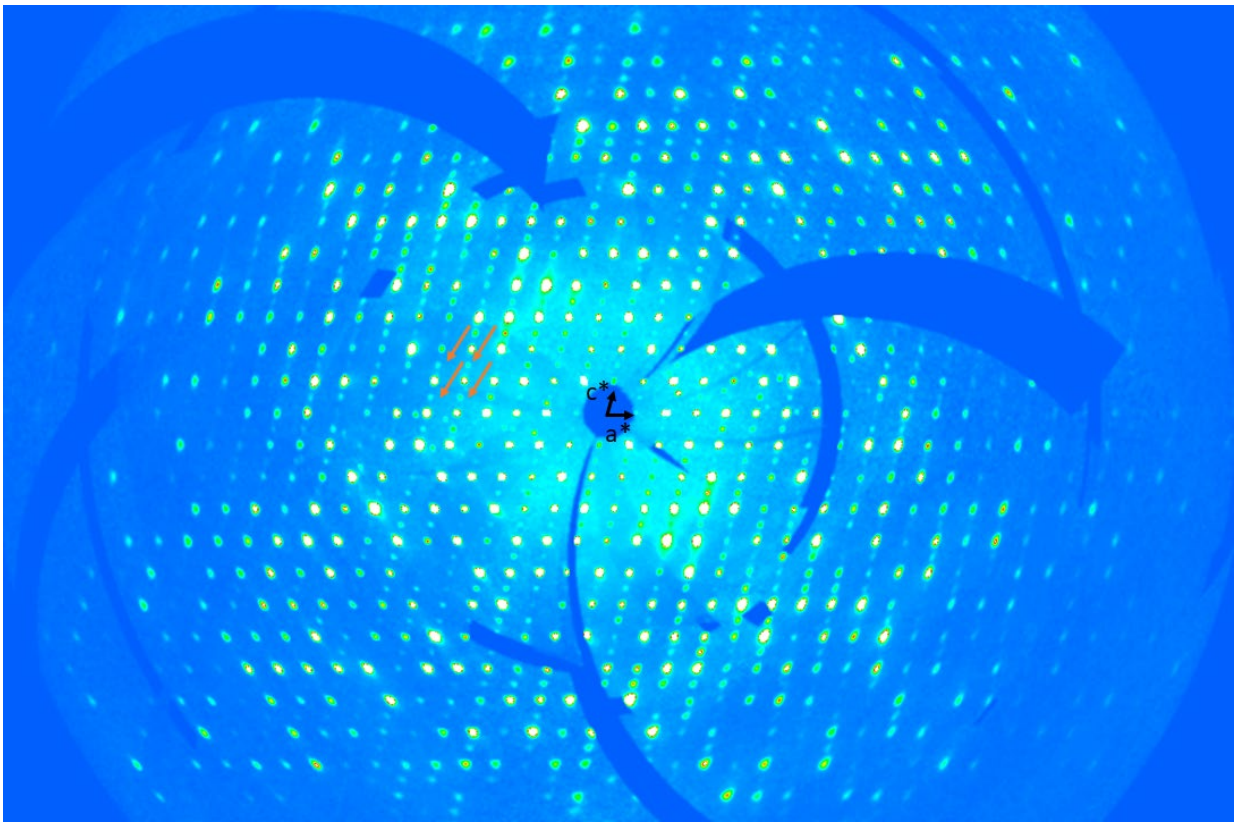
S-9-hydroxy praziquantel was isolated from incubation of enantiomerically pure S-praziquantel. The reciprocal space of **1b•M3** was inspected for the *h*0*l* layer, clearly revealing Bragg spots *h*=2*n* and *l*=*n* (Supplemental Fig.13).



Supplemental Fig. 11. ORTEP diagram with 50% probability of the asymmetric unit of **1a•M3 praziquantel**; restraints and constraints applied in the refinement of **M3 praziquantel**



Supplemental Fig. 12. Electron density map F_o of **M3 praziquantel** (contoured at the 3.33σ level)



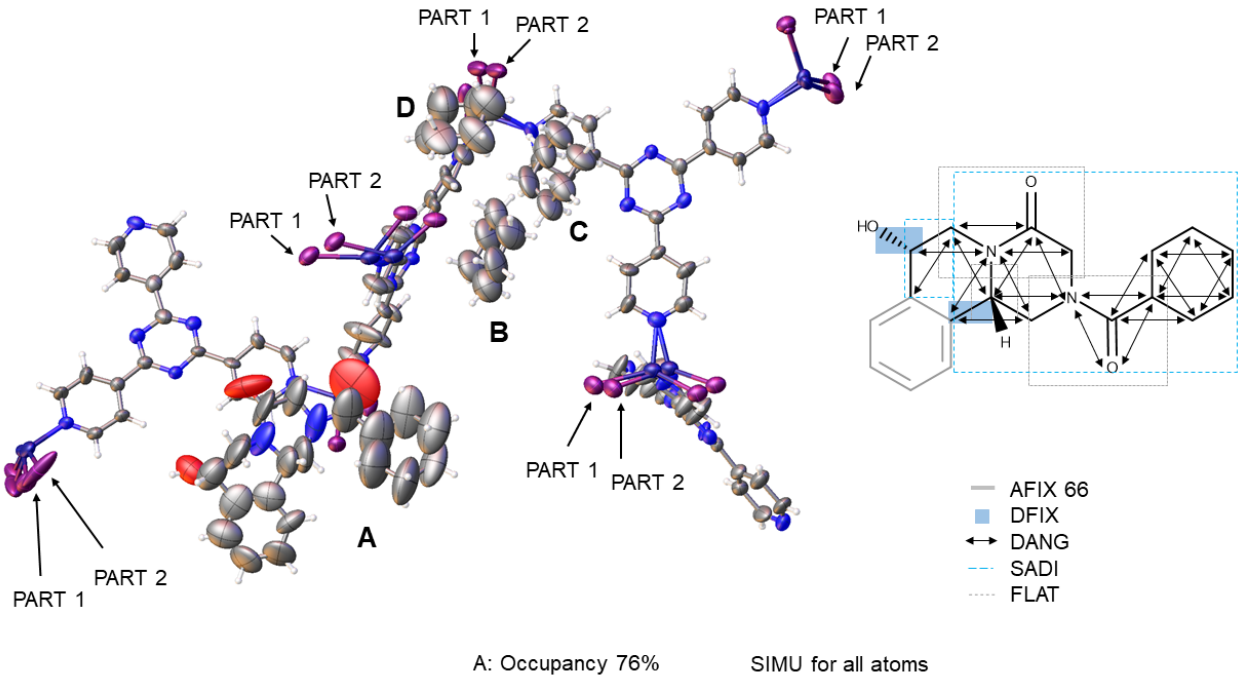
Supplemental Fig. 13. Reciprocal *h*0*l* layer after soaking ***S*-9-hydroxy praziquantel** into the crystalline sponge. Reflexes at *h*=2*n* and *l*=*n* (highlighted with orange arrows) clearly visible.

Crystallographic data for 1a•*S*-7-hydroxy *S*-praziquantel (M4) (after solvent masking)

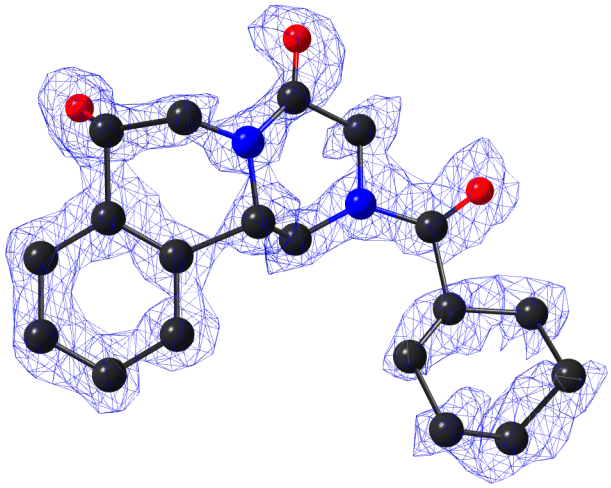
Crystal size: 198 × 142 × 89 μm^3, refined formula: $C_{100.54}H_{94.49}I_{12}N_{25.51}O_{2.27}Zn_6$, formula weight ($M_r$) = 3611.29 $^g/_{mol}$, colorless needle, crystal system: Monoclinic, space group *C*2, Z = 4, 24684 unique reflections merged from recorded 54687 ones (2.573° < θ < 67.08°) were used for structural analysis (R_{int} = 0.0145). Lattice parameters, R-factor on $F^2 > 2\sigma(F^2)$, weighted R-factor, and goodness-of-fit are as follows: a = 35.2848(5) Å, b = 14.7872(2) Å, c = 32.3789(4) Å, β = 103.223(1)°, V = 16446.2(4) Å^3, R = 0.0499, *w*R_2 = 0.1491, S = 1.089. Calculated density is 1.458 gcm^{-3}. Linear absorption coefficient (μ) is 18.979 mm^{-1}. Residual electron density (max/min) is 0.94/-1.07 eÅ^{-3}. The Flack parameters for *S*-7-hydroxy *S*-praziquantel before and after using solvent masking were χ = 0.092(8) and χ = 0.191(7), respectively. CCDC number 2107216. The ORTEP diagram of the asymmetric unit of the framework and M4 praziquantel is shown in Supplemental Fig. 14.

The framework exhibits disorder of five ZnI_2 parts refined using the disorder model. One guest molecule (A) with an occupancy of 76% and three additional cyclohexane molecules (C, D and E) can be found in the asymmetric unit. The benzene ring of molecule A was fixed using AFIX 66 and the non-aromatic rings had to be restrained using SADI, DFIX, DANG and FLAT (Supplemental Fig. 14). SIMU was applied for all atoms of the analyte molecule. A solvent mask was calculated and 272 electrons and 50 electrons were found in a volume of 1040 A^3 and 147 A^3, respectively, in two voids per asymmetric unit. This is consistent with the presence of 5.5 and 1 cyclohexane molecules per asymmetric unit. The electron density map clearly showed the introduction of an additional atom at position 7 of the non-aromatic part of the structure (Supplemental Fig. 15). The distance of the C-O bond had to be restrained using DFIX.

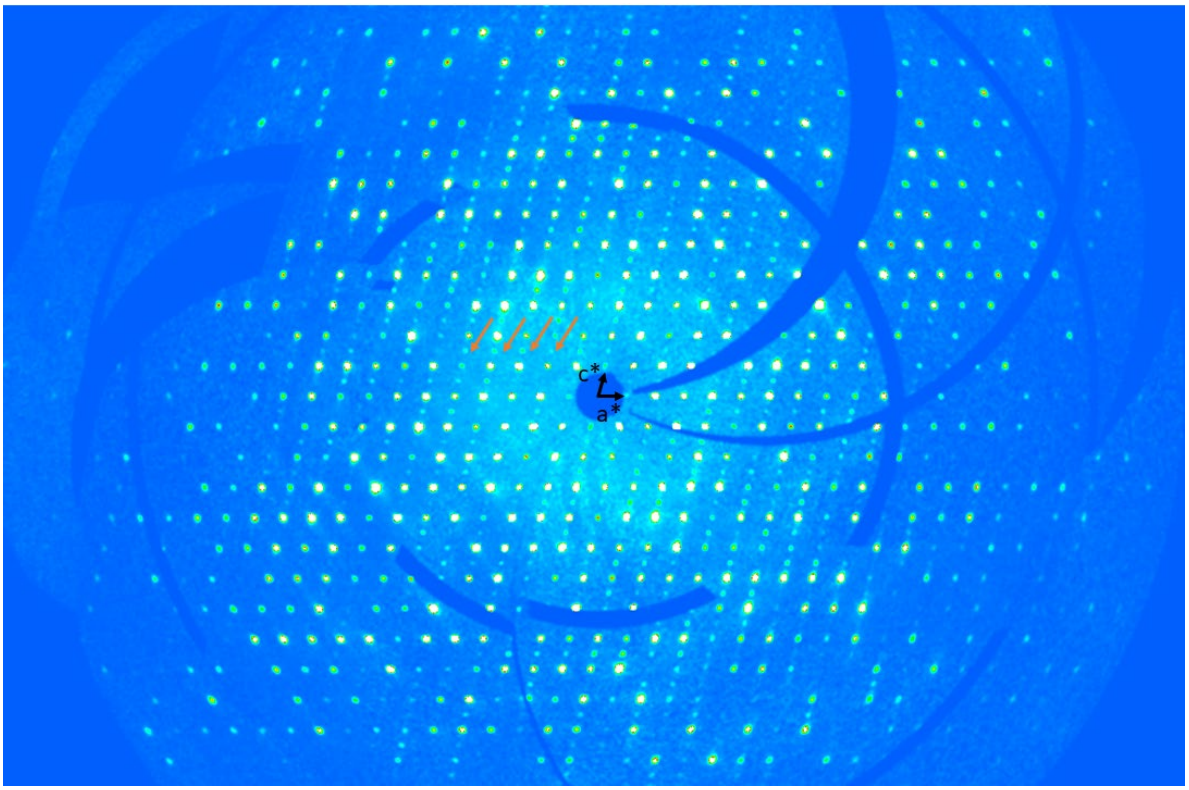
S-7-hydroxy *S*-praziquantel was isolated from incubation of enantiomerically pure S-praziquantel. The reciprocal space of **1b•M4** was inspected for the *h*0*l* layer, clearly revealing Bragg spots *h*=2*n* and *l*=*n* (Supplemental Fig.16).



Supplemental Fig. 14. ORTEP diagram with 50% probability of the asymmetric unit of **1a•M4 praziquantel**; restraints and constraints applied in the refinement of **M4 praziquantel**



Supplemental Fig. 15. Electron density map F_o of **M4 praziquantel** (contoured at the 2.11σ level)



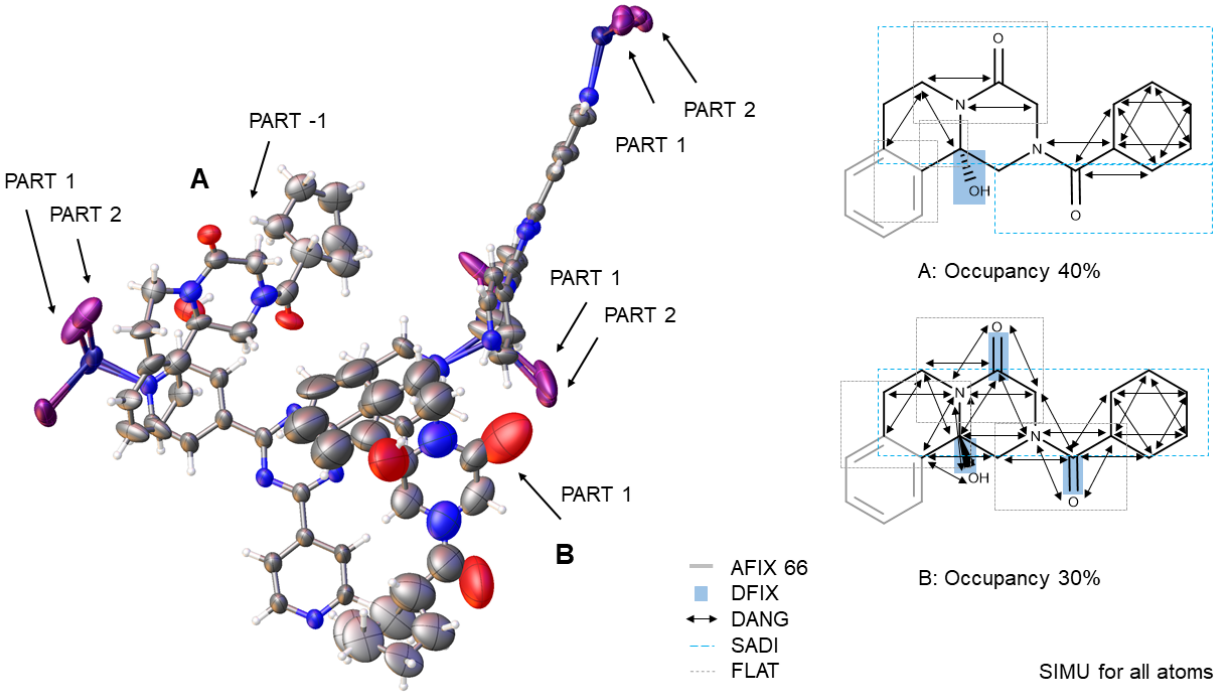
Supplemental Fig. 16. Reciprocal *h*0*l* layer after soaking ***S*-7-hydroxy *S*-praziquantel** into the crystalline sponge. Reflexes at *h*=2*n* and *l*=*n* (highlighted with orange arrows) visible.

Crystallographic data for 1a•*S*-11*b*-hydroxy praziquantel and *R*-11*b*-hydroxy praziquantel (*S*-/*R*-M6)

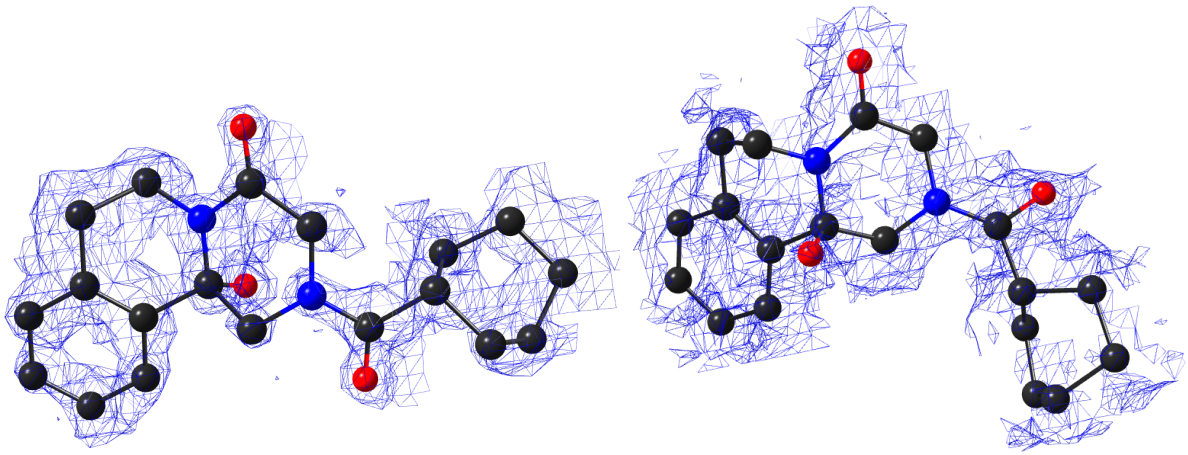
Crystal size: 339 × 172 × 90 µm^3, refined formula: $C_{45.46}H_{35.94}I_6N_{12.99}O_{1.49}Zn_3$, formula weight ($M_r$) = 1745.61 $^g/_{mol}$, yellow needle, crystal system: Monoclinic, space group *C*2/*c*, Z = 8, 14979 unique reflections merged from recorded 53191 ones (2.610° < θ < 74.592°) were used for structural analysis (R_{int} = 0.0248). Lattice parameters, R-factor on $F^2 > 2\sigma(F^2)$, weighted R-factor, and goodness-of-fit are as follows: a = 34.4208(4) Å, b = 15.0229(1) Å, c = 30.2656(3) Å, β = 100.421(1)°, V = 15392.2(3) Å^3, R = 0.0592, wR_2 = 0.1890, S = 1.090. Calculated density is 1.507 gcm^{-3}. Linear absorption coefficient (µ) is 20.264 mm^{-1}. Residual electron density (max/min) is 1.67/-0.99 eÅ^{-3}. CCDC number 2104985. The ORTEP diagram of the asymmetric unit of the framework and *S*-/*R*-M6 praziquantel is shown in Supplemental Fig. 17.

The framework is refined without restraints. The framework exhibits disorder of three ZnI_2 part refined using the disorder model. Two guest molecules (A) and (B) can be found in the asymmetric unit. Guest A is disordered at special position (inversion) and refined using PART -1 command and s.o.f. = 0.5x(FVAR#5). This leads to an analyte occupancy of 40%. An additional guest molecule (B), disordered with one ZnI_2 moiety, was observed, and is refined as PART 1 with a fixed occupancy of 30%. AFIX 66 was applied to the benzene rings, and DFIX, SADI, DANG and FLAT were used for some bonds and angles of the guest (Supplemental Fig. 17). The guest molecules were refined by applying SIMU. Additional electron density was observed for both guest molecules in position 11b in either *S* or *R* configuration of the non-aromatic ring system and was assigned as hydroxylation (+16 Da) (Supplemental Fig. 18). The introduced oxygens were restrained using the command DFIX (A, B) and DANG (B).

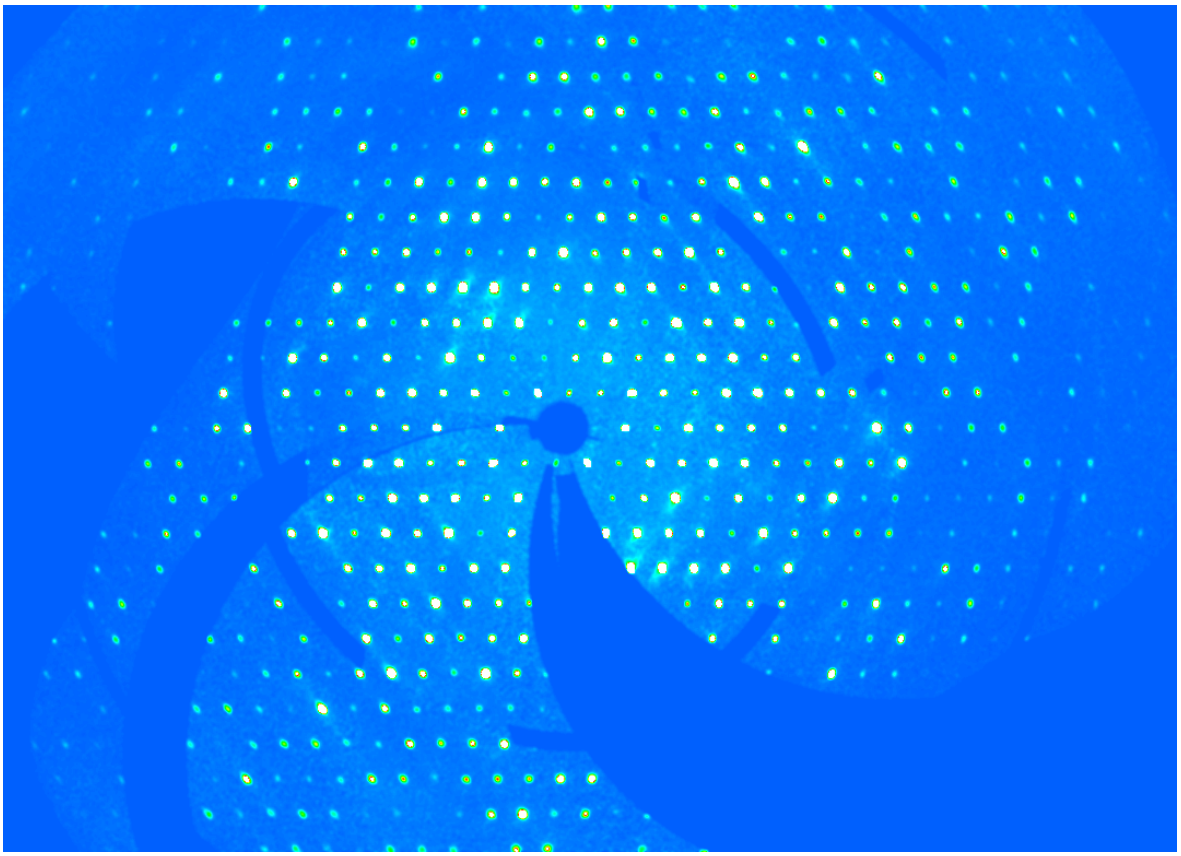
The reciprocal space of **1a•*S*-/*R*-M6** was inspected for the *h*0*l* layer, revealing the absence of Bragg spots *h*=2*n* and *l*=*n* (Supplemental Fig.19) and confirming the centrosymmetric space group *C*2/*c*.



Supplemental Fig. 17. ORTEP diagram with 50% probability of the asymmetric unit of **1a•*S*-/*R*-M6 praziquantel**; restraints and constraints applied in the refinement of ***S*-/*R*-M6 praziquantel**



Supplemental Fig. 18. Electron density map F_o of ***S*-M6 praziquantel** (contoured at the 1.89σ level) and ***R*-M6 praziquantel** (contoured at the 2.78σ level)



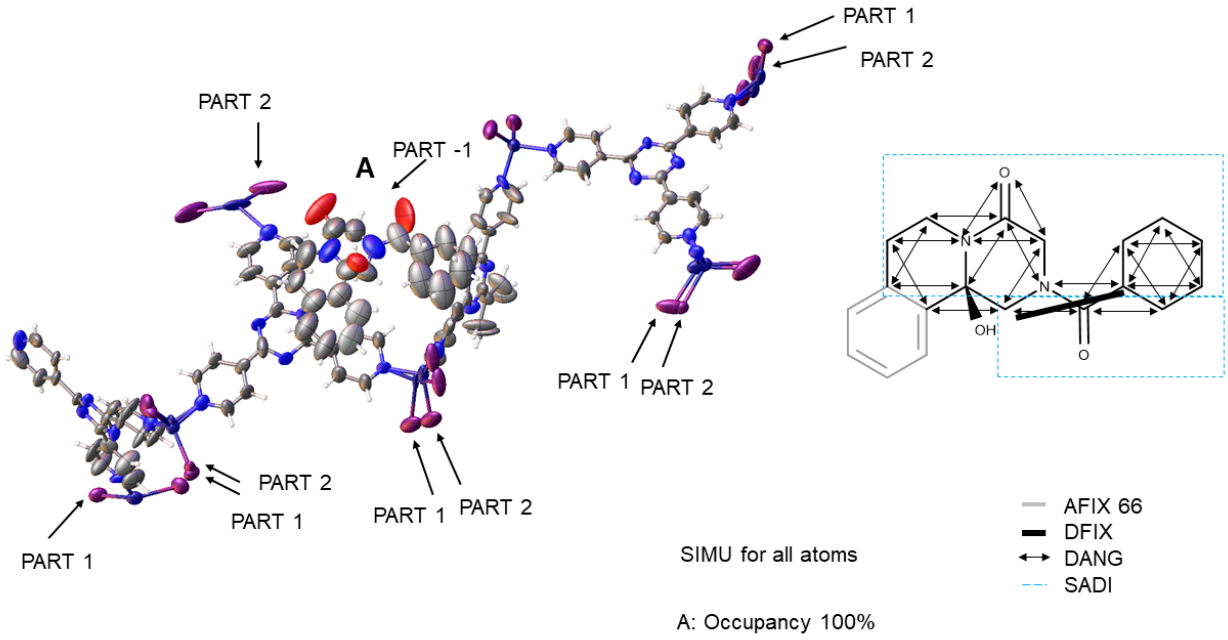
Supplemental Fig. 19. Reciprocal *h*0*l* layer after soaking ***S*-11b-hydroxy praziquantel** and ***R*-11b-hydroxy praziquantel** into the crystalline sponge. Absence of *h*=odd and *l*=odd reflexes is clearly visible.

Crystallographic data for 1a•*R*-11*b*-hydroxy praziquantel (*R*-M6) (after solvent masking)

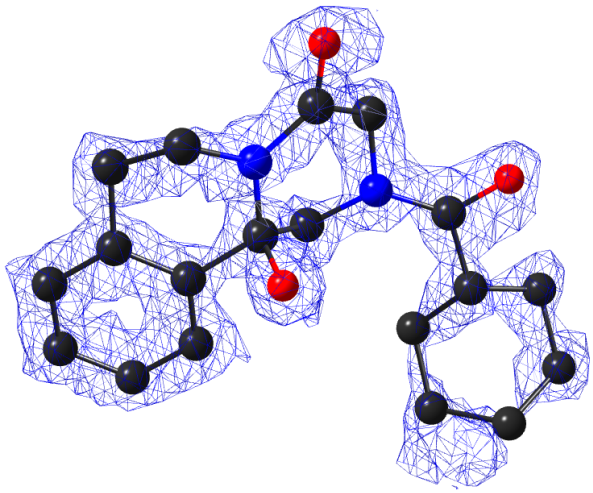
Crystal size: 236 × 168 × 103 μm^3, refined formula: $C_{81.5}H_{59.78}I_{12}N_{25}O_{1.5}Zn_6$, formula weight ($M_r$) = 3328.34 $^g/_{mol}$, light yellow needle, crystal system: Monoclinic, space group *C*2, Z = 4, 28422 unique reflections merged from recorded 185893 ones (2.575° < θ < 67.078°) were used for structural analysis (R_{int} = 0.0369). Lattice parameters, R-factor on $F^2 > 2\sigma(F^2)$, weighted R-factor, and goodness-of-fit are as follows: a = 35.2910(6) Å, b = 14.8009 (2) Å, c = 32.3794 (5) Å, β = 103.507(2)°, V = 16445.2(5) Å^3, R = 0.0732, wR_2 = 0.2394, S = 1.092. Calculated density is 1.344 gcm^{-3}. Linear absorption coefficient (μ) is 18.930 mm^{-1}. Residual electron density (max/min) is 1.75/-0.96 eÅ^{-3}. The Flack parameters for *R*-11b-hydroxy praziquantel before and after using solvent masking were χ = 0.166(11) and χ = 0.221(8), respectively. CCDC number 2129663. The ORTEP diagram of the asymmetric unit of the framework and *R*-M6 praziquantel is shown in Supplemental Fig. 20.

The framework is refined using the constraint EADP for some disordered ZnI_2 moieties and carbon atoms. DFIX and SADI was used for some tpt parts. Five ZnI_2 moieties are disordered and refined using the disorder model. One guest molecule (A) can be found in the asymmetric unit near the center of symmetry (*C*2 axis) and is refined using PART -1 command with s.o.f. = 0.5. The analyte occupancy was modeled to 100%. AFIX 66 was applied to the benzene ring, and SADI and DANG were used for some bonds and angles of the guest (Supplemental Fig. 20). The guest molecule was refined by applying SIMU. A solvent mask was calculated and 422 electrons were found in a volume of 1538 A^3 in one void per asymmetric unit. This is consistent with the presence of 8.5 cyclohexane molecules per asymmetric unit. Additional electron density was observed in position 11b of the non-aromatic ring system and was assigned as hydroxylation (+16 Da) (Supplemental Fig. 21).

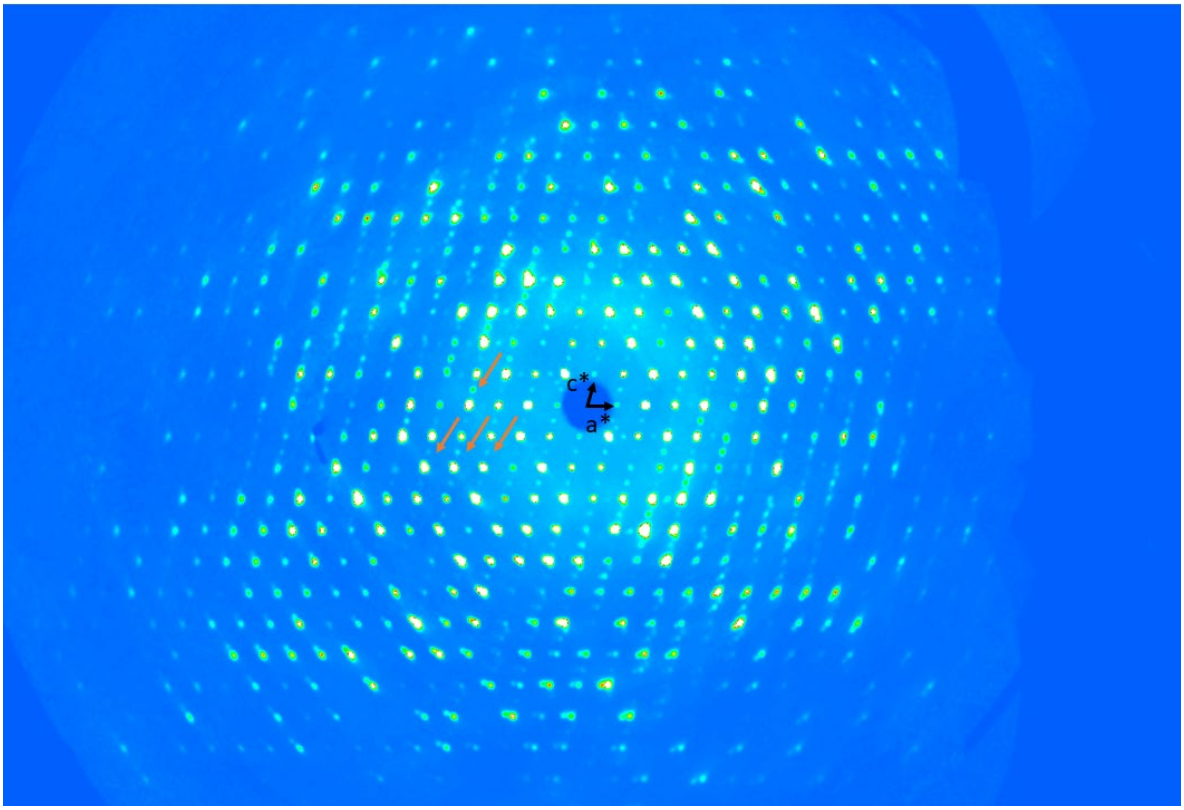
The reciprocal space of **1a•*R*-M6** was inspected for the *h*0*l* layer, revealing Bragg spots *h*=2*n* and *l*=*n* (Supplemental Fig.22).



Supplemental Fig. 20. ORTEP diagram with 50% probability of the asymmetric unit of **1a•*R*-M6 praziquantel**; restraints and constraints applied in the refinement of ***R*-M6 praziquantel**

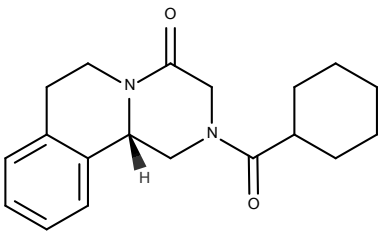
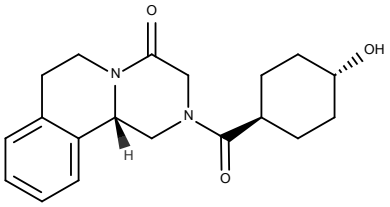
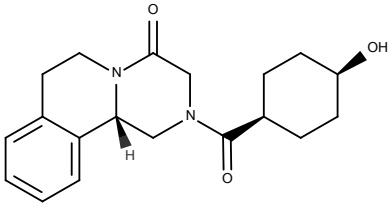
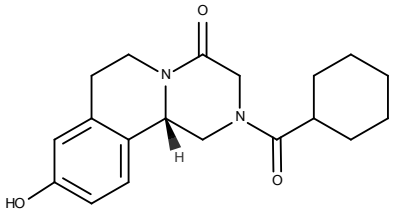
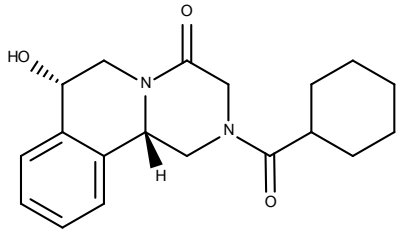


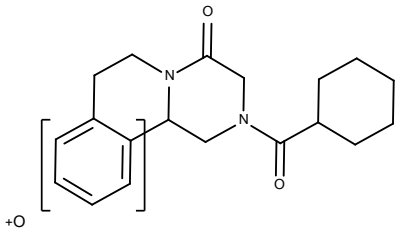
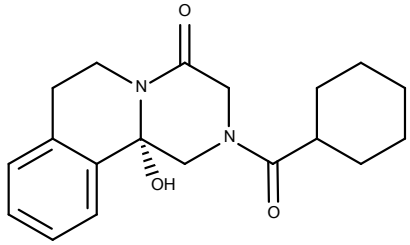
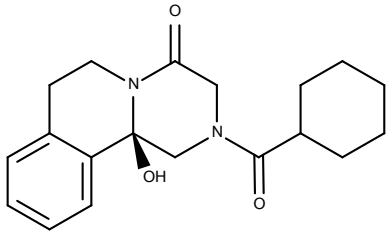
Supplemental Fig. 21. Electron density map F_o of ***R*-M6 praziquantel** (contoured at the 2.73σ level)



Supplemental Fig. 22. Reciprocal *h*0*l* layer after soaking ***R*-11b-hydroxy praziquantel** into the crystalline sponge. Reflexes at *h*=2*n* and *l*=*n* (highlighted with orange arrows) visible.

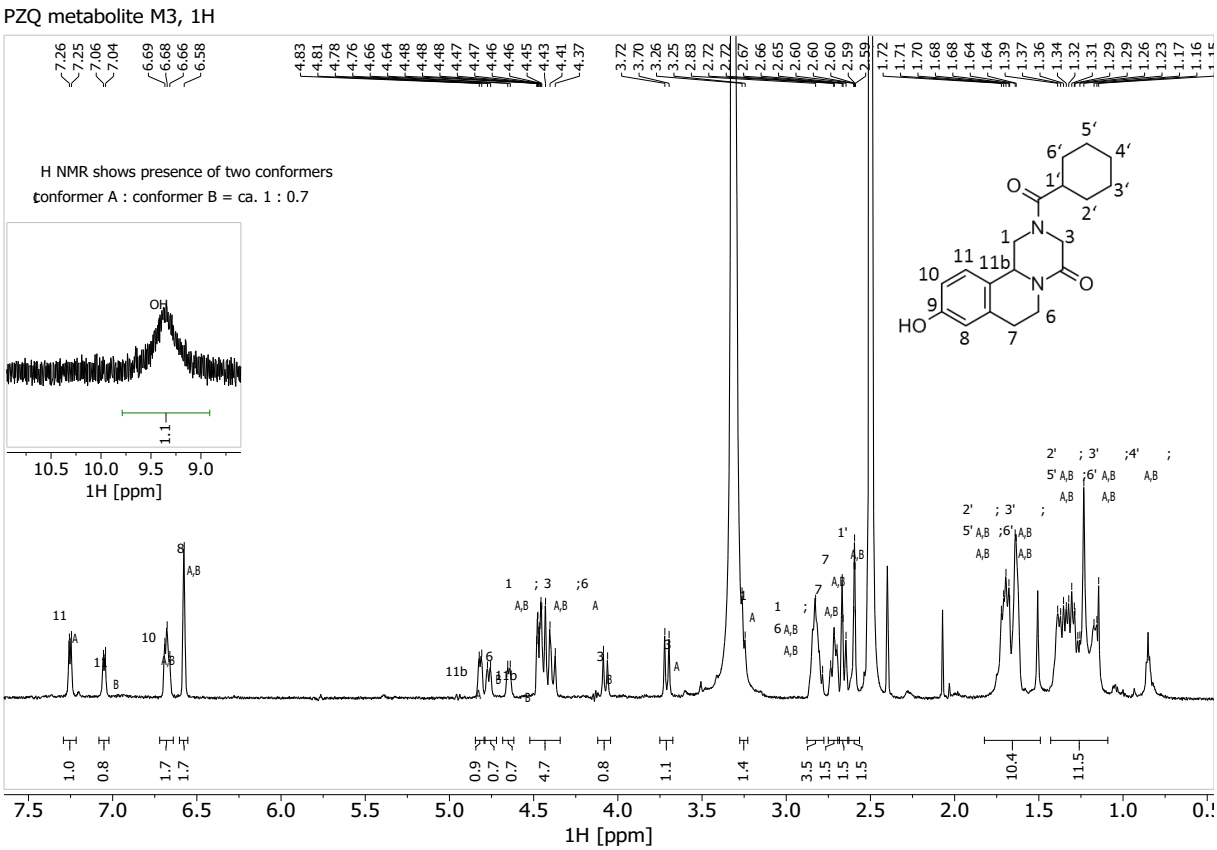
Ultra-performance liquid chromatography - quadrupole time-of-flight mass spectrometry

ID	Name	Retention time [min]	Elemental Composition (ion formula)	$[M+H]^+$ [m/z]	Main fragment ions [m/z]	Mass error [mDa]	Structure proposal
S-PZQ	*S*-praziquantel	11.19	$[C_{19}H_{24}N_2O_2 + H]^+$	313.1915			
			$[C_{12}H_{14}N_2O + H]^+$		203.1181	0.2	
			$[C_{12}H_{12}N_2O + H]^+$		201.1024	0.2	
			$[C_{11}H_{11}NO + H]^+$		174.0917	0.3	
			$[C_{10}H_{11}N + H]^+$		146.0966	0.1	
			$[C_9H_9N + H]^+$		132.0810	0.1	
			$[C_{10}H_8 + H]^+$		129.0698	-0.2	
M1	*S-trans*-4'-hydroxy praziquantel	5.06	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1861			
			$[C_{19}H_{22}N_2O_2 + H]^+$		311.1756	0.2	
			$[C_{12}H_{14}N_2O + H]^+$		203.1180	0.1	
			$[C_{11}H_{11}NO + H]^+$		174.0916	0.2	
			$[C_{10}H_{11}N + H]^+$		146.0966	0.1	
			$[C_9H_9N + H]^+$		132.0811	0.3	
			$[C_{10}H_8 + H]^+$		129.0698	-0.1	
M2	*S-cis*-4'-hydroxy praziquantel	5.33	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1864			
			$[C_{19}H_{22}N_2O_2 + H]^+$		311.1758	0.4	
			$[C_{12}H_{14}N_2O + H]^+$		203.1181	0.2	
			$[C_{12}H_{12}N_2O + H]^+$		201.1025	0.3	
			$[C_{11}H_{11}NO + H]^+$		174.0916	0.2	
			$[C_{10}H_{11}N + H]^+$		146.0966	0.1	
			$[C_9H_9N + H]^+$		132.0810	0.2	
			$[C_{10}H_8 + H]^+$		129.0698	-0.1	
M3	*S*-9-hydroxy praziquantel	7.05	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1860			
			$[C_{12}H_{14}N_2O_2 + H]^+$		219.1129	0.1	
			$[C_{11}H_{11}NO_2 + H]^+$		190.0862	-0.1	
			$[C_{10}H_{11}NO + H]^+$		162.0906	-0.7	
			$[C_9H_9NO + H]^+$		148.0753	-0.4	
			$[C_8H_8O + H]^+$		121.0637	-1.1	
M4	*S*-7-hydroxy *S*-praziquantel	7.13	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1857			
			$[C_{19}H_{22}N_2O_2 + H]^+$		311.1753	-0.1	
			$[C_{12}H_{12}N_2O + H]^+$		201.1017	-0.5	
			$[C_{10}H_9N + H]^+$		144.0801	-0.7	
			$[C_9H_7N + H]^+$		130.0646	-0.5	
			$[C_9H_6 + H]^+$		115.0531	-1.2	

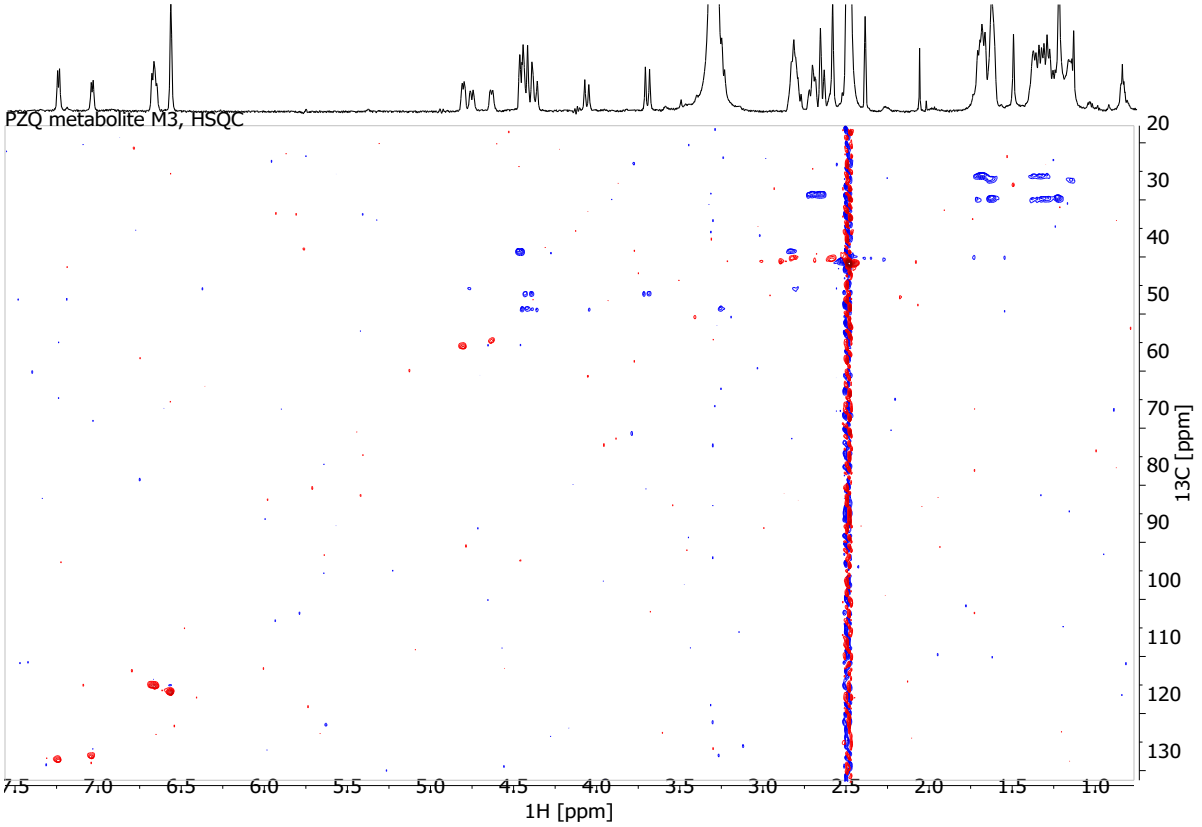
M5	?-hydroxy praziquantel	7.37	$[C_{19}H_{24}N_2O_3 + H]^+$ $[C_{12}H_{14}N_2O_2 + H]^+$ $[C_{11}H_{11}NO_2 + H]^+$ $[C_{10}H_{11}NO + H]^+$ $[C_9H_9NO + H]^+$	329.1859	 219.1127 190.0860 162.0906 148.0748	 0.0 -0.3 -0.7 -0.9	
M6	*S*-11b-hydroxy praziquantel	7.49	$[C_{19}H_{24}N_2O_3 + H]^+$ $[C_{19}H_{22}N_2O_2 + H]^+$ $[C_{12}H_{12}N_2O + H]^+$ $[C_{10}H_9N + H]^+$ $[C_9H_7N + H]^+$ $[C_9H_6 + H]^+$	329.1859	 311.1754 201.1023 144.0812 130.0653 115.0545	 0.0 0.1 0.4 0.2 0.3	
M6	*R*-11b-hydroxy praziquantel	11.85	$[C_{19}H_{24}N_2O_3 + H]^+$ $[C_{19}H_{22}N_2O_2 + H]^+$ $[C_{12}H_{12}N_2O + H]^+$ $[C_{10}H_9N + H]^+$ $[C_9H_7N + H]^+$ $[C_9H_6 + H]^+$	329.1858	 311.1753 201.1022 144.0808 130.0651 115.0542	 -0.1 0.0 0.0 0.0 -0.1	

Supplemental Table 1. Mass spectral analysis of hydroxylated metabolites formed *in vitro* from ***S*-praziquantel**

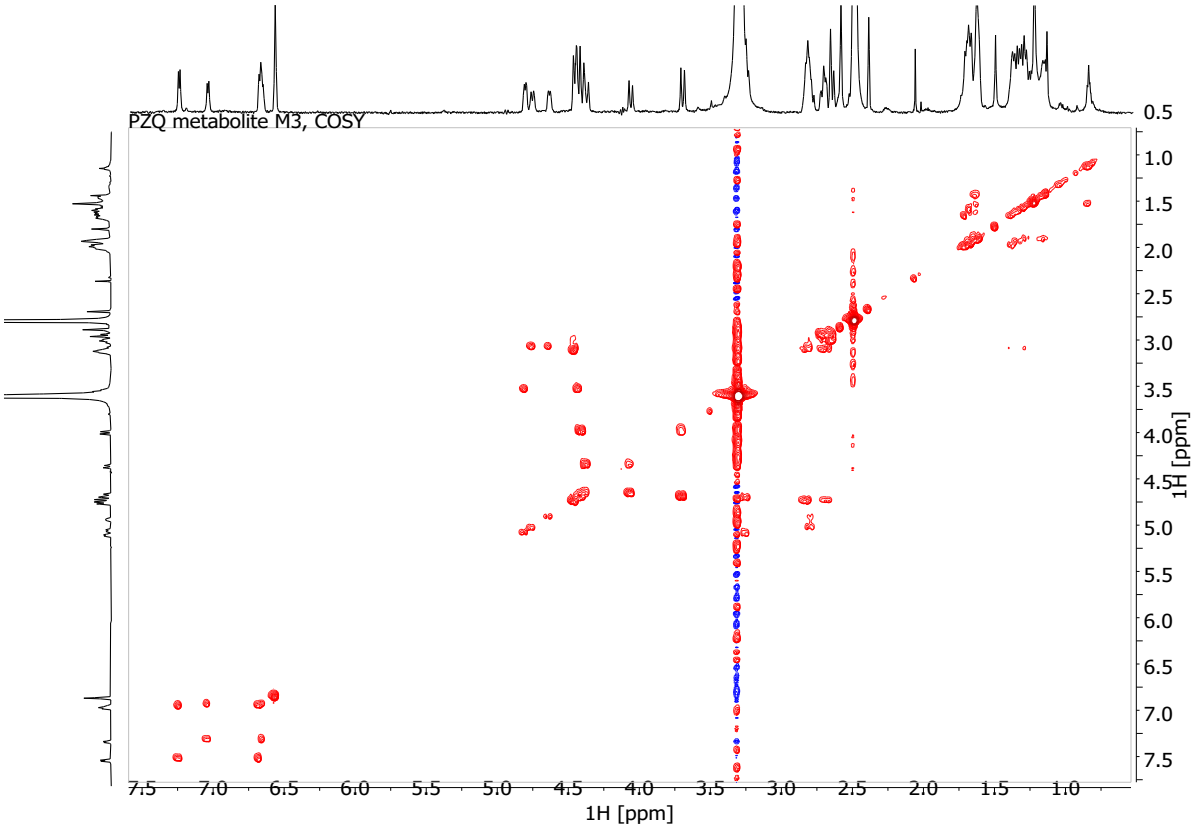
Nuclear Magnetic Resonance



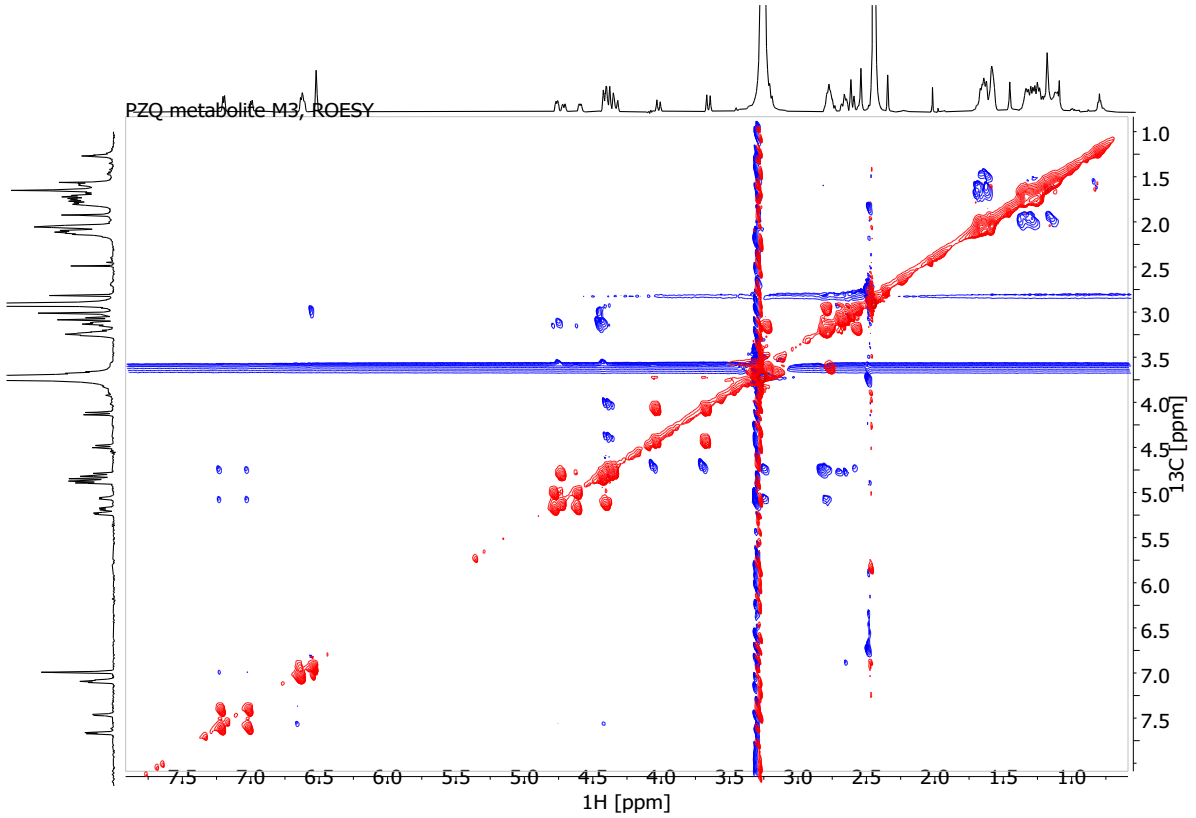
Supplemental Fig. 23. Assigned ^{1}H NMR spectrum of ***S*-9-hydroxy praziquantel** (M3)



Supplemental Fig. 24. Edited heteronuclear single quantum coherence (HSQC) NMR spectrum of ***S*-9-hydroxy praziquantel** (M3). Red: CH/CH_3, blue: CH_2

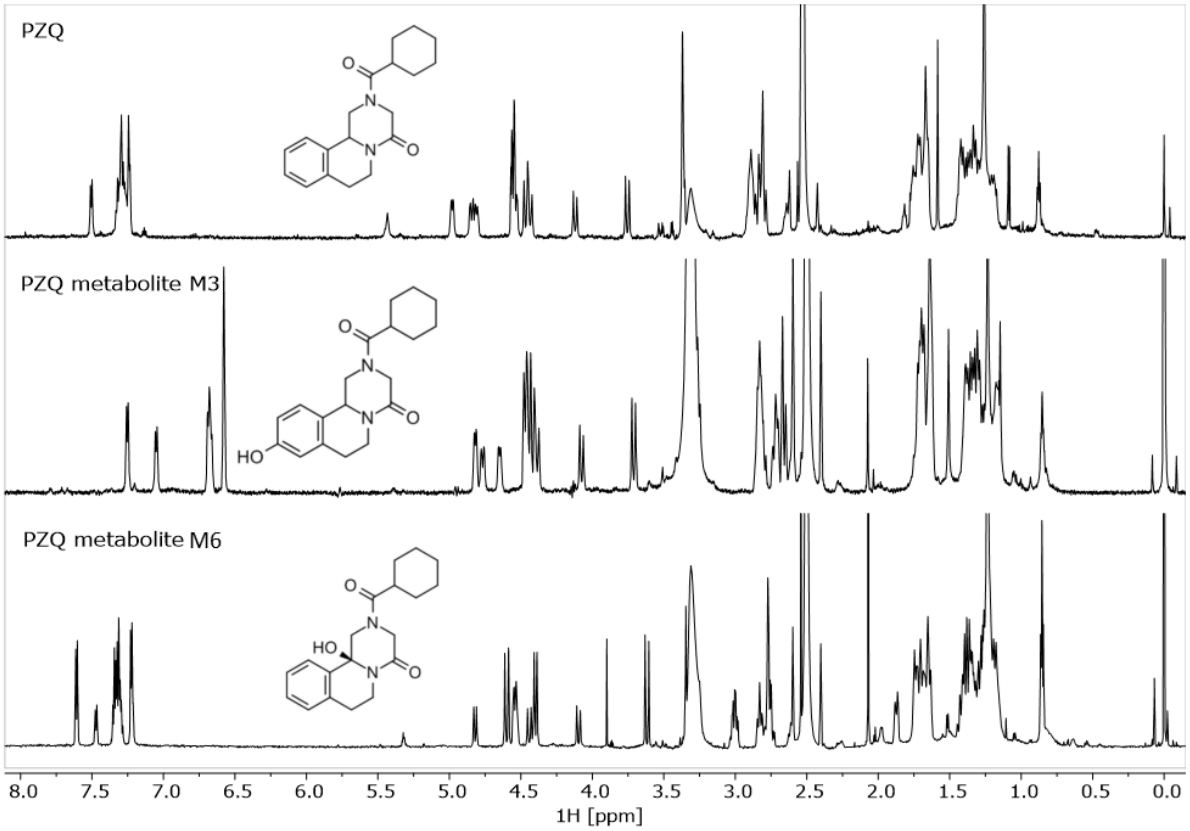


Supplemental Fig. 25. Edited homonuclear correlation spectroscopy (COSY) NMR spectrum of ***S*-9-hydroxy praziquantel** (M3)



Supplemental Fig. 26. Edited rotating frame Overhauser enhancement effect spectroscopy (ROESY) NMR spectrum of ***S*-9-hydroxy praziquantel** (M3). Blue: cross-peaks due to spatial proximity,

red: cross-peaks due to chemical exchange, i.e. interconversion of conformers



Supplemental Fig. 27. Comparison of ^{1}H NMR spectra of **praziquantel** (PZQ), **9-hydroxy praziquantel** (M3) and **11b-hydroxy praziquantel** (M6) (^{1}H NMR spectra of PZQ and M6 from Vendrell-Navarro *et al.*, 2020)

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