Supplemental Data

Manuscript full title

Insights into Praziquantel metabolism and potential enantiomeric CYP-mediated drug-drug interaction

Authors

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Nonstandard abbreviations

AIC, Akaike information criterion; CL_{int}, intrinsic clearance; $CL_{po,}$ oral clearance; CV, coefficient of variation; CYP, cytochrome P450; e.r., enantiomeric ratio; f_u, fraction unbound; hHeps, human hepatocytes; HLM, human liver microsomes; K_{dep}, first-order depletion constant; K_i, inhibition constant; K_m, Michaelis constant; NMR, Nuclear magnetic resonance spectroscopy; ODT, orally disintegrating tablet; P_{app}, apparent permeability; PBPK, physiologically based pharmacokinetic (modeling); PK, pharmacokinetics; PZQ, praziquantel; n, number of intra-assay replicates; N, number of inter-assay replicates; n.c., non converged; rhCYP, recombinant human CYP450; SD, standard deviation; V_{max}, maximum velocity of the metabolic reaction.

Figure S1. Assigned 1H NMR spectrum of the oxidized PZQ metabolite M6.

The double substituted amide functionality gives rise to two prominent conformers. Assignment is given for the higher populated conformer.

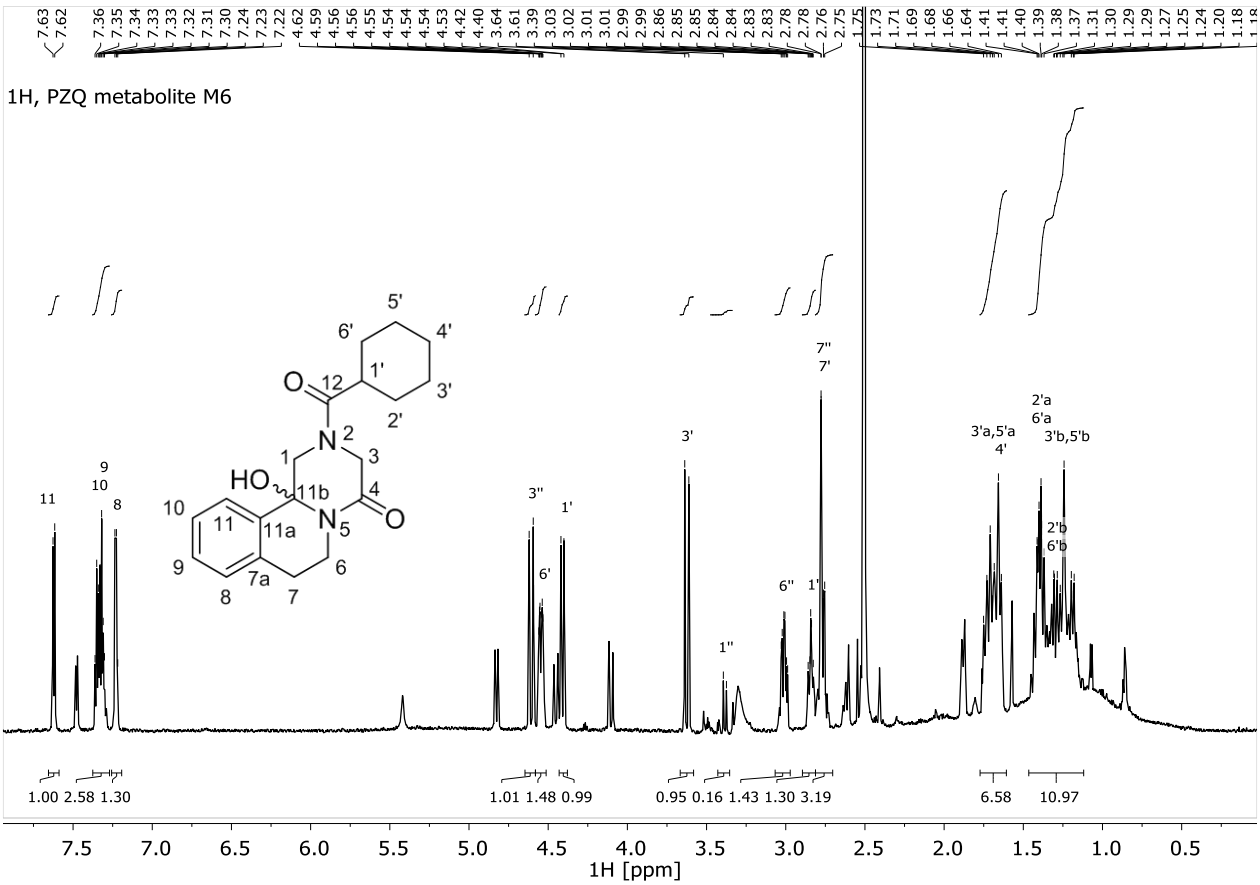


Figure S2. Edited HSQC NMR spectrum of oxidized PZQ metabolite M6.

Primary and tertiary carbon (CH and CH_3) are indicated in dark grey, secondary carbon (CH_2) in light grey.

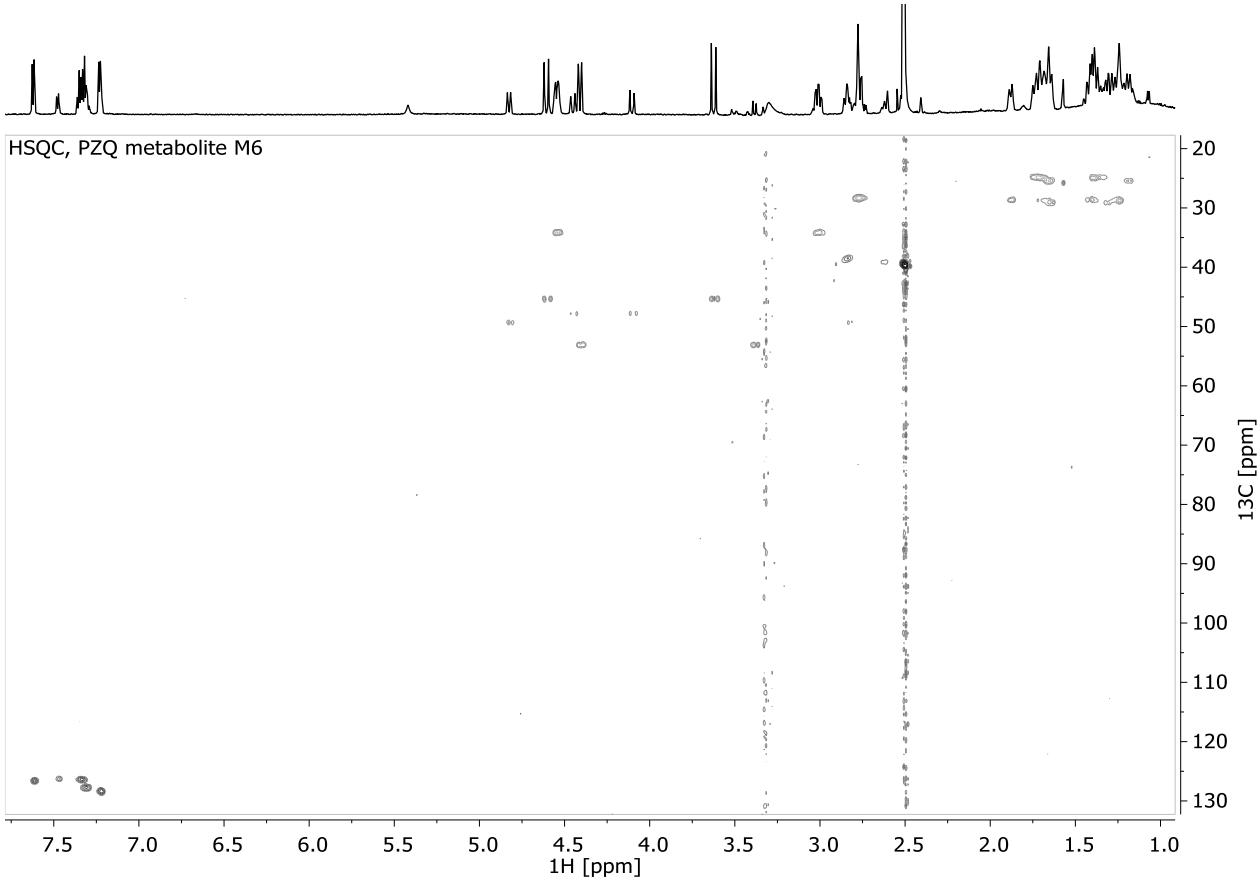


Figure S3. Edited COSY NMR spectrum of oxidized PZQ metabolite M6.

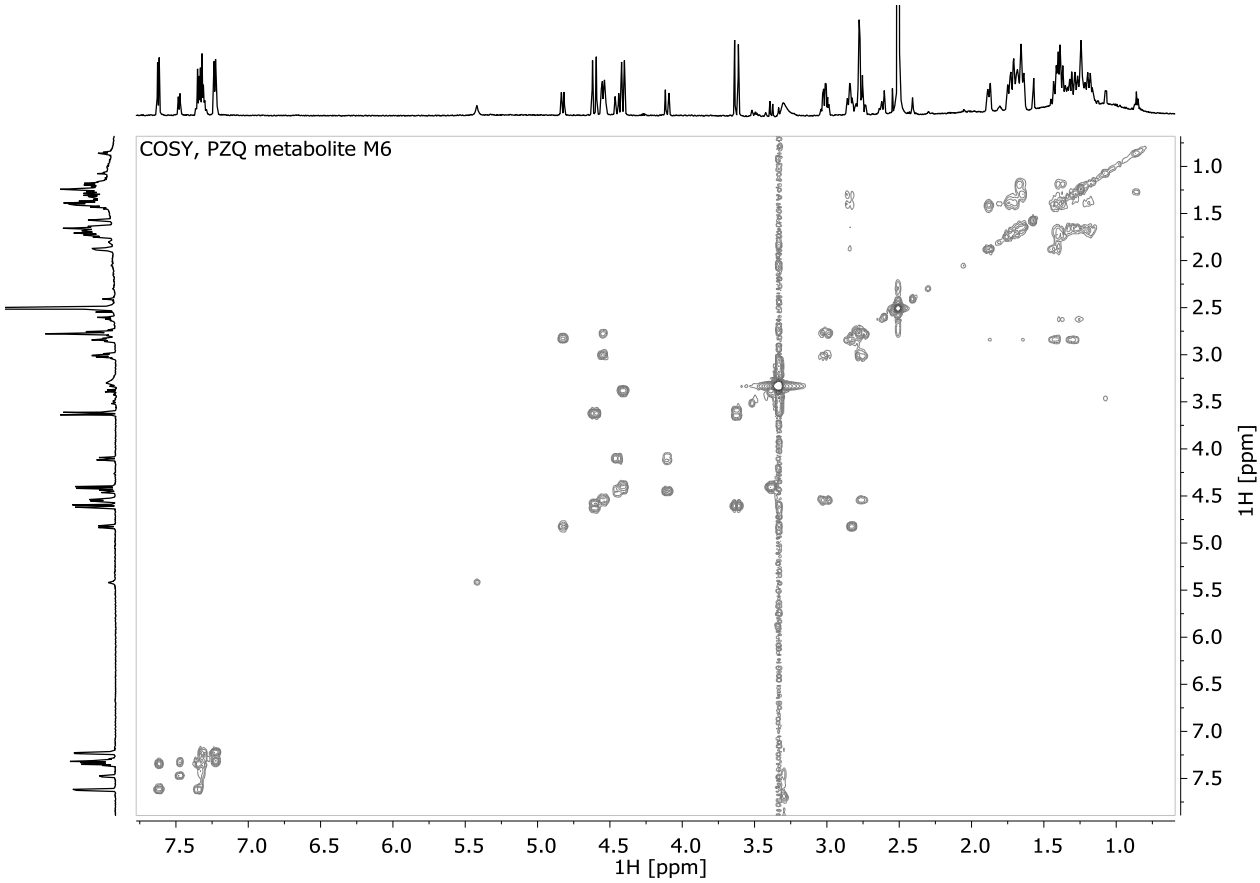


Figure S4. Comparison of 1H NMR spectra of M6 metabolites with parent PZQ.

Stacked plot of PZQ (top), oxidized *R*-PZQ metabolite R-M6 (center) and oxidized *S*-PZQ metabolite S-M6 (bottom) sample. The peaks indicated with asterisk are not related to M6. Additional peaks in S-M6 spectra compared to R-M6 are due to differences in the purification eluents and NMR solvents.

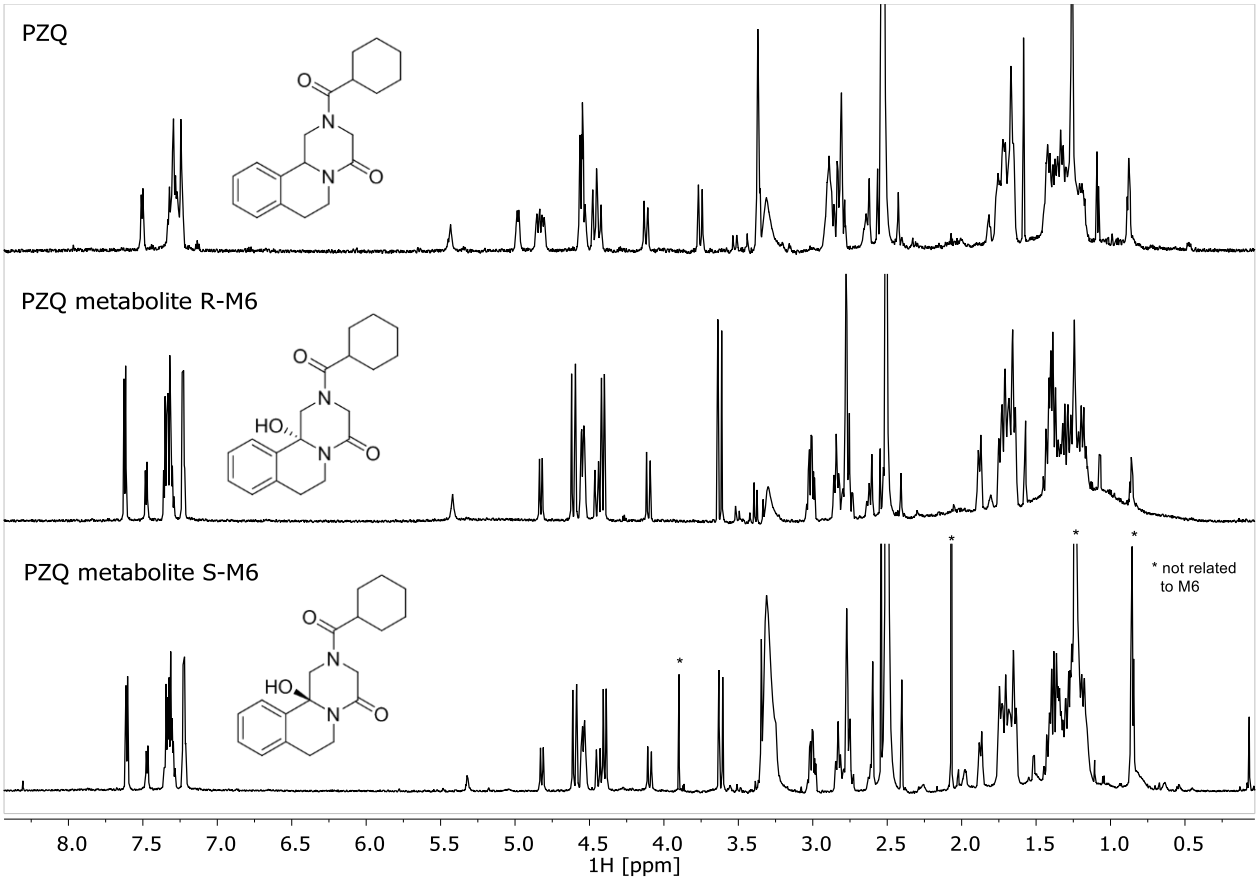


Table S1. Mass spectral analysis of *in vitro* mono-oxidized metabolites formed *in vitro* from R-PZQ

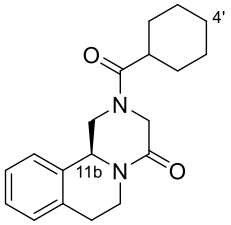
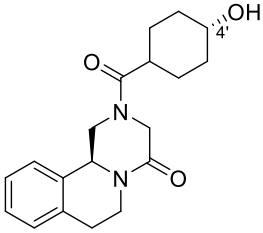
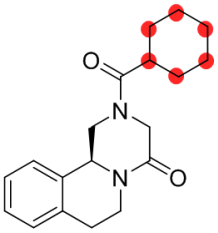
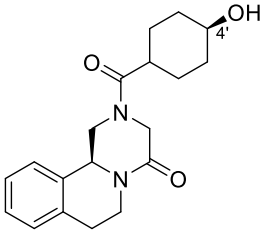
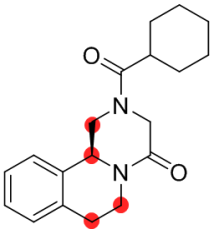
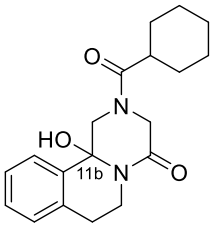
Substrate / Metabolite	Retention time [min]	Elemental Composition (ion formula)	$[M+H]^+$ [m/z]	Main fragment ions [m/z]	Structure proposal
R-PZQ	5.10	$[C_{19}H_{24}N_2O_2 + H]^+$	313.1916	203 201 174 132 105	
R-M2 (R-trans-4'-OH-PZQ)	3.15	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1867	311 203 174 146 132	
R-M3	3.37	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1864	311 203 174 132	
R-M4 (R-cis-4'-OH-PZQ)	3.40	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1869	311 203 174 132 105	
R-M5	3.99	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1879	219 190 148 130	
R-M6	4.45	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1916	311 219 201 199 174 173 132 105	

Table S2. Mass spectral analysis of mono-oxidized metabolites formed *in vitro* from S-PZQ

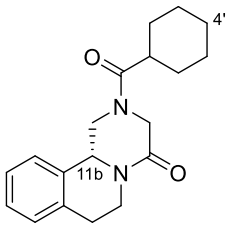
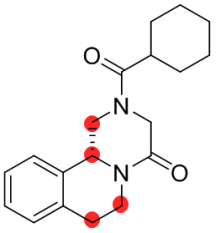
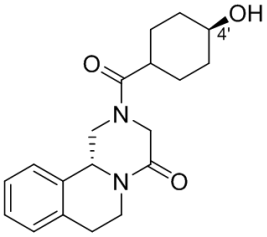
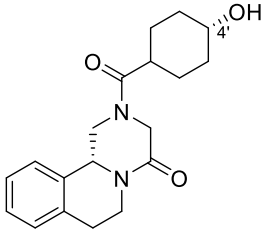
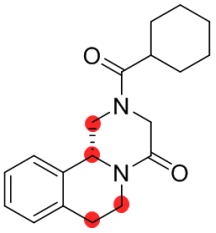
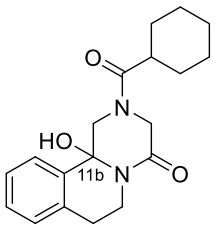
Substrate / Metabolite	Retention time [min]	Elemental Composition (ion formula)	$[M+H]^+$ [m/z]	Main fragment ions [m/z]	Structure proposal
S-PZQ	5.10	$[C_{19}H_{24}N_2O_2 + H]^+$	313.1931	203 201 184 174 132 103	
S-M1	2.93	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1870	219 201 132	
S-M2 (S-trans-4'-OH-PZQ)	3.15	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1885	311 203 184 174 146 132	
S-M4 (S-cis-4'-OH-PZQ)	3.40	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1872	311 203 174 146 132	
S-M5	3.99	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1871	219 201 190 148 130	
S-M6	4.45	$[C_{19}H_{24}N_2O_3 + H]^+$	329.1876	311 219 201 199 173 174 132 105	

Table S3. Clearance and formation rate of praziquantel enantiomers and its metabolites in hHeps.

Incubations performed at 5-10 µM (n=2). Mean and standard deviation of mean are given (N=2, unless indicated otherwise).

Substrate		Formation rate of products [pmol/min/10e6 cell]					
Substrate	Clint [µL/min/ 10e6 cells]	R-PZQ	S-PZQ	R-cis-4'-OH-PZQ	R-trans-4'-OH-PZQ	S-cis-4'-OH-PZQ	S-trans-4'-OH-PZQ
R-PZQ	19 ± 10 (N=6)	< 0.1	< 0.1	11 ± 2	9 ± 1	< 0.1	< 0.1
S-PZQ	16 ± 8 (N=6)	< 0.1	< 0.1	0.2 ± 0	< 0.1	4 ± 1	2 ± 0
R-cis-4'-OH-PZQ	8 ± 2	< 0.1	< 0.1	< 0.1	29 ± 7	< 0.1	< 0.1
R-trans-4'-OH-PZQ	1 ± 0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
S-cis-4'-OH-PZQ	3 ± 1	< 0.1	< 0.1	< 0.1	0.3 ± 0.1	< 0.1	6 ± 3
S-trans-4'-OH-PZQ	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

Table S4. *In vitro* clearance of PZQ enantiomers at 100% e.r. in incubations with recombinant CYP enzymes.

Incubations performed at 1 µM, i.e. for rac-PZQ this means a concentration of 0.5 µM of each PZQ enantiomer. Samples were analysed by reverse phase LC-MS/MS for enantiomers tested pure (100% e.r.), and using enantioselective LC-MS/MS for the racemate (50% e.r.) in order to measure the decline of each enantiomer in the mixture. Mean, standard deviation of mean are given (n=2, N=3,4). Values formatted in italic and gray are below the threshold of relevance (< 75% remaining compound after incubation time).

Test item	CL_{int} + SD [µL/min/pmol CYP]				
	CYP 1A1	CYP 1A2	CYP 2B6	CYP 2C8	CYP 2C9
R-PZQ	4.79 ± 0.06	1.67 ± 0.24	*0.06 ± 0.04*	*0.11 ± 0.02*	1.95 ± 0.25
S-PZQ	1.33 ± 0.21	0.17 ± 0.08	*0.02 ± 0.02*	*0.13 ± 0.02*	0.96 ± 0.11
rac-PZQ	2.35 ± 0.02	0.57 ± 0.11	*0.02 ± 0.02*	*0.14 ± 0.03*	1.34 ± 0.10
	CYP 2C19	CYP 2D6	CYP 3A4	CYP 3A5	CYP 3A7
R-PZQ	4.91 ± 0.77	0.45 ± 0.06	2.31 ± 0.20	1.22 ± 0.15	0.08 ± 0.03
S-PZQ	6.21 ± 1.05	0.17 ± 0.06	2.00 ± 0.36	1.71 ± 0.17	0.34 ± 0.03
rac-PZQ	5.10 ± 0.46	0.34 ± 0.05	2.19 ± 0.03	1.57 ± 0.11	0.14 ± 0.02

Table S5. Comparison of *in vitro* clearance mediated by recombinant CYP enzymes of PZQ enantiomers at 100% e.r. or 50% e.r.

Enantiomers were tested either separately (1.0 µM at 100% e.r.) or mixed as racemate (1.0 µM of racemate, 0.5 µM of each enantiomer at 50% e.r.). Mean, standard deviation of mean, and number or inter-assay replicates is given.

rhCYP Isoform	Test item	CL_{int} of 100% e.r. enantiomer at 1.0 µM and [µL/min/pmol CYP]		CL_{int} of racemate at 1.0 µM, i.e. 50% e.r. enantiomer at 0.5 µM [µL/min/pmol CYP]		Ratio CL_{int} at 50% e.r./ CL_{int} at 100% e.r.
		mean ± SD_{inter}	N	mean ± SD_{inter}	N	mean ± SD_{inter}
CYP 1A1	R-PZQ	4.79 ± 0.10	3	3.64 ± 0.49	3	0.8 ± 0.1
	S-PZQ	1.33 ± 0.36	3	0.64 ± 0.55	3	0.5 ± 0.5
CYP 1A2	R-PZQ	1.67 ± 0.42	3	1.82 ± 0.87	2	1.1 ± 0.6
	S-PZQ	0.17 ± 0.13	3	0.42 ± 0.31	3	2.5 ± 2.7
CYP 2C9	R-PZQ	1.95 ± 0.43	3	2.79 ± 1.56	3	1.4 ± 0.8
	S-PZQ	0.96 ± 0.19	3	1.47 ± 1.18	2	1.5 ± 1.2
CYP 2C19	R-PZQ	4.91 ± 1.33	3	5.02 ± 1.44	2	1.0 ± 0.4
	S-PZQ	6.21 ± 1.81	3	6.27 ± 2.43	2	1.0 ± 0.5
CYP 2D6	R-PZQ	0.45 ± 0.08	2	0.70 ± 0.12	2	1.6 ± 0.4
	S-PZQ	0.17 ± 0.08	2	0.37 ± 0.04	2	2.2 ± 1.1
CYP 3A4	R-PZQ	2.31 ± 0.28	2	2.10 ± 0.21	2	0.9 ± 0.1
	S-PZQ	2.00 ± 0.51	2	2.55 ± 0.35	2	1.3 ± 0.4
CYP 3A5	R-PZQ	1.22 ± 0.21	2	1.54 ± 1.11	3	1.3 ± 1.0
	S-PZQ	1.71 ± 0.23	2	1.74 ± 1.22	3	1.0 ± 0.7
CYP 3A7	R-PZQ	0.08 ± 0.06	5	0.11 ± 0.13	2	1.4 ± 1.9
	S-PZQ	0.34 ± 0.07	5	0.46 ± 0.20	3	1.4 ± 0.7

Figure S5. Correlation between the intrinsic Clearance of PZQ enantiomers at 100% e.r. (1.0 µM) or at 50% e.r. (0.5 µM of each enantiomer)

Incubations performed at 1 µM (i.e. rac-PZQ as mixture of 0.5 µM of each PZQ enantiomer). Mean and standard error of mean are represented (n=2, N=3,4). Both data from R-PZQ and S-PZQ are represented in the graph. For both enantiomers, CL_{int} values of the compound tested as racemate (0.5 µM as 50% e.r.) are similar (< 2-fold difference) to the CL_{int} values of the compound tested pure (1.0 µM as 100% e.r.).

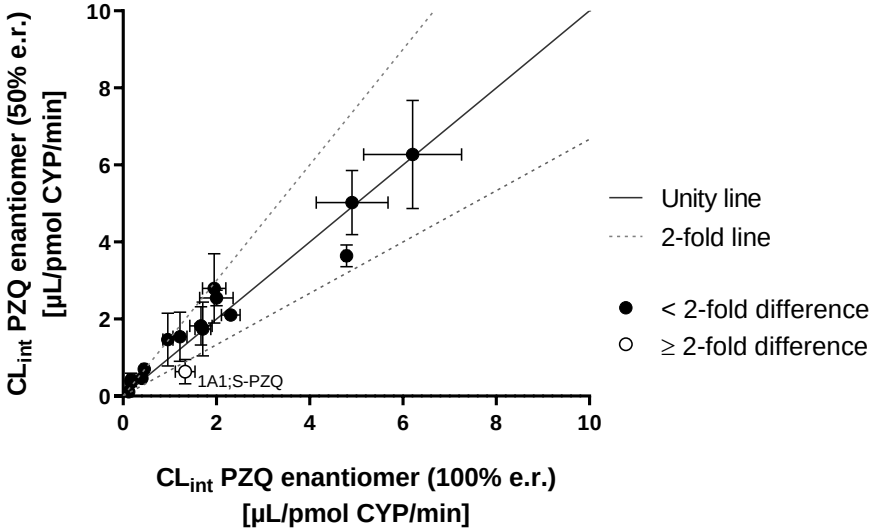


Table S6. Enzymatic kinetic constants of rhCYPs for PZQ enantiomers.

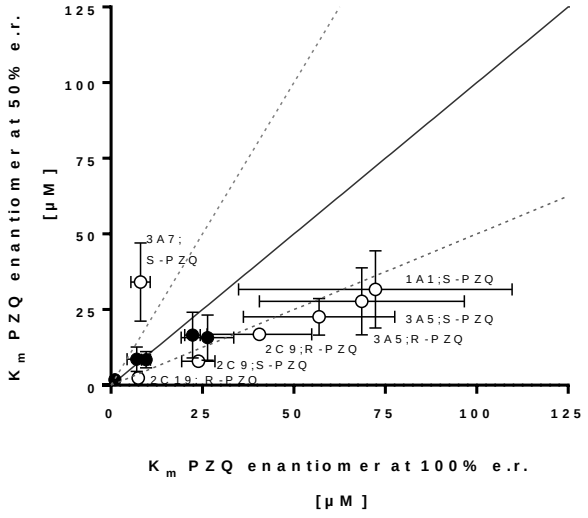
Affinity (Km value) and turnover rate (Vmax value) are given for PZQ enantiomers tested either enantiomerically pure (100% e.r.) or as racemate (50% e.r.). Inter-assay mean and standard deviation of mean is given (N= 3-4). Low clearance cases with an associated CV above 80% are described as not converged (n.c.) and labelled in gray and italic. For cases where only one replicate was performed, intra-assay standard deviation is indicated as SDintra.

rhCYP Isoform	Test item	**K_m enantiomer at 100% e.r. mean ± SD_{inter} [µM]**	**K_m enantiomer at 50% e.r. mean ± SD_{inter} [µM]**	**V_{max} enantiomer at 100% e.r mean ± SD_{inter} [pmol/pmol CYP/min]**	**V_{max} enantiomer at 50% e.r. mean ± SD_{inter} [pmol/pmol CYP/min]**
CYP 1A1	R-PZQ	9.6 ± 2.1	8.4 ± 4.7	45.6 ± 9.3	32.1 ± 17.7
	S-PZQ	*n.c. (72.3 ± 64.8)*	31.7 ± 22.2	80.2 ± 52.6	24.1 ± 13.5
CYP 1A2	R-PZQ	7.0 ± 4.6	8.6 ± 5.7	17.5 ± 11.8	19.1 ± 11.0
	S-PZQ	*n.c. (33.2 ± 56.4)*	*n.c. (44.0 ± 59.1)*	*n.c. (13.4 ± 22.8)*	*n.c. (22.4 ± 30.2)*
CYP 2C9	R-PZQ	40.6 ± 24.7	16.8 ± 0.0	130.0 ± 89.8	53.0 ± 12.4
	S-PZQ	23.9 ± 7.9	7.9 ± 2.6	36.9 ± 15.2	14.3 ± 6.9
CYP 2C19	R-PZQ	7.4 ± 1.4	2.4 ± 2.0	41.9 ± 9.0	14.7 ± 12.4
	S-PZQ	1.1 ± 0.1	1.8 ± 1.3	8.9 ± 0.6	16.1 ± 12.7
CYP 2D6	R-PZQ	*n.c. (32.7, SD_{intra} 30.2)*	*n.c. (15.2 ± 15.3)*	*n.c. (18.0, SD_{intra} 15.6)*	*n.c. (10.0 ± 8.6)*
	S-PZQ	*n.c. (204, SD_{intra} 399)*	*n.c. (24.0 ± 24.6)*	*n.c. (56.8, SD_{intra} 107.0)*	*n.c. (7.4 ± 6.2)*
CYP 3A4	R-PZQ	22.3 ± 3.7	16.6 ± 10.8	60.0 ± 13.7	38.2 ± 27.1
	S-PZQ	26.4 ± 12.5	15.7 ± 10.6	78.2 ± 41.9	44.0 ± 30.4
CYP 3A5	R-PZQ	56.9 ± 20.7	22.6 ± 10.5	92.1 ± 39.6	49.6 ± 25.5
	S-PZQ	68.6 ± 28.0	27.7 ± 19.2	127.0 ± 48.9	68.6 ± 47.0
CYP 3A7	R-PZQ	*n.c. (0.8, SD_{intra} 0.8)*	*n.c. (30.1, SD_{intra} 38.5)*	*n.c. (0.2, SD_{intra} 0.2)*	*n.c. (4.2, SD_{intra} 5.0)*
	S-PZQ	8.1, SD_{intra} 3.7	34.1, SD_{intra} 18.3	4.5, SD_{intra} 1.9	18.1, SD_{intra} 9.2

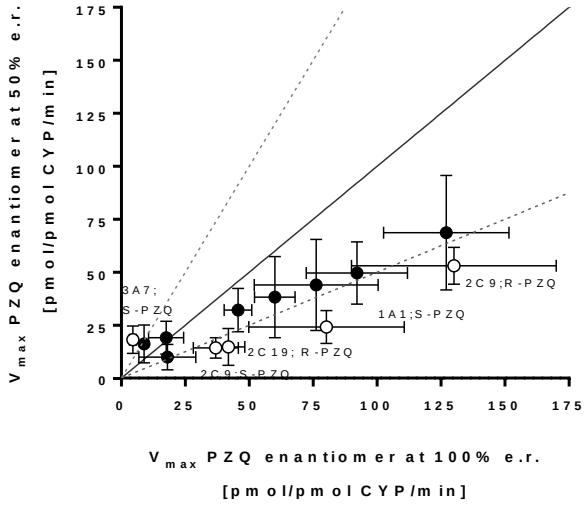
Figure S6. Correlation between Michaelis-Menten parameters for PZQ enantiomers at 100% or at 50% e.r..

Low clearance cases which resulted in high deviations (CV above 80%) were excluded (i.e. S-PZQ for CYP 1A2, R,S-PZQ for CYP 2D6, R-PZQ for 3A7). Mean and standard deviation of mean are represented. Unity line (continuous) and 2-fold difference line (dashed) represented. Cases showing a > 2-fold difference are formatted as empty circle and labelled with CYP isoform and PZQ enantiomer.

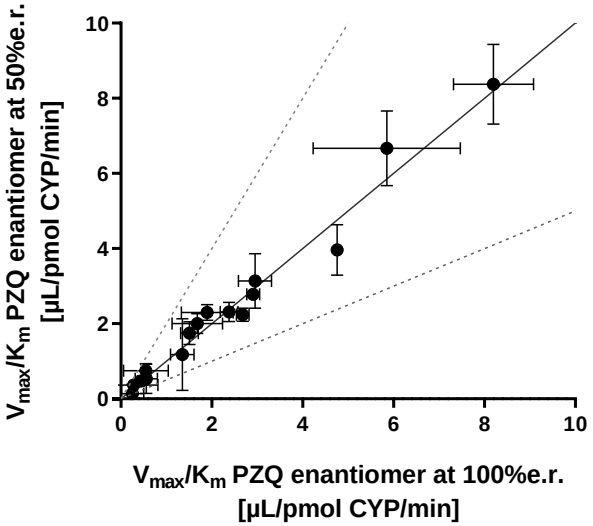
a) K_m at 100% or 50 % e.r.



b) V_{max} at 100% or 50 % e.r.



c) V_{max}/K_m at 100% or 50 % e.r.



d) V_{max}/K_m vs Clint (1 µM) at 100% e.r.

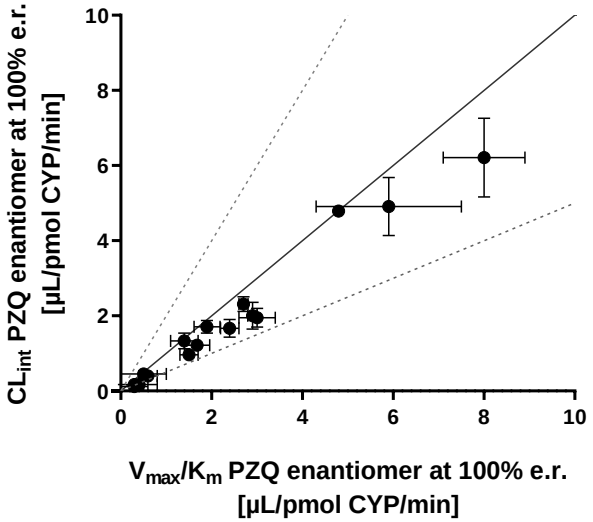


Table S7. Definition of parameters used in kinetic equations.

Magnitude	Abbreviation	Unit	Additional specifications	Equation reference
Compound concentration	S	µM	Substrate (general)	1, 3, 5, 7
	R_PZQ, S_PZQ	µM	Differentiation of each PZQ enantiomer	10, 11
	I	µM	Inhibitor (general)	4-10
CYP concentration	C_{rhCYP}	pmol CYP/µL	At single concentration	2
		nmol CYP/L	Unit option to calculate Michaelis-Menten Vmax	
Time	t	min		1, 10, 11
Depletion constant	k_{dep}	min^{-1}		1, 2, 3, 5, 7-11
Intrinsic clearance	CL_{int}	µL/min/pmol CYP		2
Michaelis Menten constant	K_m	µM		3-5, 7, 9
	$K_{m,obs}$	µM	Observed Km, competitive inhibition	4
	$K_{m,app}$	µM	Km under linear-mixed inhibition	
Maximum depletion rate not normalized to rhCYP	ν_{max}	µmol substrate/L/min		3, 5, 6-8
	$\nu_{max,inh}$	µmol substrate/L/min	ν_{max} under non-competitive inhibition	6, 7
	$\nu_{max,app}$	µmol substrate/L/min	ν_{max} under linear-mixed inhibition	
Michaelis Menten maximum rate	V_{max}		Normalized to C_{rhCYP}	
Inhibition constant	K_i	µM		
Inhibitory alpha constant	α	dimensionless		8, 9

Table S8. Equations for the dynamic non-linear models

R_PZQ and S_PZQ are respectively the time-dependent concentrations of the R-PZQ and S-enantiomer of PZQ.

Equations used in the competitive model	
$\frac{d\ S_PZQ}{dt} = -v_{max,\ S_PZQ} * \frac{S_PZQ}{k_{m,\ obs,S_PZQ}+S_PZQ}$	**(1)**
$\frac{d\ R_PZQ}{dt} = -v_{max,\ R_PZQ} * \frac{R_PZQ}{k_{m,\ obs,R_PZQ}+R_PZQ}$	**(2)**
$k_{m,obs,S_PZQ} = K_{m,\ S_PZQ} * \left(1 + \frac{R_PZQ}{K_{i,R_PZQ}}\right)$	**(3)**
$k_{m,obs,R_PZQ} = K_{m,\ R_PZQ} * \left(1 + \frac{S_PZQ}{K_{i,S_PZQ}}\right)$	**(4)**
Equations used in the non-competitive model	
$\frac{d\ S_PZQ}{dt} = -v_{max,inh,\ S_PZQ} * \frac{S_PZQ}{k_{m,\ S_PZQ}+S_PZQ}$	**(5)**
$\frac{d\ R_PZQ}{dt} = -v_{max,inh,\ R_PZQ} * \frac{R_PZQ}{k_{m,,R_PZQ}+R_PZQ}$	**(6)**
$v_{max,inh\ S_PZQ} = v_{max,\ S_PZQ} \Big/ \left(1 + \frac{R_PZQ}{K_{i,R_PZQ}}\right)$	**(7)**
$v_{max,inh\ R_PZQ} = v_{max,\ R_PZQ} \Big/ \left(1 + \frac{S_PZQ}{K_{i,S_PZQ}}\right)$	**(8)**

Table S9. Interaction kinetics based on linear static models

Kinetic values K_m, V_{max} and K_i are given as inter-assay mean values with standard deviation (mean ± SD_{inter}). Akaike information criterion (AIC) values expressed as the relative quality of competitive model over the non-competitive model in %. R-squared statistical value informs on how close the data for this PZQ enantiomer fits to a competitive inhibitor (R2 as competitor). Min-max range of AIC and R2 values are reported (N=2-3). Ratios of kinetic values are calculated using mean of corresponding values.

CYP	**Test item**	**K_m** [µM]	**V_{max}** [pmol/pmol CYP/min]	**K_i** [µM]	**Probability of competitive model by AIC** [%]	**R2 as competitor**	**Ratio K_m 100% e.r./ static model** (mean)	**Ratio V_{max} 100% e.r./ static model** (mean)	**Ratio $K_{i,R}/K_{i,S}$**
1A1	R-PZQ	7.8 ± 1.6	31 ± 3	68 ± 7	40 – 54	0.55 – 0.57	0.8	0.7	-
	S-PZQ	*n.c.*	*n.c.*	29 ± 0	59 – 97	0.63 – 0.93	-	-	
2C9	R-PZQ	46 ± 10	144 ± 13	33 ± 15	77 – 85	0.73 – 0.80	1.1	1.1	0.9
	S-PZQ	24 ± 3	34 ± 7	36 ± 19	50 – 100	0.70 – 0.96	0.9	0.9	
2C19	R-PZQ	8.4 ± 1.0	69 ± 20	6.9 ± 0.6	73 – 98	0.92 – 0.95	1.1	1.6	2.3
	S-PZQ	2.7 ± 0.6	23 ± 4	3.0 ± 1.1	76 – 77	0.88 – 0.96	2.4	2.6	
3A4	R-PZQ	30 ± 20	51 ± 34	29 ± 10	91 – 93	0.92 – 0.94	1.3	0.8	1.4
	S-PZQ	24 ± 6	63 ± 6	21 ± 8	93 – 99	0.83 – 0.86	0.9	0.8	
3A5	R-PZQ	20 ± 4	34 ± 6	39 ± 24	86 – 100	0.66 – 0.92	0.3	0.4	1.4
	S-PZQ	24 ± 2	47 ± 6	27 ± 4	61 – 91	0.83 – 0.95	0.4	0.4	
3A7	R-PZQ	*n.c.*	*n.c.*	147	-	-	-	-	-
	S-PZQ	209	97	*n.c.*	-	-	-	-	-

Table S10. Interaction kinetics based on non-linear dynamic model.

Low clearance cases with an associated CV above 80% are described as not converged (n.c.) and labelled in gray and italic.

rhCYP isoform	Test item	K_m [µM]	V_{max} [pmol/pmol CYP/min]	K_i [µM]	Ratio K_m 100% er/ dynamic model	Ratio V_{max} 100% er/ dynamic model	Ratio $K_{i,R}/K_{i,S}$
1A1	R-PZQ	6.8 ± 4.3	25 ± 6	23 ± 7	0.7	0.5	0.9
	S-PZQ	25 ± 8	35 ± 27	25 ± 3	0.3	0.4	
2C9	R-PZQ	30 ± 3	77 ± 8	31 ± 8	0.7	0.6	1.4
	S-PZQ	16 ± 4	20 ± 6	22 ± 5	0.7	0.5	
2C19	R-PZQ	6.4 ± 1.5	40 ± 5	9.4 ± 0.4	0.9	0.9	3.9
	S-PZQ	2.8 ± 0.4	17 ± 1	2.4 ± 0.7	2.5	2.0	
3A4	R-PZQ	25 ± 14	42 ± 28	18 ± 6	1.1	0.7	0.9
	S-PZQ	24 ± 8	36 ± 14	21 ± 2	0.9	0.5	
3A5	R-PZQ	22 ± 1	28 ± 1	27 ± 6	0.4	0.3	1.7
	S-PZQ	19 ± 5	32 ± 4	15 ± 1	0.3	0.3	
3A7	R-PZQ	*n.c.*	*n.c.*	153			-
	S-PZQ	178	75	*n.c.*			

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