Binding site interactions of modulators of Breast Cancer Resistance Protein, Multidrug Resistance Associated Protein 2 and P-glycoprotein activity

Feng Deng, Leo Ghemtio, Evgeni Grazhdankin, Peter Wipf, Henri Xhaard, Heidi Kidron

SUPPORTING INFORMATION

SUPPORTING INFORMATION 1

Homology modelling protocol

MODELLER (v9.18) (Šali and Blundell, 1993) was used as the homology modelling program.

Alignment

MRP2

Sequences and their alignment were fetched from Ensembl database (release 89) (Zerbino *et al.*, 2018). To include all the MRP genes of interest (MRP1 cow and human; MRP2 human, MRP3 human) we took the deepest node from the genetic tree containing all these.

Selection returned around 200 sequences. The tree was opened in Jalview (Waterhouse *et al.*, 2009) and a few outliers were removed manually, totalling in 196 sequences.

Correspondence of the used Uniprot entries (Bateman *et al.*, 2017) to Ensembl is shown in Table S1 for convenience.

Table S1: Mapping of Uniprot entries to Ensembl database.

Uniprot entry	***Ensembl entry***
MRP1_HUMAN	ENSP00000382342_Hsap
MRP2_HUMAN	ENSP00000359478_Hsap
MRP3_HUMAN	ENSP00000285238_Hsap
MRP1_BOVIN	ENSBTAP00000028094_Btau

P-gp

Sequences of pdb ids 3g5u, 4q9k, 5ko2 were aligned to MDR1_HUMAN (from Uniprot) with Clustal Omega (v.1.2.4) (Sievers *et al.*, 2011).

Modelling

MRP2

Template: 5uja (pdb id).

Model sequence: ENSP00000359478_Hsap, corresponding to MRP2_HUMAN.

Alignment: ENSEMBL_ABCC1_cow__ABCC2_human.ali with one residue exchanged (V to M at position 986 by MODELLER counting, in ENSBTAP00000028094_Btau corresponding to 5uja). Sequence identity: 55.4% (5uja)."

Modelling: Slow refinement, 200 models.

Assessment: The best model was selected by the global DOPE score.

MODELLER restraint violations were mostly few, except for the Phi/Psi pair group, which had around 20 violations. However, since there was approximately the same number of violations in this group when modelling self (5uja to 5uja), this should be acceptable.

P-gp

Templates: A-chains of 3g5u, 4q9k, 5ko2 (pdb ids).

Model sequence: MDR1_HUMAN (from Uniprot).

Alignment: MDR1_HUMAN__5ko2_3g5u_4q9k.ali. Sequence identities at 89.1% (3g5u), 88.8% (4q9k), 88.9% (5ko2).

Modelling: Standard protocol, 100 models.

Assessment: The best model was selected by the global DOPE score.

Restraint violations were comparable to self on individual templates (tested with 5ko2).

References

Bateman, A. *et al.* (2017) 'UniProt: the universal protein knowledgebase', *Nucleic Acids Research*. Oxford University Press, 45(D1), pp. D158–D169. doi: 10.1093/nar/gkw1099.

Šali, A. and Blundell, T. L. (1993) 'Comparative Protein Modelling by Satisfaction of Spatial Restraints', *Journal of Molecular Biology*, 234(3), pp. 779–815. doi: https://doi.org/10.1006/jmbi.1993.1626.

Sievers, F. *et al.* (2011) 'Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega.', *Molecular systems biology*. EMBO Press, 7(1), p. 539. doi: 10.1038/msb.2011.75.

Waterhouse, A. M. *et al.* (2009) 'Jalview Version 2—a multiple sequence alignment editor and analysis workbench', *Bioinformatics*, 25(9), pp. 1189–1191. doi: 10.1093/bioinformatics/btp033.

Zerbino, D. R. *et al.* (2018) 'Ensembl 2018', *Nucleic Acids Research*. Oxford University Press, 46(D1), pp. D754–D761. doi: 10.1093/nar/gkx1098.

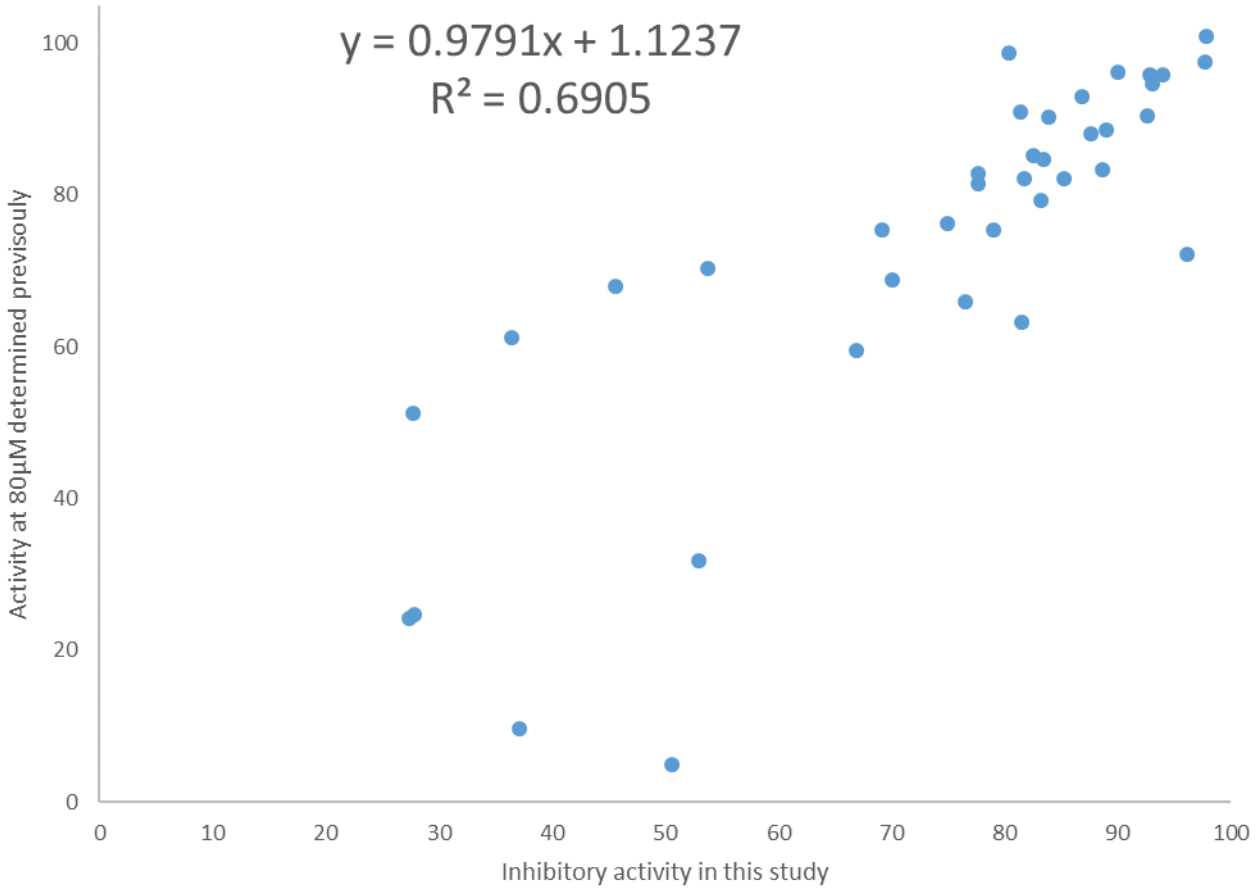
SUPPORTING INFORMATION 2

The relative inhibitory activity and the docking score of tested compounds against each transporter: 100% inhibition denotes full inhibition, 0% no inhibition and negative value indicates stimulation of probe transport. The binding affinity between the compound and the transporter are described with a docking score, where a low score indicates high affinity of the compound to the protein. SBC stands for substrate-binding cavity and NBD for nucleotide-binding domain.

	BCRP			MRP2			P-gp		
	Inhibition % (sd)	SBC score	NBD score	Inhibition % (sd)	SBC score	NBD score	Inhibition % (sd)	SBC score	NBD score
Scaffold 1									
1a	69.2 (2.8)	-7.3	-4.3	84 (2)	-7.0	-3.1	43.9 (6.9)	-6.3	-2.9
1aa	98.3 (0.2)	-7.0	-2.1	97.7 (2.6)	-5.2	-2.8	13.8 (13.2)	-6.6	-3.6
1ac	97.7 (0.3)	-7.7	-3.6	90 (1.9)	-5.5	-3.2	-33.1 (19.6)	-3.8	-3.2
1ad	92.9 (2.4)	-8.4	-0.9	50.6 (7.1)	-3.5	-4.9	67.6 (3.4)	-8.4	-2.7
1ag	88.3 (0.7)	-9.3	-1.2	53 (9.3)	-4.8	-5.2	10.1 (8.4)	-9.7	-2.9
1b	99 (0.2)	-7.5	-2.0	81.5 (3.5)	-5.2	-5.6	93.3 (2.7)	-7.8	-2.8
1d	95 (0.4)	-8.3	-1.7	66.9 (7.8)	-5.5	-6.2	80.8 (3)	-9.4	-3.0
1e	84.3 (1.4)	-7.7	-3.4	89 (3.6)	-7.5	-3.0	37.6 (9.4)	-6.6	-2.8
1f	99.1 (0.4)	-7.9	-1.9	76.5 (4)	-4.7	-3.5	91.2 (2.6)	-8.1	-2.4
1g	99.5 (0.5)	-8.3	-1.7	83.5 (4.6)	-3.9	-3.1	89.6 (2.1)	-7.7	-2.9
1i	99.6 (0.4)	-6.9	-0.3	79 (6.8)	-4.5	-3.0	90 (3.2)	-6.6	-3.4
1j	98.3 (0.5)	-9.0	-2.2	82.5 (2.5)	-5.8	-5.6	36.6 (8)	-8.4	-2.8
1k	98.4 (0.2)	-7.0	-1.5	70 (4.7)	-4.8	-3.9	48.1 (9.2)	-7.0	-3.5
1l	98.5 (0.3)	-7.4	-3.3	93.9 (1.3)	-4.9	-5.1	8.4 (13.9)	-7.2	-3.0
1q	98.5 (0.1)	-8.9	-2.1	93.1 (2)	-3.6	-3.1	27.8 (4.5)	-7.2	-2.6
Scaffold 2A									
2ab	97.6 (0.6)	-8.9	-0.9	5.4 (23.9)	-6.6	-5.8	86.9 (2.5)	-9.3	-3.0
2g	98 (0.2)	-8.3	-1.0	36.4 (13.2)	-6.0	-6.1	98.5 (1.6)	-8.5	-3.0
2h	98.2 (0.1)	-8.7	-2.8	27.8 (17)	-5.1	-4.7	58.4 (2.7)	-8.3	-2.5
Scaffold 2B									
2ac	91.7 (0.6)	-7.6	-2.3	77.7 (4.4)	-7.6	-5.1	18.9 (6.5)	-8.1	-3.5
2ad	90 (0.4)	-6.6	-4.6	80.4 (3.7)	-10.9	-6.2	14.7 (5.7)	-8.5	-4.0
2ae	92.8 (0.6)	-7.6	-5.0	88.6 (2.2)	-8.5	-4.8	42.3 (5.9)	-7.2	-4.4
2af	15.2 (3.2)	-6.1	-4.4	27.4 (14.6)	-8.2	-5.3	-11.7 (7.4)	-6.2	-4.3
2ag	69.1 (0.8)	-9.0	-1.8	-3.1 (19.8)	-8.4	-5.2	-62.5 (12.9)	-7.7	-3.6
2ah	95.5 (1)	-8.3	-3.6	83.3 (5.5)	-9.9	-6.9	-45.5 (9.3)	-8.6	-4.1
2al	96.9 (0.3)	-8.2	-3.7	92.9 (1.8)	-10.0	-5.9	34.1 (4.8)	-8.1	-4.2
2i	58.2 (4.6)	-6.9	-4.4	27.8 (25.4)	-7.0	-6.9	-2.2 (13.7)	-7.9	-3.8
Scaffold 2C									
2an	82.6 (1.3)	-7.3	-3.3	25.4 (14.1)	-9.3	-6.2	21.1 (13.6)	-7.1	-4.1
2ao	60.8 (1.7)	-6.9	-4.9	22.2 (22.1)	-8.9	-5.3	-34.5 (14.4)	-7.6	-3.4
2au	95.5 (0.2)	-9.9	-5.0	81.7 (4.2)	-11.1	-6.5	62.3 (2.2)	-9.6	-3.3
2av	96.8 (0.3)	-8.9	-3.7	85.2 (3.9)	-9.8	-6.1	70.3 (3.3)	-9.4	-3.1
2aw	97.8 (0.1)	-8.9	-4.2	97.8 (0.7)	-9.6	-4.2	95.9 (1.5)	-9.1	-3.9
2ax	98.8 (1)	-9.0	-4.2	86.9 (2)	-10.2	-6.7	75.7 (4)	-8.6	-4.1
2az	94.7 (0.5)	-9.2	-3.6	77.7 (4.7)	-10.5	-7.4	66.7 (5.5)	-9.1	-4.0
2bb	99.5 (0.3)	-9.9	-4.7	96.2 (1.7)	-10.4	-5.7	84.8 (1.4)	-9.4	-3.3
2o	94.1 (0.5)	-7.8	-4.1	69.2 (5.9)	-6.8	-7.5	27.8 (12.5)	-8.3	-4.0
2p	84.4 (1.7)	-8.5	-3.1	26 (25.9)	-9.0	-4.7	10.2 (10.4)	-7.8	-4.9
Scaffold 3									
3aa	96.9 (0.3)	-8.3	-1.0	53.7 (13.1)	-5.0	-5.6	-87.9 (25.1)	-7.7	-3.0
3ab	98.3 (0.9)	-8.4	-2.9	45.6 (12.5)	-6.4	-7.7	-4.1 (10.8)	-8.8	-4.0
3ac	100.2 (-0.3)	-9.9	-1.9	92.6 (2.4)	-6.8	-4.7	68.2 (2.7)	-9.7	-4.4
3ad	99.3 (0.5)	-9.9	-1.2	87.6 (8.6)	-6.6	-4.8	77.1 (4)	-10.6	-4.8
3ag	85 (0.3)	-10.3	-1.5	37.1 (13.8)	-5.2	-6.5	40.5 (2.8)	-10.0	-3.3
3b	95.4 (0.8)	-7.3	-5.0	74.9 (4.6)	-4.5	-6.0	50.4 (3.5)	-7.3	-2.4
3c	85.9 (1.5)	-7.7	-1.5	11 (20)	-5.6	-4.6	-98 (21.1)	-8.5	-2.8
3j	99.5 (0.1)	-8.5	-1.9	81.4 (5)	-5.1	-6.5	-1.1 (25.4)	-9.2	-3.2

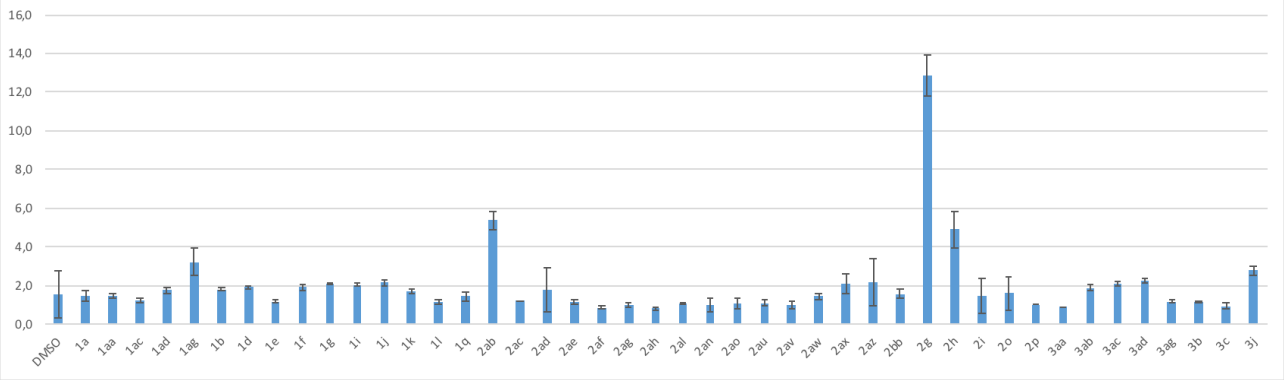
SUPPORTING INFORMATION 3

The relationship between two independent MRP2 experiments. The previous inhibitory activity (Y-axis, %) (Wissel et al., 2017; Wissel et al., 2015) and the current MRP2 inhibition data (X-axis, %) at 80µM test compound concentration.



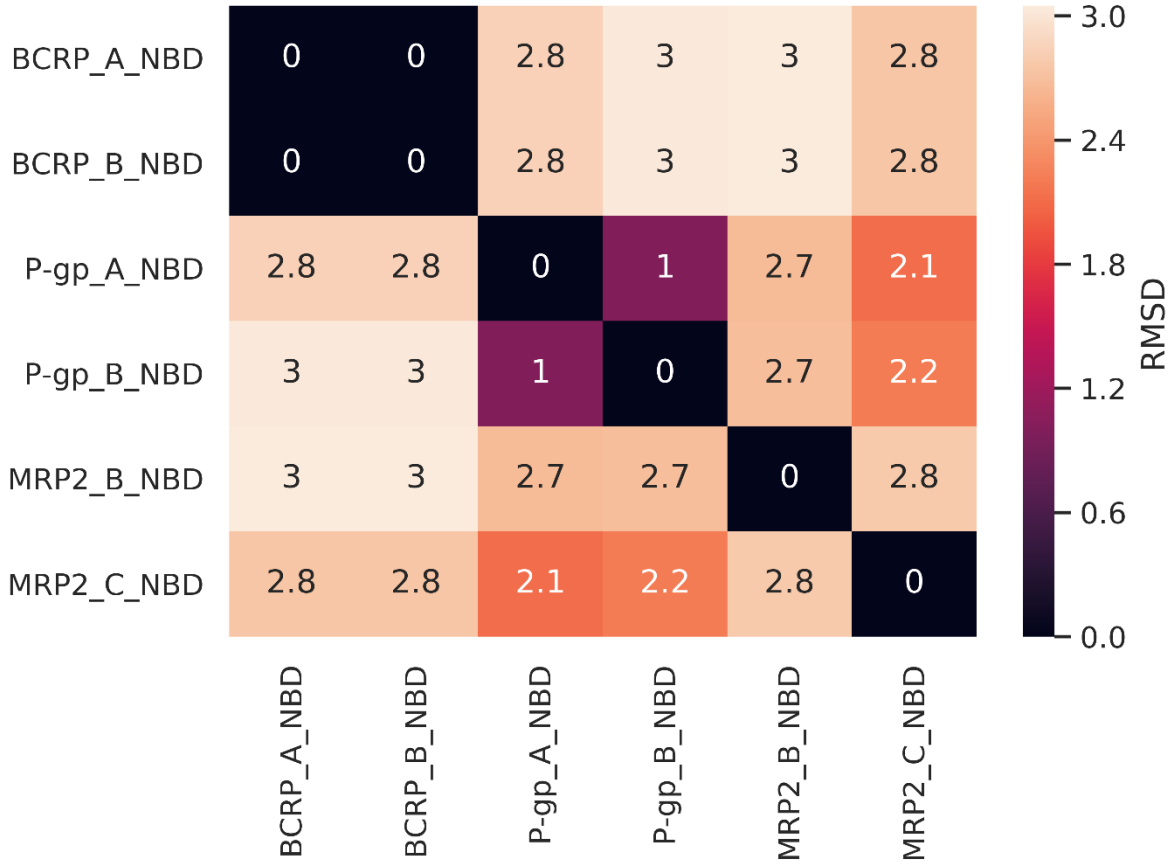
SUPPORTING INFORMATION 4

Interference by the aggregation of studied compounds in the assay was evaluated with a Nepheloskan Ascent nephelometer (Thermo Fisher Scientific, USA) with a lamp voltage of 10 and photomultiplier tube voltage of 250. In the aggregation control test, the turbidity by light scattering of vesicular transport assay solution is expressed in RNUs (relative nephelometry units). The samples contained 80µM of test compound, and were measured in triplicates. Only DMSO was added to the assay solution in the control.



SUPPORTING INFORMATION 5

RMSD value matrix for nucleotide-binding domains of BCRP (PDB ID 5NJ3), P-gp and MRP2 models. NDBs were structurally aligned with TM-align (Zhang and Skolnick 2005). The figure was generated using Matplotlib (v3.1, Hunter 2007) and Seaborn (v0.9, Waskom).



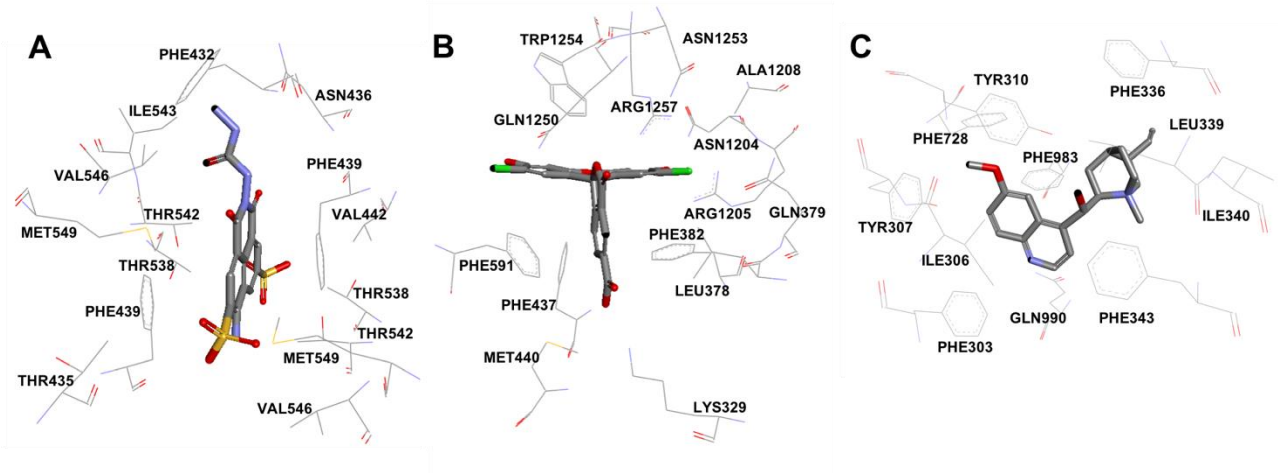
References

Hunter, J.D, 2007. Matplotlib: A 2D Graphics Environment. Computing in Science & Engineering, 9, 90–95.

Waskom, M. seaborn. Available at http://seaborn.pydata.org (accessed 04.02.2020)

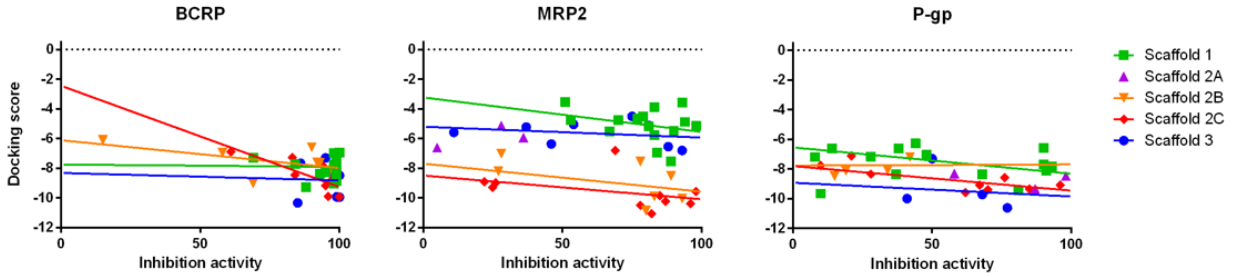
SUPPORTING INFORMATION 6

Visualization of substrates used in the in vitro assays docked to the SBC of the three transporters. A) BCRP and Lucifer Yellow, B) MRP2 and CDCF and C) P-gp and NMQ.



SUPPORTING INFORMATION 7

The relationship between inhibitory activity and SBC docking score illustrated in XY-plots. In the plots, compounds in different scaffolds are marked with distinctive color and shape: scaffold 1 (green square), scaffold 2A (purple ascending triangle), scaffold 2B (orange descending triangle), scaffold 2C (red diamond) and scaffold 3 (blue circle). Below the plots, the GraphPad Prism determined coefficient of determination (R^2) and p-value whether the slope of fit is significantly non-zero are presented. Linear fit for scaffold 2A was not attempted due to small number of data points. Stimulators are not visualized in the plots, but they were taken into consideration in the linear regression.



	R^2	p		R^2	p		R^2	p	
Scaffold 1	0.000	0.949		0.086	0.289		0.228	0.072	
Scaffold 2B	0.305	0.156		0.271	0.186		0.002	0.927	
Scaffold 2C	0.641	0.005	*	0.169	0.238		0.625	0.007	*
Scaffold 3	0.001	0.950		0.063	0.548		0.306	0.155	

SUPPORTING INFORMATION 8

Correlation of in vitro activity and number of interactions of certain nature within compound scaffolds. The type of interaction is denoted on the top of column. Slope describes the slope of linear regression between activity and number of interactions, R^2 goodness of the fit and p-value whether the slope deviates significantly from zero. The values highlighted with red color indicates statistically significant correlation.

BCRP

Scaffold 1	Acceptor	Aromatic	Backbone	Contact	Donor	Hydrophobic	Polar	Sidechain
slope	-0.007	-0.030	0.005	0.026		-0.016	0.038	0.022
R2	0.022	0.100	0.002	0.035		0.010	0.376	0.022
p-value	0.599	0.252	0.875	0.501		0.725	0.015	0.596
Scaffold 2B								
slope		-0.010	0.008	-0.002		-0.010	0.008	-0.002
R2		0.272	0.031	0.008		0.146	0.192	0.006
p-value		0.185	0.679	0.837		0.351	0.278	0.855
Scaffold 2C								
slope		-0.020	0.007	-0.051	-0.049	-0.072	-0.002	-0.074
R2		0.129	0.007	0.169	0.694	0.302	0.001	0.253
p-value		0.309	0.817	0.238	0.003	0.100	0.947	0.138
Scaffold 3								
slope	0.012	0.008	0.104	-0.038		-0.062	0.023	-0.038
R2	0.023	0.008	0.258	0.008		0.056	0.014	0.008
p-value	0.720	0.836	0.199	0.831		0.572	0.780	0.831

MRP2

Scaffold 1	Acceptor	Aromatic	Backbone	Charged	Contact	Donor	Hydrophobic	Polar	Sidechain
slope	0.004	-0.017	-0.027	0.002	-0.095	0.011	-0.039	-0.053	-0.092
R2	0.043	0.057	0.045	0.001	0.325	0.044	0.133	0.134	0.422
p-value	0.457	0.393	0.450	0.930	0.026	0.455	0.181	0.180	0.009
Scaffold 2B									
slope	-0.010	0.004	-0.006	0.001	0.027	0.006	0.018	0.009	0.028
R2	0.641	0.020	0.031	0.001	0.104	0.113	0.172	0.100	0.203
p-value	0.017	0.739	0.676	0.932	0.436	0.416	0.307	0.445	0.263
Scaffold 2C									
slope	0.004	0.003	0.019	0.006	0.055	-0.003	0.023	0.032	0.055
R2	0.130	0.015	0.339	0.121	0.538	0.015	0.269	0.653	0.538
p-value	0.307	0.735	0.078	0.325	0.016	0.739	0.125	0.005	0.016
Scaffold 3									
slope	-0.006	0.003	0.035	0.008	0.034	0.013	-0.021	0.033	0.012
R2	0.048	0.004	0.502	0.092	0.226	0.338	0.120	0.235	0.044
p-value	0.604	0.881	0.049	0.466	0.234	0.131	0.401	0.224	0.616

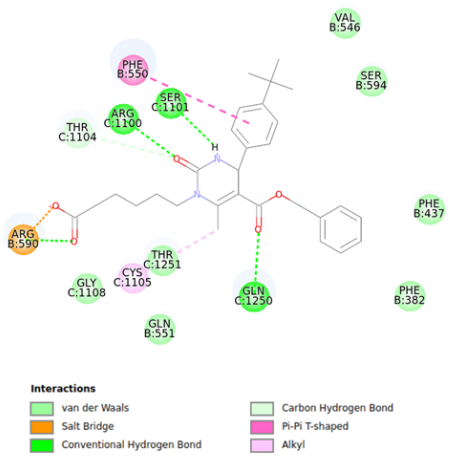
P-gp

Scaffold 1	Acceptor	Aromatic	Backbone	Contact	Donor	Hydrophobic	Polar	Sidechain
slope	0.003	-0.014	-0.010	-0.013	-0.004	-0.009	-0.002	-0.011
R2	0.045	0.084	0.049	0.052	0.339	0.040	0.017	0.047
p-value	0.450	0.293	0.426	0.412	0.023	0.477	0.646	0.440
Scaffold 2B								
slope		0.029	0.000	0.029	-0.004	0.020	0.003	0.022
R2		0.413	0.000	0.198	0.082	0.168	0.025	0.140
p-value		0.086	0.987	0.269	0.491	0.314	0.706	0.361
Scaffold 2C								
slope	0.001	-0.011	0.004	0.002	0.015	-0.009	0.011	0.002
R2	0.036	0.069	0.034	0.003	0.626	0.046	0.302	0.003
p-value	0.600	0.462	0.611	0.887	0.006	0.551	0.100	0.887
Scaffold 3								
slope	-0.001	0.020	0.004	0.025		0.021	0.004	0.025
R2	0.014	0.356	0.048	0.729		0.773	0.375	0.729
p-value	0.781	0.119	0.604	0.007		0.004	0.107	0.007

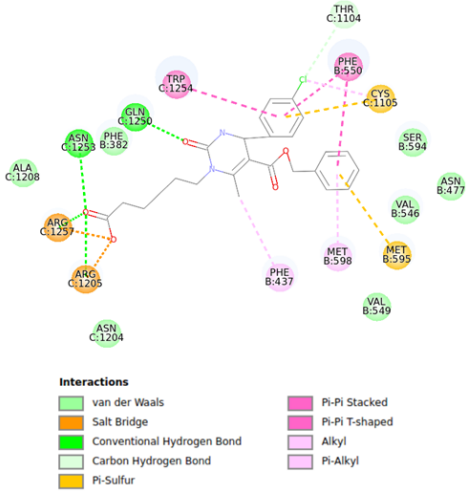
SUPPORTING INFORMATION 9

Adjacent residues of MRP2 interacting scaffold 2C compounds 2aw, 2ax, 2az, 2bb and 2o

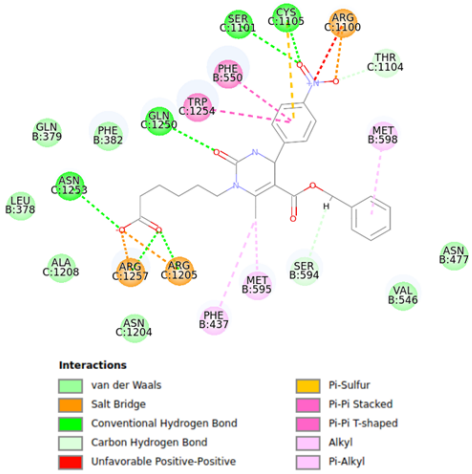
Inhibitor-2aw



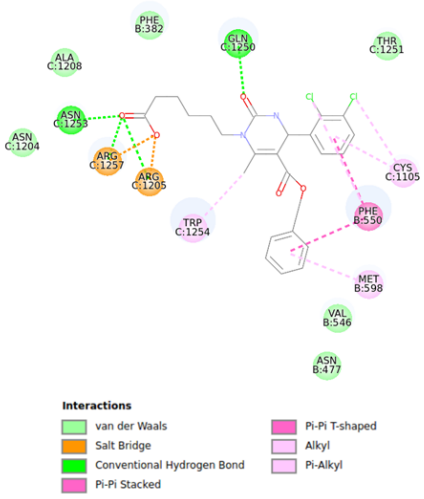
Inhibitor-2ax



Inhibitor-2az



Inhibitor-2bb



Inhibitor-2o