

Virtual screening of ABCC1 transporter nucleotide-binding domains as a therapeutic target in multidrug resistant cancer

Kanin Rungsardthong, Sergio Mares- Sámano & Jeffrey Penny*

University of Manchester, School of Pharmacy & Pharmaceutical Sciences, Stopford Building, M13 9PT, UK; Jeffrey Penny – Email: Jeffrey.penny@manchester.ac.uk; Phone: +44 (0)1612758344, Fax: +44 (0)1612758349; *Corresponding author

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Abstract:

ABCC1 is a member of the ATP-binding Cassette super family of transporters, actively effluxes xenobiotics from cells. Clinically, ABCC1 expression is linked to cancer multidrug resistance. Substrate efflux is energised by ATP binding and hydrolysis at the nucleotide-binding domains (NBDs) and inhibition of these events may help combat drug resistance. The aim of this study is to identify potential inhibitors of ABCC1 through virtual screening of National Cancer Institute (NCI) compounds. A three-dimensional model of ABCC1 NBD2 was generated using MODELLER whilst the X-ray crystal structure of ABCC1 NBD1 was retrieved from the Protein Data Bank. A pharmacophore hypothesis was generated based on flavonoids known to bind at the NBDs using PHASE, and used to screen the NCI database. GLIDE was employed in molecular docking studies for all hit compounds identified by pharmacophore screening. The best potential inhibitors were identified as compounds possessing predicted binding affinities greater than ATP. Approximately 5% (13/265) of the hit compounds possessed lower docking scores than ATP in ABCC1 NBD1 (NSC93033, NSC662377, NSC319661, NSC333748, NSC683893, NSC226639, NSC94231, NSC55979, NSC169121, NSC166574, NSC73380, NSC127738, NSC115534), whereas approximately 7% (7/104) of docked NCI compounds were predicted to possess lower docking scores than ATP in ABCC1 NBD2 (NSC91789, NSC529483, NSC211168, NSC318214, NSC116519, NSC372332, NSC526974). Analyses of docking orientations revealed P-loop residues of each NBD and the aromatic amino acids Trp653 (NBD1) and Tyr1302 (NBD2) were key in interacting with high-affinity compounds. On the basis of docked orientation and docking score the compounds identified may be potential inhibitors of ABCC1 and require further pharmacological analysis.

Keywords: Homology, ABCC1, Flavonoid, Pharmacophore, Docking, Nucleotide-binding domain

Abbreviation: ABC: ATP-binding cassette, DHS: dehydrosilybin, MDR: multidrug resistance, NBD: nucleotide-binding domain, PDB: protein data bank

Background:

Multidrug resistance (MDR) imposes a serious constraint on successful treatment of cancer. A major factor contributing to MDR is overexpression of efflux transporters of the ATP-binding cassette (ABC) superfamily, which act as energy-dependent drug efflux pumps [1-3]. Anticancer drugs within the major categories of chemotherapeutics, including vinca

alkaloids, anthracyclines, epipodophyllotoxins and folate-based antimetabolites [4] are ABCC1 substrates and high levels of ABCC1 expression has been associated with poor clinical outcome in the treatment of retinoblastoma and neuroblastoma [5, 6]. ABCC1 expression can also affect the response of non-small cell lung cancer [7], breast cancer [8-10], and ovarian cancer [11] to chemotherapy. ABCC1 is a 190-kDa glycoprotein

consisting of 2 nucleotide-binding domains (NBDs) and 3 transmembrane domains [12-14]. ABCC1-mediated substrate translocation to the cell exterior is energised by ATP binding and hydrolysis at the NBDs [15, 16]. Since ATP binding and hydrolysis are fundamentally important to active drug efflux, targeting agents to the NBDs to disrupt the catalytic cycle is a potential strategy for addressing ABCC1-mediated cancer multidrug resistance. *In vitro* studies reveal that naturally occurring flavonoids (e.g. in fruits, vegetables, tea), bind to recombinant ABCC1 NBDs [17]. Therefore, in this study, flavonoids and flavonoid-based compounds were used as input ligands for pharmacophore generation to identify potential modulators of ABCC1 NBDs using both ligand (pharmacophore)- and structure (molecular docking)-based approaches.

Methodology:

Retrieval of compounds from the NCI Database

The library of compounds for screening against the ABCC1 transporter was obtained from the National Cancer Institute (NCI) database in SDF format from <http://cactus.nci.nih.gov/download/nci>. NCI compounds were prepared for screening using the *LigPrep* process (Schrodinger Suite 2009, Schrodinger LLC NY). The ligands were parametrised using OPLS_2005 force field and tautomers and ionisation states expected to occur between pH 5.0 and 9.0 [18, 19]. Following energy minimisation, the total number of compounds generated was 309,520. Compounds demonstrating good drug-like properties i.e. predicted high oral bioavailability and potentially low toxicity, were selected using the *QikProp* filter based on the modification of Lipinski's rule of five [20, 21]. This step yielded a total of 167,350 potential drug-like compounds.

Pharmacophore Generation and Screening

The pharmacophore hypotheses for ABCC1 NBD1 and ABCC1 NBD2 were constructed using the basic structures of flavonoids known to interact at each NBD (Genistein, Apigenin, Naringenin, Galangin, Quercetin, Dehydrosilybin (DHS), Silybin, 6-prenyl DHS, 6-geranyl DHS, 8-prenyl DHS, 8-geranyl DHS) [17]. The biological activities were reported as dissociation constants (K_D), which were subsequently converted to pK_D ($-\log(K_D)$) for each NBD. Pharmacophores were generated using PHASE (Pharmacophore Alignment and Scoring Engine) software, version 3.1, 2009 (Schrodinger Suite 2009, Schrodinger LLC NY) [22].

To identify the best-optimised hypothesis for pharmacophore-based screening, all hypotheses for ABCC1 NBD1 and ABCC1 NBD2 were subjected to an analysis scoring function (survival score). Compounds identified from the 167,350 drug-like compounds were required to match at least five pharmacophoric features to be considered appropriate for structure-based molecular docking studies, with distance tolerances for pharmacophoric features set at 2 Å.

Generation of the homology model of ABCC1 nucleotide-binding domain 2

Homology modelling was used to construct the three-dimensional model of ABCC1 NBD2 using template amino acid sequences of ABC transporters for which X-ray crystal structures are available. An X-ray crystal structure of ABCC1

NBD1 (PDB code: 2CBZ) is available in the protein data bank (PDB) [23]. The full-length human ABCC1 protein sequence (ID: P33527) was obtained from the National Centre for Biotechnology Information (NCBI). The amino acid sequence of the C-terminal NBD of ABCC1 (Arg1292 – Asp1527) was subsequently extracted as a target sequence. Template sequences homologous to the ABCC1 NBD2 sequence were retrieved using a BLAST search. An amino acid multiple sequence alignment of ABCC1 NBD2 and the five protein templates was generated using ClustalW [24]. The Esript programme was used to generate secondary structures of the five templates [25]. ABCC1 NBD2 homology models were generated using Modeller 9 version 7 [26]. Stereochemical analyses of the refined models were carried out using Ramachandran plots by molprobiy website [27]. Refined models were subjected to 1000 energy minimisation steps using GROMACS (Version 4.0) [28].

Molecular Docking Screening

PrepWizard was used to assign bond orders, add hydrogen atoms and delete water molecules for ABCC1 NBD1 (X-ray crystal structure) and NBD2 (homology model). Energy minimisation was implemented using OPLS_2005 forcefield (Schrodinger Suite 2009, Schrodinger LLC NY). Compounds identified using pharmacophore-based screening were docked into ABCC1 NBD1 and ABCC1 NBD2 using Glide (Grid-Based Ligand Docking with Energetics) software package v5.5 [29]. In this study, initially standard precision (SP) docking was carried out and ligand conformations demonstrating the highest scores (i.e. top 10% of docking scores) were subsequently redocked using the extra precision (XP) docking algorithm to obtain high quality refinement ligand poses. Visualisation of ligands was carried out by the Maestro graphical interface.

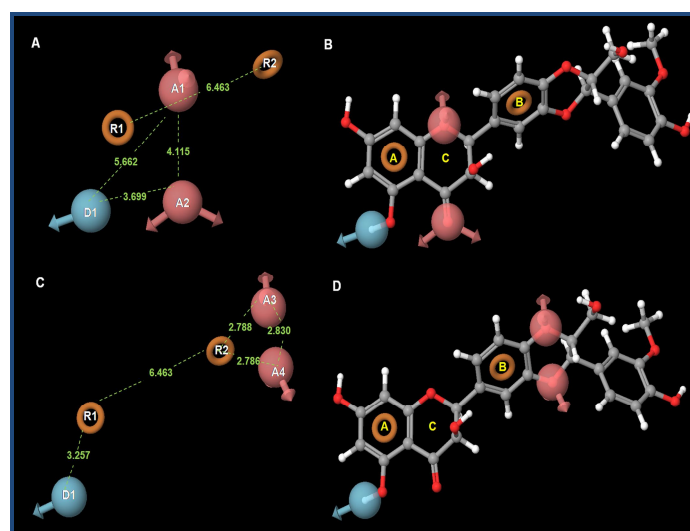


Figure 1: Geometry of ABCC1 NBD1 and ABCC1 NBD2 pharmacophore hypotheses. Geometry of the pharmacophore hypotheses of AADRR.65 for ABCC1 NBD1 (A) and mapped onto the flavonoid dehydrosilybin (B). Red spheres; H-bond acceptor feature, Orange torus; aromatic ring feature, blue sphere; H-bond donor feature. Pharmacophore hypotheses of AADRR.1277 for ABCC1 NBD2 (C) and mapped onto the flavonoid dehydrosilybin (D). Distances, in Angstroms, between pharmacophore features are shown by green dotted lines.

Results and Discussion:

A total of 5,953 hypotheses were constructed for ABCC1 NBD1 and 6,505 for ABCC1 NBD2. Each hypothesis possessed five pharmacophoric features categorised as AADDD, AAADR, AAADD, AAAAD, AAAAR AADRR, AAARR, AADDR, or ADDRR (A, hydrogen bond acceptor; D, hydrogen bond donor; R, aromatic ring).

For ABCC1 NBD1, the best-optimised hypothesis was AADRR.65 (survival score: 5.952). The spatial distribution of pharmacophore sites was two aromatic rings (R1 and R2), whose centres were separated by 6.463 Å (Figure 1A). The rings mapped to ring A and B of flavonoids (Figure 1B). Two H-bond acceptors, A1 and A2 (Figure 1A), were separated by 4.115 Å; A1 mapped to the oxygen atom at position 1 of flavonoids, whereas A2 mapped to the oxygen atom of the carbonyl group at ring C of flavonoids (Figure 1B). The H-bond donor D1 was located adjacent to H-bond acceptors A1 and A2 at a distance of 3.699 Å and 5.662 Å, respectively (Figure 1A). D1 mapped to the hydrogen atom of the hydroxyl group at position 5 of ring A of flavonoids (Figure 1B).

The best-optimised pharmacophore hypothesis for ABCC1 NBD2 was AADRR.1277 (survival score: 5.955). AADRR.1277 possessed two aromatic rings, R1 and R2 separated by 6.463 Å (Figure 1C), which mapped to rings A and B of flavonoids (Figure 1D). The hydrogen bond donor D1, 3.257 Å from the centre of R1, was allocated to the hydrogen atom at position 5 of ring of flavonoids (Figure 1D). Two H-bond acceptors, A3 and A4, were separated by 2.830 Å and mapped to the oxygen atoms at positions 3' and 4' respectively of ring B of flavonoids (Figure 1D).

AAARR.65 (NBD1) and AADRR.1277 (NBD2) exhibited partial structural similarity with each other, in terms of positions of the R1 aromatic rings and R2 aromatic rings, (Figure 1, R1 and R2 of each pharmacophore). These pharmacophoric features mapped precisely to rings A and B of flavonoids (Figure 1B (NBD1), 1D (NBD2)). There was also consistency between the position of the H-bond donor feature in each pharmacophore (Figure 1A, D1 and Figure 1C, D1) and the location of the H-bond donor at position 5 of ring A of flavonoids. These findings reflect the basic characteristic structural features of flavonoids (i.e. rings A and B) reported to inhibit ABCC1 activity *in vitro* [30-33]. Tawari *et al.* (2008) generated an ABCC1-targeted pharmacophore, based on non-flavonoid-like pyrrolopyrimidines and indopyrimidines, that was validated using compounds with high potency against ABCC1, including dehydrosilybin. The aromatic ring feature (Figure 1, R1) of AADRR.65 and AADRR.1277 was also present in the pharmacophore generated by Tawari *et al.* (2008), as was the hydrogen bond acceptor feature (A2) in ABCC1 NBD1 (Figure 1) [34]. Pharmacophore-based screening of the NCI database with AADRR.65 and AADR.1277 identified 3,163 and 1,113 hit compounds respectively.

Generation of the homology model of ABCC1 nucleotide-binding domain 2

Sequences homologous to ABCC1 NBD2 were retrieved using a BLAST search filtered for known 3D structures. The *Escherichia coli* haemolysin B NBD (PDB code: 1MT0) [35] demonstrated highest sequence identity, 37%, with ABCC1 NBD2 whilst

Salmonella typhimurium MsbA (PDB code: 3B60) [36] and *Plasmodium yoelii* MDR protein 2 (PDB code: 2GHI) [37] both demonstrated 36% sequence identity. The bacterial ABC transporter Sav1866 (PDB code: 2HYD) [38] and the *Lactococcus lactis* ABC transporter NBD (PDB code: 1MV5) [39] possessed 35% and 34% amino acid identity respectively. Multiple sequence alignment of human ABCC1 NBD2 with the structural template sequences demonstrated the highly conserved regions within the proteins, namely the Walker-A and -B motifs, Signature sequence, Q-loop, D-loop and H-loop (Figure 2).

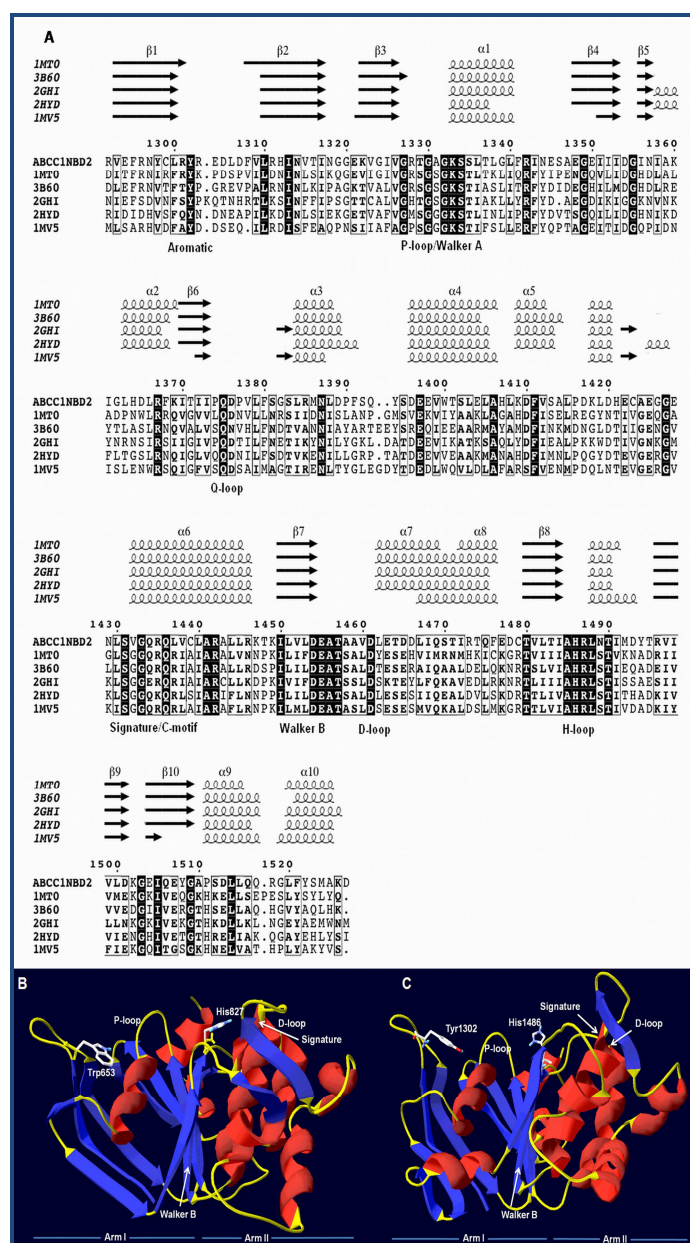


Figure 2: Multiple sequence alignment of human ABCC1 NBD2 and five homologous templates (A) and a three-dimensional model of ABCC1 NBD1 (B) and NBD2 (C).

The ABCC1 NBD2 homology model is comprised of Arm I and Arm II which together possessed ten β-sheets and ten α-helices (Figure 2) consistent with the secondary structures of the template sequences. Arm I, the ABC-β subdomain, is composed of nine β-sheets and two α-helices containing the conserved P-

loop, Walker B, H-loop and D-loop sequences. The P-loop sequence is located within a linker region between $\beta 3$ and $\alpha 1$ and the Walker B sequence is localised in $\beta 7$. Arm II represents the ABC- α subdomain and contains eight α helices ($\alpha 3$ - $\alpha 10$) with the Signature sequence (C-motif) located at $\alpha 6$ of the ABC- α subdomain. The homology model with the highest percentage

of amino acids within the favoured regions, 97.9%, and allowed regions, 99.6%, as identified by Ramachandran plot analysis, was used in subsequent docking studies. Within the homology model only a single amino acid, Asp1389, was not within the allowed region.

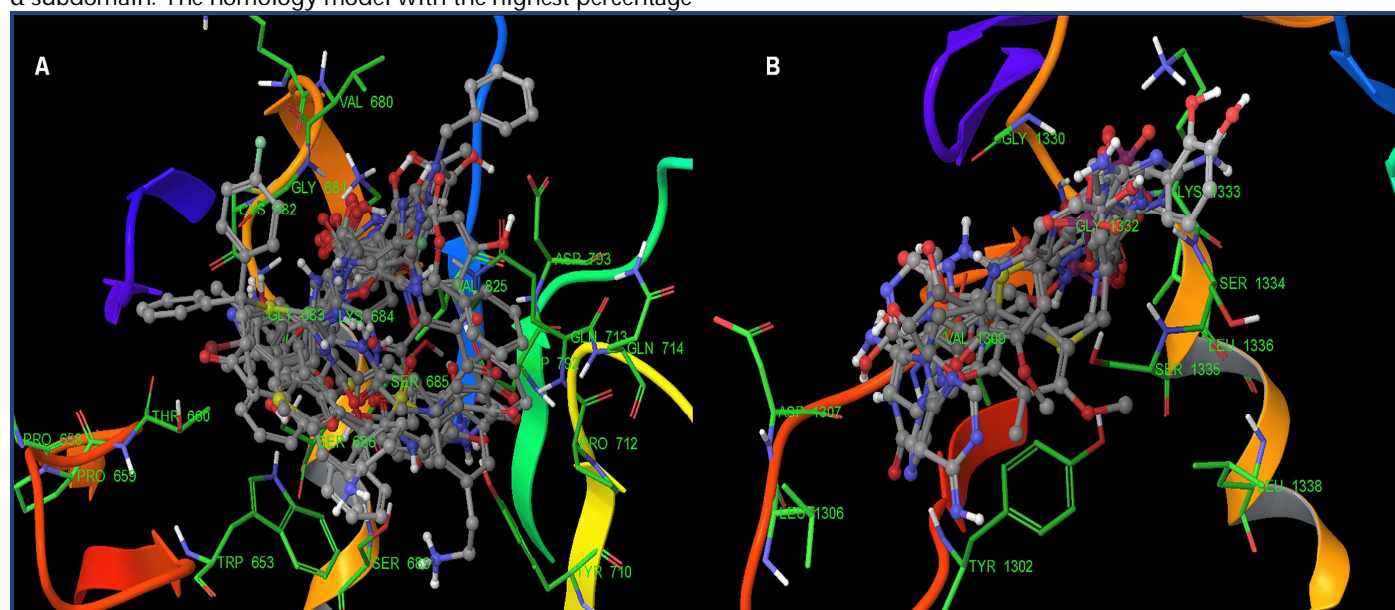


Figure 3: Predicted docking orientation and molecular interaction of NCI compounds within ABCC1 NBD1 (A) and NBD2 (B).

Molecular Docking Screening

The ligand conformation possessing the lowest docking score is representative of a conformation with favourable binding energy (i.e. high binding affinity). The docking scores of compounds docked into ABCC1 NBD1 (using XP docking mode) ranged from -7.33 to 0.77. Approximately 5% (13/265) of these compounds possessed a lower docking score than ATP (-6.19), suggesting they may bind with a higher affinity compared to ATP. Docking studies of these compounds, NSC93033, NSC662377, NSC319661, NSC333748, NSC683893, NSC226639, NSC94231, NSC55979, NSC169121, NSC166574, NSC73380, NSC127738 and NSC115534, within ABCC1 NBD1 revealed a propensity for them to be partially accommodated in the ATP binding site within the binding cavity. The oxygen and hydrogen atoms of the above compounds were predicted to form extensive hydrogen bonds with Gly681, Lys684, Ser685 and Ser686 within the P-loop. Hydrophobic interactions were also identified between the compounds and the aromatic amino acid Trp653 (Figure 3).

The predicted docking score of NCI compounds docked into the homology model of ABCC1 NBD2 ranged from -7.36 to -1.60. Approximately 7% (7/104) of these compounds were predicted to possess a lower docking score than ATP (ATP docking score; -6.17), and may bind with relatively high affinity to the NBD. These compounds were NSC91789, NSC529483, NSC211168, NSC318214, NSC116519, NSC372332 and NSC526974. The predicted docking orientation of the high-affinity compounds within ABCC1 NBD2 overlapped with that of ATP and the oxygen atoms and hydrogen atoms were predicted to form a network of hydrogen bonds with Lys1333, Ser1334 and Ser1335 within the P-loop. Hydrophobic contacts stabilised the interaction with the aromatic amino acid Tyr1302 (Figure 3).

The predicted ATP-binding site is a narrow pocket which restricts the binding orientation of selected NCI compounds, i.e. restricted along a site defined by aromatic residues (Trp653 in NBD1) and Tyr1302 in NBD2) and the P-loop cavity. Our studies have shown that Trp653 (NBD1) and Tyr1302 (NBD2) are significant for ligand-binding since, in both NBDs, they interact with hydrophobic moieties of compounds with predicted high-binding affinity. Importantly, NCI compounds with predicted low binding affinities (high docking scores) demonstrated fewer or no hydrophobic interactions with these key aromatic amino acids (data not shown). These findings are consistent with the important stabilising hydrophobic interactions reported between the adenine ring of ATP and Trp653 (NBD1) and Tyr1302 (NBD2) in ABCC1 [40]. The P-loop in both NBDs was predicted to be the main site for hydrogen bond interactions between docked NCI compounds and ABCC1 NBDs. This is expected since the P-loop region contains a large proportion of polar amino acids and is the well-recognised site of hydrogen bond interaction between the phosphate groups of the endogenous ligand ATP and NBDs [16, 35, 41]. In addition, this finding is supported by the study of Badhan (2006) in which the *in silico* interaction of flavonoids with ABCB1 (P-glycoprotein) NBD2 reports the 3-hydroxyl and 5-hydroxyl groups and carbonyl oxygen are involved in hydrogen bonding with the P-loop [42].

Currently, flavonoid-based compounds are the only group of chemical structures reported to modulate ABC transporters by binding to NBDs [17, 43-45]. Our studies show all screened NCI compounds possessed the pharmacophoric features of flavonoid-based compounds as the primary chemical scaffold. Although some hits differed structurally from flavonoid-based compounds, they possessed the same pharmacophoric features and predicted molecular interactions within ATP-binding sites,

namely hydrogen bonding at the P-loop and hydrophobic interactions at aromatic acid residues (Trp653 in NBD1, and Tyr1302 in NBD2).

Conclusion:

We have identified compounds from the NCI repository predicted to bind with high affinity at the ATP-binding sites within the ABCC1 efflux transporter. These compounds may show potential as modulators of the catalytic cycle of ABCC1 and consequently could inhibit ABCC1 transporter-mediated drug efflux.

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