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Research Article

ANTICANCER DRUGS AS POTENTIAL INHIBITORS OF ACRAB-TOLC OF MULTIDRUG RESISTANT *ESCHERICHIA COLI*: AN *IN SILICO* MOLECULAR MODELING AND DOCKING STUDY

PRANJALI GUPTA, NISHANT RAI, PANKAJ GAUTAM*

Department of Biotechnology, Graphic Era University, Dehradun - 248 001, Uttarakhand, India. Email: gautampankaj76@gmail.com

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ABSTRACT

Objective: Overexpression of AcrAB-TolC protein complex is often associated with the virulence of multidrug-resistant bacteria. Development of an effective efflux pump inhibitors (EPI) can be a major strategy to enhance the effectivity of current antibiotics and to restrain the menace of antibiotic resistance among bacteria. Molecular docking based assessment of anticancer drugs as EPI with comparable docking scores of known putative EPI.

Methods: Molecular docking of target proteins (AcrA, AcrB and TolC) of *Escherichia coli* was carried out with four putative and seven selected anticancer drugs using iGEMDOCK software separately.

Results: All the four putative inhibitors (norepinephrine, reserpine, verapamil and trimethoprim) used in the present study binds to AcrA (−41.9 kcal/mol, −56.75 kcal/mol, −76.69 kcal/mol and −45.20 kcal/mol respectively), AcrB (−74.61 kcal/mol, −135.97 kcal/mol, −126.66 kcal/mol and −87.57kcal/mol respectively) and TolC (−78.49 kcal/mol, −90.22 kcal/mol, −89.42 kcal/mol and −62.57 kcal/mol respectively) with high affinity and seven drug ligands (etoposide, paclitaxel, tamoxifen, mitomycin and thalidomide, vinblastine methotrexate) showed comparable docking energies (AcrA [−36.44 kcal/mol, −78.23 kcal/mol, −17.04 kcal/mol, −42.96 kcal/mol, −44.94 kcal/mol, −67.96 kcal/mol and −20.15 kcal/mol respectively], AcrB [−128.11 kcal/mol, −132.86 kcal/mol, −104.85 kcal/mol, −98.91 kcal/mol, −96.47 kcal/mol, −108.79 and −106.36 kcal/mol respectively], TolC [−68.42 kcal/mol, −88.29 kcal/mol, −64.69 kcal/mol, −68.28 kcal/mol, −59.36 kcal/mol, −77.28 kcal/mol and −74.52 respectively]) with putative inhibitors.

Conclusion: Paclitaxel and vinblastine showed high affinity for all units of AcrAB-TolC of *E. coli.*

Keywords: AcrAB-TolC, Efflux pump, Efflux pump inhibitor, Multidrug resistance, Molecular modeling and docking, *Escherichia coli*.

INTRODUCTION

Multidrug resistance (MDR) is fast emerging as a major health problem in humans and the management of bacterial diseases such as tuberculosis, typhoid is increasingly becoming more challenging worldwide in the current scenario [1,2]. At international level MDR related outbreaks are fairly frequent in endemic areas like South America, Africa and Southeast Asia (India, Pakistan, Vietnam and Burma) [3], moreover the situation has deteriorated with the increased global mobility and overuse of antibiotics [4]. Use of the multidrug combination is being practiced by clinicians as there is a lack of new antibiotics attacking new targets sites in pathogenic organism [6].

AcrAB-TolC is tripartite efflux system of RND family in Gram-negative bacteria having three components; a periplasmic AcrA fusion protein, a transporter protein AcrB and an outer membrane protein TolC [7]. Different clinically relevant MDR strains of Gram-negative bacteria like *Escherichia coli* [8], *Salmonella typhimurium*, *Pseudomonas aeruginosa* expresses AcrAB-TolC system in their membrane [9]. Over-expressed efflux system expels a range of antibiotics, dyes, detergents and biocides. Among the major substrate of AcrAB-TolC system is quinolones, chloramphenicol, tetracyclins, acriflavine, ethidium bromide, and bile salts. Different deletion strains of *Acinetobacter baumannii* [10] *Mycobacterium smegmatics* [11] *S. typhimurium* [12] renders them susceptible to common antibiotics, therefore these efflux protein systems are major targets for the development of drugs inhibiting the growth of MDR pathogenic strains.

Efflux pumps are the central membrane protein for the import and export of various substrates including antibiotics, dyes, and biocides. Efflux proteins overexpression within the bacterial membrane was found correlated with its ability to acquire drug resistance over time [13] Different efforts to develop new efflux pump inhibitors (EPI) are the part of strategy to restrict the dissemination of multidrug-resistant bacterium [14]. A number of EPIs are showing good experimental inhibition of bacterium, but their use as an effective drug is limited for humans because of high toxicity [15].

Drug repurposing can address problems related with EPIs toxicity as drug pharmacokinetics, phamacodynamics, and their toxicity is already available. Drug repositioning provides an opportunity to reduce the time for new drug discovery by almost one-third and we already have a number of successful repositioned drugs as antidepressant (sildenafil, duloxetine, bupropion, dapoxetine, fluoetine, milnacipran, sibutramine), neurological drugs; (atomoxetine, chlorpromazine, galantamine, lidocaine) and non-neurological drugs (celecoxib, eflornithine, finasteride, mecamylamine, minoxidil, raloxifene, raloxifene, topiramate) [16]. Various pharmaceutical companies are viewing drug repositioning as an opportunity in the backdrop of the high cost and slow processes involved in drug discovery. Anticancer drugs are repurposing as antibacterial agents, especially against MDR strain is still an unexplored area. Few examples are available in the literature where anticancer drugs have been repositioned for any bacterial diseases. Relation between cancer and MDR was hard to conceive until the report was published regarding enhanced sensitivity of antibiotics during anticancer drug administration (celecoxib and tirapazamine [TPZ]).

Celecoxib; a specific inhibitor of cyclooxygenase-2, increases the sensitivity of bacteria to the antibiotics (ampicillin, kanamycin, chloramphenicol, and ciprofloxacin) in combinations but it showed no antimicrobial activity alone. Celecoxib exerts antimicrobial effects with antibiotics by inhibiting MDR1 efflux pump in resistant bacteria which use these efflux pumps to expel antibiotics out of cell [17].

TPZ; an anticancer prodrug, was found effective against fluoroquinolone-resistant *E. coli* strains, methicillin-resistant *Staphylococcus aureus* strains and pathogenic *Clostridium difficile*. TPZ shows antibacterial potential in aerobic as well as anaerobic condition (hypoxic environment) [18].

Raloxifene; a drug used in breast cancer in post-menopausal woman, inhibits the production of a pigment (pyocyanin) in *P. aeruginosa.* Pyocyanin is responsible for the characteristic blue-green color of bacterial colony and acts as a signal molecule for quorum sensing and virulence [19].

In the present *in silico* study known different putative EPIs like norepinephrine (NOR) and trimethoprim (TMP), reserpine, verapamil and a battery of mainstream anticancer drug ligands with have been tested for their potential application as an antibacterial agent, especially against Gram-negative MDR strains of *E. coli*.

NOR is a catecholamine with multiple roles, including as a hormone, and a neurotransmitter is currently used as putative EPI [20]. Reserpine is an indole alkaloid, antipsychotic and antihypertensive drug that has been used for the control of high blood pressure and for the relief of psychotic symptoms and currently used as EPI [21]. Verapamil is an L-type calcium channel blocker of the phenylalkylamine class. It has been used in the treatment of hypertension, cardiac arrhythmia, and most recently, cluster headaches. Currently used as efflux pump-mediated resistance in chemotherapy [22]. TMP is a bacteriostatic antibiotic used mainly in the prevention and treatment of urinary tract infection, it is putative EPI of Gram-negative bacteria and plays a role in virulence [23]. A number of anticancer drug ligands were tested, but only seven showed good docking score with AcrAB-TolC efflux system of *E. coli.*

Etoposide (VP16); a podophyllotoxin derivative that mediates inhibition of DNA topoisomerase 2, is currently used for a number of cancer malignancies [24]. Paclitaxel; a complex diterpene with taxen ring, mediates its action by forming stable microtubules and preventing its depolymerization to tubulin [25]. Mitomycin; a highly selective cytotoxic drug produced by *Streptomyces lavendulae,* is being used in cancer chemotherapy against a number of cancer malignancies (gastric, cervical, and pancreatic [26]. Mitomycin mediates cytotoxicity and apoptosis in host cell by cross linking itself to DNA strand after the reduction step and subsequent alkylation.

Methotrexate; a dihydrofolate reductase inhibitor, mediates its cytotoxicity by limiting the cofactors (N^5-N^{10} methylene tetrahydrofolate and N^{10} formyl tetrahydrofolate) for thymidylate formation, which halts DNA synthesis and subsequently causes apoptosis in host cell [27]. Thalidomide; a derivative of glutamic acid and initially used to treat morning sickness in pregnant women, mediates its cytotoxicity by inhibiting angiogenesis in solid tumors [28]. Thalidomide causes immunomodulation by inhibiting tumor necrosis factor-alpha that is involved in angiogenesis and adhesion [29]. Vinblastine; a dimeric alkaloid isolated from plant *Catharantus roseus*, mediates its pharmacological action through the depolymerization of microtubule [30]. Thus in the present study we report experimental evidences based on *in silico* study regarding the use of mainstream anticancer drugs as EPI inhibitors in MDR bacterial (*E. coli*) strains.

METHODS

Physiochemical characterization

Physiochemical properties of each AcrA (PDB Id: 2F1M), AcrB (PDB Id: 1IWG) and TolC (PDB Id: 1EKG) of *E. coli* were calculated by ProtParam. Details of ligands were obtained from Pubchem [31]. Major characteristics determined by ProtParam include instability index, aliphatic index, grand average of hydropathicity (GRAVY) value and theoretical pI (web.expasy.org/protpram).

Binding site analysis of AcrA, AcrB and TolC subunits

Active sites identification on different subunits AcrA (gi|83286920), AcrB(gi|399001) and TolC (gi|109940038) of AcrAB-TolC were determined by AADS; an automated active site identification, docking, and scoring protocol for proteins with known structures developed by SCFBio at IIT, Delhi (India) (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp) (Table 1) [32].

Anticancer drug ligands

All anticancer drugs structure (etoposide, methotrexate, mitomycin, paclitaxel, tamoxifen, thalidoamide and vinblastine) used in present studies were downloaded from zinc database [33] (www.zincdocking.org) and their molecular structures were obtained from Pubchem (pubchem.ncbi.nlm.nih.gov).

Molecular docking

Docking studies were performed by iGEMDOCK [34] iGEMDOCK is an integrated virtual screening environment which utilizing post-screening analysis with pharmacological interactions. Multiple ligands library can be uploaded at the same time with the given protein target and software generates integrated docking, post analysis and graphical visualization showing the favorable docked poses within the protein target. Data related to free energy (E), hydrogen bonding (H), van der waal (vdW) and electrostatic functions can be obtained.

RESULTS

Physiochemical characterization

Instability index for the target proteins; AcrA, AcrB and TolC was calculated as 29.31, 28.60 and 34.53 respectively by ProtPram. All these proteins have *in vivo* half-life more than 16 hrs as the value of instability index falls below 40 [35].

Aliphatic index is a measure of the relative volume of protein occupied by alanine, valine, isoleucine, and leucine amino acids and depicts the thermostability of the protein [36]. Aliphatic index for AcrA, AcrB and TolC, was estimated as 91.18, 101.52 and 86.57 respectively.

GRAVY score states the hydophobicity or hydrophilicity of the protein in question [37]. GRAVY value was calculated as −0.256, −0.266 and −0.427 for AcrA, AcrB and TolC respectively thus AcrA and TolC are hydrophilic whereas AcrB was found to be hydrophobic in nature.

Theoretical isoelectric point for AcrA, AcrB and TolC was determined as 7.69, 5.39 and 5.46 respectively.

Table 1: Details of binding cavities determined by the AADS protocol, each cavity is lined by the amino acids residues (top five cavities of each AcrA, AcrB and TolC)

Cavities on subunit AcrA	
Cavity 1	Kiyrlapqnvdshtgef
Cavity 2	Vaksyrnqglpitdefh
Cavity 3	Gedrknvatiyfplqsh
Cavity 4	Ygqniatrvslkdpe
Cavity 5	Lpiqtydageknsrvfh
Cavities on subunit AcrB	
Cavity 1	Stmfvaeynqprdligkw
Cavity 2	Ndftqsaplgeimyvwr
Cavity 3	Frtygelpamnsdqvki
Cavity 4	Qrwpfdisymtkeglnav
Cavity 5	Eqrtaigkmnlwdpyfsv
Cavities on subunit TolC	
Cavity 1	Tnayevqksldi
Cavity 2	Akygntdqirsfv
Cavity 3	Tyqigasnkdrvlf
Cavity 4	Tqnesiadyrlv
Cavity 5	Tsdglyqirkwpnahvef

Model assessment

Quality assessment of generated models (AcrA, AcrB and TolC) was verified by Swiss Model assessment server. Ramachandran plot values were calculated to be 86.7% (AcrA), 90.5% (AcrB) and 90.4% (TolC) (Fig. 1) with the QMEAN-Z score −2.02 (AcrA), −2.29 (AcrB) and −3.79 (TolC) respectively. Ramachandran plot values suggest suitability of these models for the molecular docking.

Molecular docking

Putative EPI (NOR, reserpine, verapamil, TMP) showed good binding energies (−41.9 kcal/mol, −56.75 kcal/mol, −76.69 kcal/mol and −45.2 kcal/mol respectively) with receptor target AcrA. Similarly receptor target AcrB (NOR, reserpine, verapamil, TMP) showed good binding energies (−74.61 kcal/mol, −135.97 kcal/mol, −126.66 kcal/mol, −87.57 kcal/mol). Whereas the binding energy of drugs: NOR, reserpine, verapamil, TMP with TolC (−78.49 kcal/mol, −90.22 kcal/mol, −89.42 kcal/mol, −62.57 kcal/mol) (Table 2). Different docked poses of putative EPI with protein subunits AcrAB and TolC have been illustrated in Fig. 2.

Anticancer drugs (etoposide, paclitaxel, tamoxifen, mitomycin thalidoamide, vinblastine, methotrexate and showed good binding energies (−36.44 kcal/mol, −78.23 kcal/mol, −17.04 kcal/mol, −42.96 kcal/mol, −44.95 kcal/mol, −67.97 kcal/mol, and −20.15 kcal/mol a respectively) with the receptor target AcrA. In a separate docking experiment AcrB showed high binding energies (−128.11 kcal/mol, −132.86 kcal/mol, −104.85 kcal/mol, −98.91 kcal/mol, −96.47 kcal/mol, −108.79 kcal/mol and −106.36 kcal/mol) with etoposide, paclitaxel, tamoxifen, mitomycin thalidoamide, vinblastine, methotrexate respectively, whereas the binding energy of drugs; etoposide, paclitaxel, tamoxifen, mitomycin thalidoamide, vinblastine, methotrexate with TolC was determined as −68.42 kcal/mol, −88.29 kcal/mol, −64.69 kcal/mol, −68.28 kcal/mol, −59.36 kcal/mol, −77.28 kcal/mol and −74.52 ol respectively (Table 3). Different docked poses of drug ligands with protein subunits AcrAB and TolC have been illustrated in Fig. 3.

Table 2: Comparative docking energies of AcrAB and Tolc of putative EPI and anticancer drugs as ligands

Compound	Total energy (kcal/mol)		
	AcrA	AcrB	TolC
NOR	−41.9	−74.61	−78.49
Reserpine	−56.75	−135.97	−90.22
Verapamil	−76.69	−126.66	−89.42
TMP	−45.2	−87.57	−62.57
Etoposide	−36.44	−128.11	−68.42
Paclitaxel	−78.23	−132.86	−88.29
Tamoxifen	−17.04	−104.85	−64.69
Mitomycin	−42.96	−98.91	−68.28
Thalidoamide	−44.94	−96.47	−59.36
Vinblastine	−67.96	−108.79	−77.28
Methotrexate	−20.15	−106.36	−74.52

TMP: Trimethoprim, NOR: Norepinephrine, EPI: Efflux pump inhibitors

DISCUSSION

Computational methods in pharmacology and drug discovery have taken a centre stage since the prolific growth of various databases and continuous addition of new information along with conventional *in vivo* and *in vitro* approach to test the given hypothesis. Few publications are available which provide evidences regarding the use of anticancer drugs to enhance the antibiotics potency in experimental conditions. The exact mechanism of enhanced potency of antibiotic after anticancer drug administration is still unknown.

In present study *in silico* docking score of putative EPIs (NOR, reserpine, verapamil and TMP) have been compared with the test ligand (etoposide, paclitaxel, tamoxifen, mitomycin and thalidomide, vinblastine and methotrexate) to check their potential as prospective EPI. Repurposing of anticancer drugs as EPI in multidrug resistant bacteria (*E. coli, S. typhimurium* and *Salmonella typhi*) provides an opportunity to counter the menace of antibiotic resistance in clinical pathogen.

With AcrA protein subunit docking energies of test ligands: Paclitaxel, vinblastine (−78.23 kcal/mol, −67.96 kcal/mol respectively) showed comparable values with the known putative inhibitors: Verapamil (−76.69 kcal/mol) whereas obtained docking energies of drugs mitomycin and thalidomide (−42.96 kcal/mol and −44.94 kcal/mol) were found close to that of TMP and NOR (−45.2 kcal/mol and −41.9 kcal/mol respectively) Table 2. Other anticancer drugs used like etoposide, tamoxifen and methhotrexate produced less negative energies (−36.44 kcal/mol, −17.04 kcal/mol and −20.15 kcal/mol respectively) with protein subunit AcrA and were not comparable with any of the putative EPI docking energies. All putative EPI showed overlapping amino acids (Table 2) in binding pockets on AcrA subunit.

With AcrB protein subunit tested drug ligands produced highest negative docking energies as compared to other protein targets; AcrA and TolC. Docking scores of etoposide, paclitaxel, vinblastine and tamoxifen (−128.11 kcal/mol, −132.86 kcal/mol, −108.79 kcal/mol and −104.85 kcal/mol respectively) were comparable with putative EPIs: Reserpine and verapamil (−135.97 kcal/mol and −126.66 kcal/mol respectively) whereas dock score of other drug ligands: Thalidomide, methotrexate and mitomycin (−96.47 kcal/mol, −106.36 kcal/mol and −98.91 kcal/mol) showed close values to putative TMP and NOR

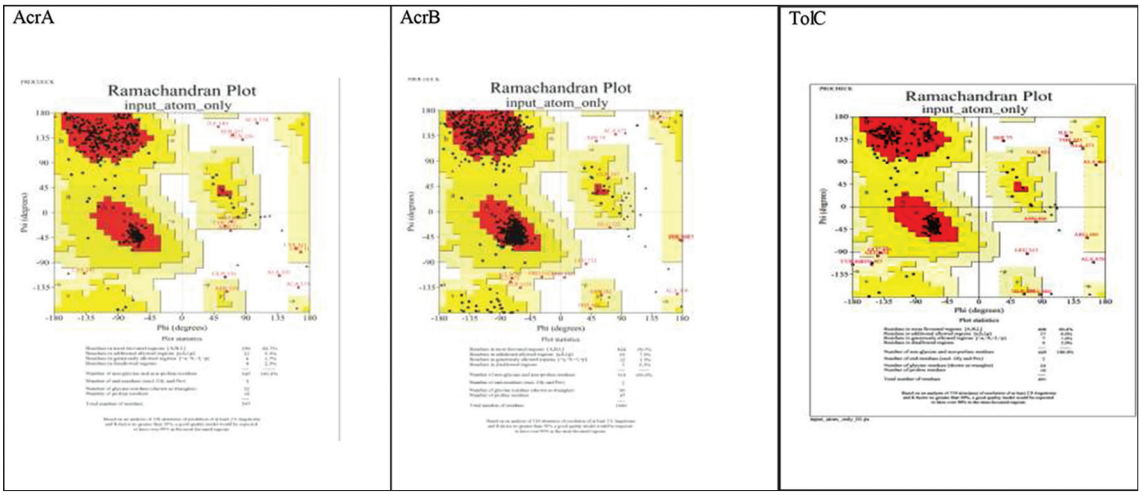


Fig. 1: Ramachandran plot of, (a) AcrA, (b) AcrB, (c) TolC

Table 3: Interaction analysis table of anticancer drugs used as ligands, amino acid numbers and energies

Anticancer compounds	Interaction analysis											
	AcrA				AcrB				TolC			
Etoposide	H-M SER 219 −3.2	H-M SER 220 −6.9	H-S SER 220 −4.3	H-M SER 273 −7	H-M GLN 34 −6 H-S SER 389 −2.5	H-M TYR 35 −2.5	H-S THR 37 −3.4	H-M GLY 296 −7	H-M GLU 1 −7.4	H-M ALA 194 −5.4		
Paclitaxel	H-M GLN 218 −6.6	H-M ILE 274 −2.5	H-M LEU 276 −10.5	H-S ASP 101 −2.5 H-M VAL 172 −4.8	H-S GLN 108 −3.5 H-M GLY 173 −5.5	H-M GLN 125 −2.5	H-M VAL 127 −5.5	H-M THR 109 −6.9	H-S THR 109 −3.3	H-M GLU 409 −10.3		
Mitomycin	H-M GLN 218 −4.5 H-S SER 273 −3.5	H-M SER 219 −3.6	H-S SER 219 −3.3	H-S ASP 222 −4.9	H-M PRO 40 −2.5 H-S SER 135 −3.6	H-M ALA 42 −3.5 H-S THR 295 −2.5	H-M SER 134 −2.6	H-S SER 134 −3.5	H-M GLU 1 −6	H-S GLU 1 −3.5	H-M TYR 113 −2.5	H-M ALA 194 −6.9
Thalidoamide	H-M GLN 218 −2.5 H-S SER 220 −4	H-S GLN 218 −4.2 H-M SER 273 −4.3	H-M SER 219 −4.3 H-M ILE 274 −6.4	H-M SER 220 −5.7	H-M ILE 38 −3.5 H-M THR 295 −3.5	H-M ALA 42 −3.5 H-S THR 295 −6	H-M SER 132 −5.7 H-M GLY 296 −4.4	H-M ALA 294 −5 H-M ALA 297 −3.1	H-S ASN 2 −8.1	H-S ASN 416 −8.1		
Vinblastine	H-M LEU 276 −5.5				H-M ILE 235 −3.5				H-S GLN 5 −2.6	H-S GLN 8 −3.5	H-S GLN 9 −3.5	H-S ASN 417 −3.5
Methotrexate	H-S SER 220 −2.5	H-M ASP 222 −4.3	H-M SER 273 −7	H-M ALA 47 −3.5 H-M VAL 87 −7 H-M ARG 767 −3.5	H-M ASN 81 −3.5 H-S GLN 88 −4.5	H-S ASP 83 −3.7 H-S LYS 89 −7	H-M THR 87 −4 H-S ASP 163 −2.5	H-S TYR 190 −5.1	H-M ALA 194 −7			

H-S: hydrogen bond with side chain, H-M: Hydrogen bond with main chain

Table 4: Interaction analysis table of putative EPI

Compounds	Interaction analysis											
	AcrA				AcrB				TolC			
NOR	H-S GLN 218 −9.5	H-M SER 219 −2.5	H-S SER 220 −2.5	H-M ASP 222 −3.5	H-M SER 84 −3.5 H-S GLN 577 −6.4	H-S SER 84 −5 H-S ASN 623 −3.4	H-M THR 85 −3.5 H-S GLU 722 −2.5	H-S THR 85 −2.5	H-M LEU 3 −4.9 H-S GLN 8 −4.9	H-M GLN 5 −7 H-S GLU 409 −5.2	H-M TYR 7 −3.5 H-S ASN 416 −2.5	H-M GLN 8 −3.5
Reserpine	H-M GLN 218	H-M SER 219	H-S SER 219	H-S ASP 222	H-M THR 37 −2.6				H-M GLN 5 −4.3	H-S GLN 5 −3.5	H-M GLN 8 −3.5	H-S GLN 9 −3.5
Verapamil	H-S	H-S	H-M	H-M	H-M				H-M	H-S	H-S	H-M

Contd...

Table 4: Contd...

Compounds	Interaction analysis											
	AcrA				AcrB				TolC			
TMP	ASP 222 −2.5 H-S GLN 218 −2.9	SER 273 −3.5 H-M SER 219 −7	GLY 296 −4.3 H-M SER 220 −7	PRO 669 −3.5 H-S SER 220 −3.5	VAL 672 −3.3 H-M ILE 38 −3.5 H-S ASP 101 −2.5	H-M ALA 39 −3.9	H-S SER 96 −3.5	H-S ASP 99 −3.8	GLN 5 −7 H-M GLU 1 −3.5	GLN 8 −3.3 H-M TYR 113 −7	GLU 409 −3.5	ALA 414 −3.5

H-S: Hydrogen bond with side chain, H-M: Hydrogen bond with main chain, TMP: Trimethoprim, NOR: Norepinephrine, EPI: Efflux pump inhibitors

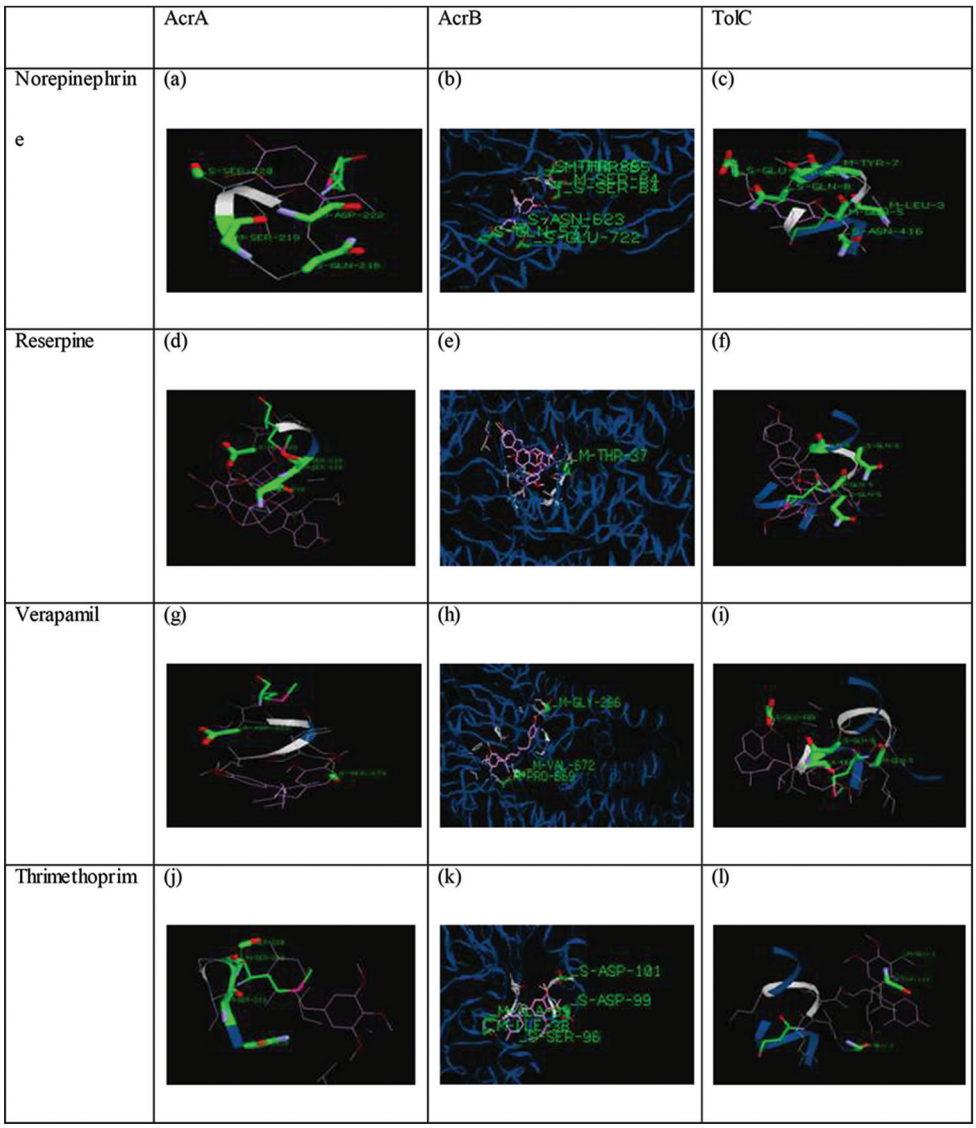


Fig. 2: Different docked poses of putative efflux pump inhibitors with protein subunits (a) AcrA, (b) AcrB and (c) TolC

(−87.57 kcal/mol and −74.61 kcal/mol respectively). All putative EPI showed non-overlapping amino acids (Table 2) in different binding pockets on AcrB subunit.

With TolC protein subunit the calculated docking energies of drug ligands: Paclitaxel, vinblastine and methotrexate (−88.29 kcal/mol, −77.28 kcal/mol and −74.52 kcal/mol respectively) were found comparable to putative EPI: Reserpine and verapamil (−90.22 kcal/mol and −89.42 kcal/mol respectively) whereas other tested drugs: Etoposide, tamoxifen, mitomycin and thalidomide (−68.42 kcal/mol, −64.69 kcal/mol, −68.28 kcal/mol and −59.36 kcal/mol respectively) showed proximity with putative EPIs: NOR and TMP (−78.49 kcal/mol, −62.57 kcal/mol

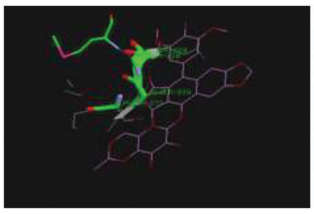
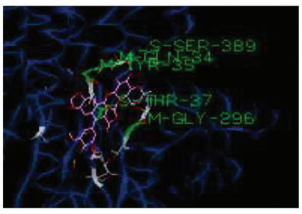
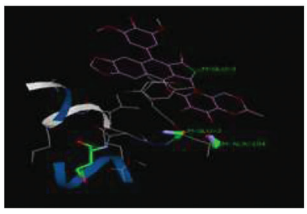
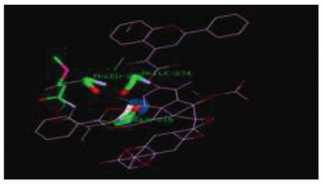
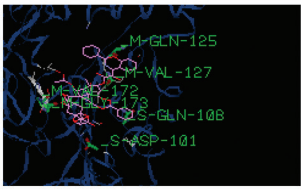
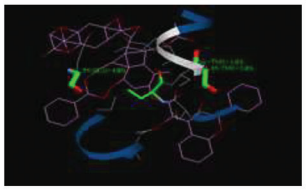
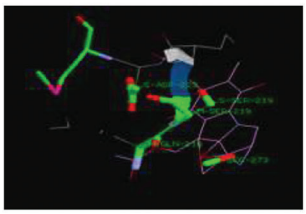
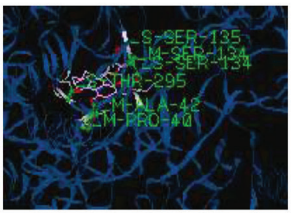
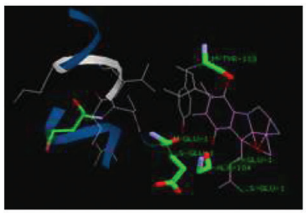
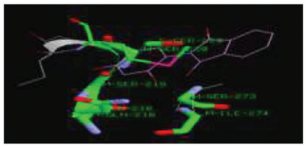
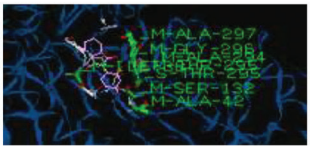
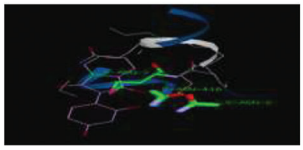
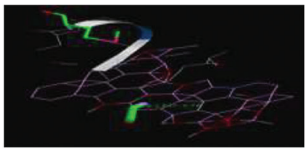
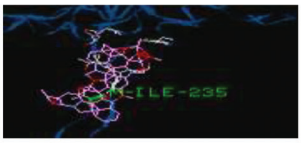
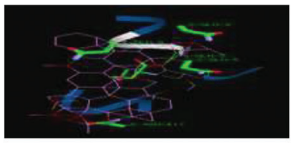
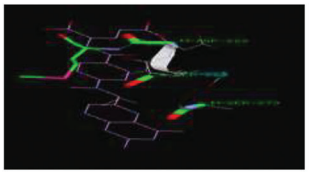
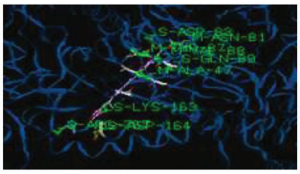
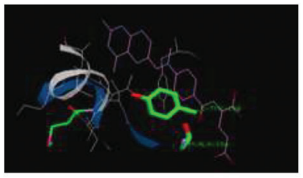
	AcrA	AcrB	TolC
Etoposide	(a) 	(f) 	(k) 
Paclitaxel	(b) 	(g) 	(l) 
Mitomycin	(d) 	(i) 	(n) 
Thalidomide	(e) 	(j) 	(o) 
Vinblastine	(p) 	(q) 	(r) 
Methotrexate	(s) 	(t) 	(u) 

Fig. 3: Different docked poses of anticancer drugs as ligands with protein subunits (a) AcrA, (b) AcrB and, (c) TolC

respectively). All putative EPI showed overlapping amino acids (Table 2) in binding pockets on TolC subunit.

Thus various anticancer drug ligands used in present study show *in silico* evidences that they interact with different units of AcrAB-TolC

of *E. coli*. Each drug ligand forms hydrogen bonding with a number of amino acids present in AcrA, AcrB and TolC (Tables 4 and 3). Hydrogen bonding is central in defining the drug ligand interactions and high negative energy reflects thermodynamically favorable bonding [38]. A number of EPI; peptidomimectics (phenylalanine arginyl b-naphthylamide) for *P. aeruginosa*, quinoline derivatives for *Enterobacter aerogenes, Klebsiella pneumoniae*, phenylpiperidine for *E. coli*, pyridopyrimidine for *P. aeruginosa*, arylpiperazine for *E. coli* are currently being used as an adjuvants along with antibiotics. Recently, a synthetic compound tetraphenyl phosphonium was evaluated as RND EPI in *S. typhimurium*. New class of broad spectrum EPI; MC-02,595 and MC-04,124 are available and higly effective against *Streptomyces coelicolor.* Isobavachalcone and diospyrone are two examples of EPI obtained from natural products [39] and found effective against *E. coli, E. aerogenes, K. pneumoniae, P. aeruginosa.*

Currently limited number of publications are available which explores the potential of anticancer drugs as EPI. Celecoxib, TPZ and raloxifene are few of those anticancer drugs which potentiates the effect of antibiotics by directly interacting with the efflux pumps of bacterial pathogens.

CONCLUSION

Out of seven tested drug ligands used in present study (etoposide, paclitaxel, tamoxifen, mitomycin and thalidomide, vinblastine and methotrexate) paclitaxel and vinblastine showed good affinity for all subunits of AcrAB-TolC efflux protein of *E. coli* and therefore can be used as EPI in combination with antibiotics whereas methotrexate, etoposide, tamoxifen, mitomycin and thalidomide produced better docking scores with AcrB and TolC comparable with values obtained with the known EPI (reserpine, TMP, NOR and verapamil). Combinations of antibiotics with paclitaxel and vinblastine may further be tested in *in vitro* and *in vivo* model to validate the hypothesis.

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