Since 2002

3D-Pharmacophore and Molecular Docking Studies for AcrAB-TolC Efflux Pump Potential Inhibitors from DrugBank and Traditional Chinese Medical Database

Thien-Vy Phan, Cao-Hoang-Hao Nguyen, Vu-Thuy-Vy Nguyen*

Department of Pharmacy, Nguyen Tat Thanh University, Ho Chi Minh, Vietnam

Abstract

BACKGROUND: Nowadays, antimicrobial resistance rates in Gram-negative bacteria are increasing rapidly and efflux pump has been found to be related to multi-drug resistance in clinical isolates. Due to the widespread resistance to several antibiotics, the AcrAB-TolC tripartite efflux pump is the primary multidrug efflux system of *Escherichia coli*. One of the most promising therapies is the combination using of antibiotics and efflux pump inhibitors (EPIs), which can be discovered by *in silico* approaches.

AIM: This study aims to create virtual screening models, which has predicted capabilities for the efflux pump inhibitory effects of candidates from the DrugBank and Traditional Chinese Medical databank.

MATERIALS AND METHODS: The pharmacophore models were developed by MOE 2015.10 software using a database of 119 EPIs discovered in 12 research publications. The binding site was found on the AcrB protein (PDB: 4DX7) by LeadIT 2.0.2 software that corresponds to the hydrophobic trap in the distal pocket.

RESULTS: The best pharmacophore models had the sensitive, specificity, and accuracy values over 90%. The potential inhibitors, which satisfied the pharmacophore model and had docking scores under −20 kJ/moL have been established. In which, traditional Chinese medical (TCM)_20290, DB00303, DB04642, DB08116, TCM_29530, and 2,5-dimethyl-3-O-D-glucopyranosyl-naphthol have the best docking scores of −32.76, −26.59, −26.14, −25.62, −24.88, and −22.82 kJ/mol, respectively.

CONCLUSIONS: Based on the virtual screening result, six compounds might be potential *E. coli* AcrAB-TolC EPIs. In the future, further *in vitro* and *in vivo* research should be required to confirm the effects of these compounds.

Introduction

In recent years, antimicrobial resistance based on a multi-drug efflux system has become more popular in both Gram-negative and positive bacteria. These systems are also known as transport proteins, which take an important role in transporting toxic agents, namely, antibiotics, biocides, and dyes to the outer environment. Some bacterial efflux pumps expelled special antibiotics, while others may transport a variety of compounds with various structural differences, creating a multidrug resistant phenotype [1], [2].

Due to the widespread, resistance to antibiotics such as chloramphenicol, fluoroquinolones, lipophilic β-lactam antibiotics, nalidixic acid, novobiocin, rifampicin, and tetracycline, *Escherichia coli* AcrAB-TolC tripartite efflux pump is currently one of the most common multi-drug efflux system [3]. This pump comprises the secondary transporter located in the inner membrane AcrB, the outer-membrane channel TolC, and the periplasmic protein adaptor AcrA, which connects two integral membrane proteins. Antibiotics enter the periplasm by diffusion through the lipid bilayer or through a porin channel and interact with the subtracted-binding pocket AcrB. The AcrB transporter, then, uses the proton gradient to extrude the compound into the TolC channel and then out of the cell [4]. Given the tripartite AcrAB-TolC complex's obvious involvement in the increase in antibiotic resistance in *E. coli*, the combination of using antibiotics and efflux pump inhibitors (EPIs) is a prospective approach to enhance the intracellular concentration of antibiotics, reduce the intrinsic bacterial resistance, widen the antibacterial spectrum, and restrict the emergence of antibiotic-resistant mutants [3], [5].

After the discovery of phenylalanine-arginine-β-napthylamide-the first EPI in 2001 [6], 100 of inhibitors were identified from various sources such as synthetic [7], [8], [9], [10], [11], [12], [13], [14], [15], medicines [16], [17], and natural compounds [18], [19], [20], [21], [22]. Frequently, the discovery of almost EPIs begins with *in vitro* antimicrobial assay on *E. coli* AcrAB or AcrB over-expressing strain, when the wild-type parent or efflux-deleted strain is used as a reference one. The result of antimicrobial assay is commonly the minimum inhibitory concentration (MIC) value. Further, testing has been done on the compound which is exhibited

little to no antimicrobial activity when used alone but has a synergistic effect with an antibacterial agent. In the studies, the substrate inhibition at the various concentrations, as well as a comparison of antibiotic efficacy in the presence and absence of the tested compound in the over-expressing strain, has been used to examine the inhibitory activity of novel EPIs. There is also known as the checkerboard assay with the result that is the value of minimal concentration of an EPIs required to decrease the MIC of an antibiotic by 2-fold (MPC$_2$), 4-fold (MPC$_4$), and the fluorometric efflux assay such as Ethidium bromide assay, Nile red efflux assay, and H33342 accumulation assay [23], [24].

Despite the increase in EPIs identification over the past decades, none of these were approved to use in infection treatment. Moreover, developing new drug is complicated process, which can take up to 15 years and cost an average of 2.6 billion dollars [25]. Beside that, the application of virtual screening on large databases can save a lot of time and money required for the discover of potential EPIs [26]. Therefore, the objectives of the research are building 3D-pharmacophore and docking models to predict potential efflux pump inhibition from DrugBank and Traditional Chinese medical (TCM) databank.

Materials and Methods

Data sources

In this study, total 119 compounds from 12 scientific articles were collected to build the *E. coli* AcrAB-TolC inhibitors pharmacophore models [7], [8], [9], [10], [16], [27], [28], [29], [30], [31], [32], and [33]. The database was divided into two set:

- The training set: Four compounds have binding affinity with AcrB efflux pump, which were determined by K$_d$ value (the equilibrium dissociation constant) below than 2 μM
- Testing set and ligand database: One hundred and fifteen compounds have the MPC$_4$ values on eight antibiotics such as levofloxacin, ciprofloxacin, chloramphenicol, erythromycin, clarithromycin, novobiocin, piperacillin, and rifampicin. Testing set was categorized into two data sets:
  - Data set 1: 42 active compounds with MPC$_4$ ≤10μM
  - Data set 2: 73 inactive compounds with MPC$_4$ >10μM

Pharmacophore approach

The pharmacophore models were built using MOE 2015.10 software (Retrieved from: https://www.

chemcomp.com/index.htm). The K$_d$ (μM) values were converted to pK$_d$ = −logK$_d$ to develop pharmacophore models. At the beginning, the Training and Testing set were minimalized energy by the *Energy Minimize* tool in MOE (Forcefield: MMFF94, Gradient: 0.0001 kcal/mol). Next, the low-energy conformations of the Training Set were generated by using the *Conformation Import* tool (Refine Conformation Limit: 10,000; Stochastic Search Failure Limit: 1000; Energy Minimization Iteration Limit: 1000; and Energy Minimization Gradient Limit: 0.0001). Then, 3D-pharmacophore models were built by the *Pharmacophore Elucidator* tool (Conformation: As-Is; Activity Field: pK$_d$), the pharmacophore queries were exported with indicators such as cover, overlap, and accuracy. Finally, the Testing set were used to evaluate previous pharmacophore queries using *Pharmacophore search* tool. The quality of pharmacophore models was assessed by basic values such as sensitivity, specificity, accuracy, true positives, false positives, true negatives, and false negatives and goodness of hit (GH) score. The model with GH score of 1 showed the best predictive ability with high selectivity and specificity which are defined by the retrieval of active and inactive compounds, respectively. The performance of the classification model was evaluated by parameters as follow:

$$\text{Accuracy} = \frac{TP + TN}{TP + FP + TN + FN}$$

Sensitivity (accuracy of positive prediction): The ratio of the true positive prediction results in all active substances.

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

Specificity (accuracy of negative prediction): The ratio of true negative prediction results in all inactive substances.

$$\text{Specificity} = \frac{TN}{TN + FP}$$

GH score for active substances (having effects):

$$GH = \frac{TP[(TP + FP) + (TP + FN)]}{2(TP + FP)(TP + FN)}$$

GH score for inactive substances (that have no effect): GH score is used for evaluating the model. The better the model is, the higher the GH score becomes [34].

$$GH = \frac{TN[(TN + FN) + (TN + FP)]}{2(TN + FN)(TN + FP)}$$

Docking study

AcrB structure

Protein code 4DX7, which was released on May 2, 2012, on Protein Databank (https://www.rcsb.org/search), was chosen for this research due to having the cocrystallization ligand (Doxorubicin), the resolution is 2.253 Å and represents wild-type strain.

Docking process

First, the protein was prepared using LigX function in MOE 2015.10 software, the process was described as follows: Verify the cocrystallized ligand, protonate, delete unbound water, and minimizing energy. Second, the binding site was determined from the cocrystallized ligand to a radius of 6.5 Å and protein is re-docked with Doxorubicin prepared the same as the ligands by LeadIT 2.0.2 software (Retrieved from: https://www.biosolveit.de). Third, ligands were drawn by Chemdraw 12.0.2 software and minimized energy 2 times by Sybyl-X 2.0 (Retrieved from: https://www.certara.com/sybyl-x-software/) (Method: Conj Grad; Termination: Energy Change 0.0001 kcal/(mol*A); Max Iteration: 10,000; Charges: Gasteriger-Huckel). Fourth, the compounds from ligand database were docked to the protein, scored, and ranked by LeadIT. The docking process was, then, performed with followed parameters: The number of poses: 10, the maximum number of solutions per iteration: 1000, the maximum number of per fragmentation: 200, and the remaining parameters were at default values. Finally, the result was evaluated by docking scores which are based on the interaction between ligand and protein such as ion bonding, hydrogen bonding, and Van der Waals, π–π bonding. The docking result indicated not only the affinity bond between protein and ligand but also the interaction between ligand and residues of the binding site. Moreover, the docking result was used to research the accordant binding cavity [35].

Database and virtual screening

Screening database was collected from TCM with 57,423 compounds (Available from: http://tcm.cmu.edu.tw/) and DrugBank database with 8,823 compounds (https://go.drugbank.com/access date 30/05/2020) which are belonging to six different groups namely "approved," "experimental," "investigational," "nutraceutical," "withdrawn," and "illicit." All TCM compounds that pass-through Lipinski's rule of five and DrugBank database were screened by the pharmacophore models. The ligand structures were prepared and minimized energy using Sybyl-X to ready for dock. Docking results were analyzed to discover the most potential inhibitors.

Results

3D-pharmacophore models

Based on the Training set, 11 pharmacophore (PH4) models were built by *Pharmacophore Elucidator*. The result of the 11 PH4 models and descriptors is shown in Table 1.

Table 1: The result of 11 pharmacophore models and descriptors

Model	Feature	Overlap scoring	Accuracy scoring	Factors	Aro/PiR	Hyd	Acc2
PH 4_1	RHHH_1	2.56	1	4	1	3	0
PH 4_2	RHHHa_1	2.38	1	5	1	3	1
PH 4_3	RHHa_1	2.36	1	4	1	2	1
PH 4_4	HHHa_5	2.18	1	4	0	3	1
PH 4_5	HHHa_1	2.16	1	4	0	3	1
PH 4_6	HHHa_4	2.11	1	4	0	3	1
PH 4_7	HHaa_3	2.09	1	4	0	2	2
PH 4_8	HHHa_2	2.06	1	4	0	3	1
PH 4_9	HHHa_3	2.05	1	4	0	3	1
PH 4_10	HHaa_2	2.01	1	4	0	2	2
PH 4_11	HHaa_1	1.90	1	4	0	2	2

The PH4 models of AcrB EPIs consisted of features: aromatic center (Aro)/ring center (PiR), hydrophobic centroid (H), and H-bond acceptor projection (Acc2).

The Testing set was used to evaluate all the PH4 models, and the results are shown in Table 2.

According to the result table, the sensitive, specificity, and accuracy of PH4_5, PH4_9, and PH4_11 models were higher than 90% and the GH scores of those were excellent than the others. Therefore, PH4_5, PH4_9, and PH4_11 models were applied to virtual screening database. The distance of three models is displayed in Figure 1.

Table 2: Evaluation result of 11 pharmacophore models

Models	Features	Active (42)		Inactive (73)		Sensitive (SE) (%)	Specificity (SP) (%)	Accuracy (ACC)	GH score (%)	
		TP	FN	FP	TN				Active	Inactive
PH 4_1	RHHH_1	38	4	16	57	90.48	78.08	82.61	80.42	85.76
PH 4_2	RHHHa_1	37	5	8	65	88.10	89.04	88.70	85.16	90.95
PH 4_3	RHHa_1	38	4	11	62	90.48	84.93	86.96	84.01	89.44
PH 4_4	HHHa_5	39	3	13	60	92.86	82.19	86.09	83.93	88.71
PH 4_5	HHHa_1	38	4	7	66	90.48	90.41	90.43	87.46	92.35
PH 4_6	HHHa_4	37	5	7	66	88.10	90.41	89.57	86.09	91.68
PH 4_7	HHaa_3	39	3	17	56	92.86	76.71	82.61	81.25	85.81
PH 4_8	HHHa_2	10	32	11	62	23.81	84.93	62.61	35.71	75.44
PH 4_9	HHHa_3	38	4	7	66	90.48	90.41	90.43	87.46	92.35
PH 4_10	HHaa_2	32	10	9	64	76.19	87.67	83.48	77.12	87.08
PH 4_11	HHaa_1	38	4	7	66	90.48	90.41	90.43	87.46	92.35

TP: True positives, FP: False positives, TN: True negatives, FN: False negatives, GH: Goodness of hit.
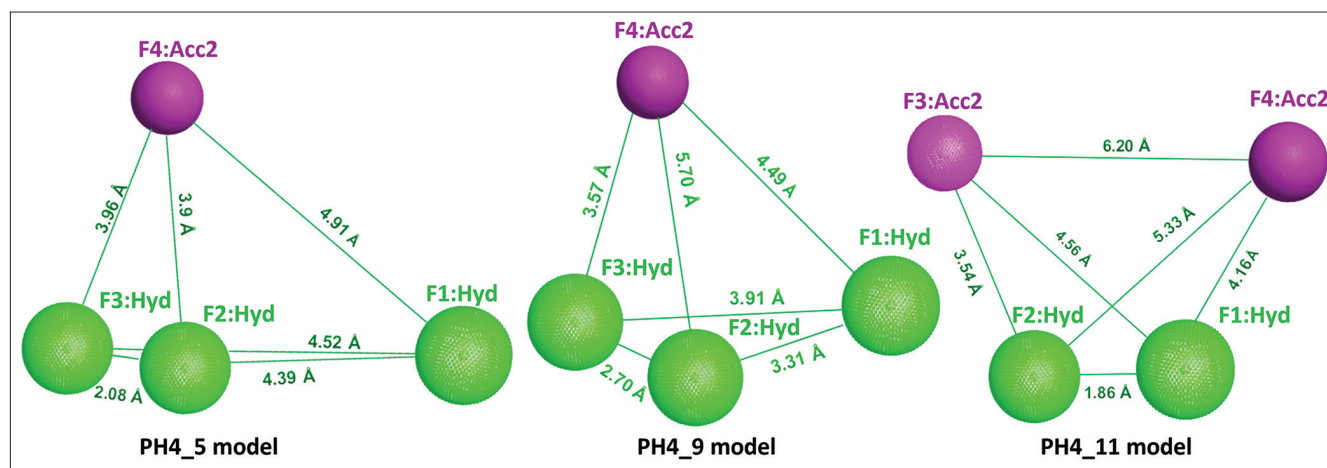
Figure 1: The distance of three models, green: hydrophobic groups (Hyd) and pink: hydrogen bonding acceptors (Acc2)

Molecular docking study

Determining binding site

The binding site was determined by *Prepare receptor* tool of LeadIT 2.0.2 and displayed in Figure 2. The binding site was contained some residues: Phe 136, Gln 151, Gln 176, Leu 177, Phe 178, Ser 180, Ile 277, Phe 610, Phe 615, and Phe 628. According to Jamshidi *et al*., in 2018, these residues corresponded to the distal pocket [36]. The residues Phe 136, Phe 178, Phe 610, Phe 615, and Phe 628 formed the hydrophobic trap.

Redocking results

Doxorubicin successfully docked into the binding site and had docking scores of −21.86 kJ/mol.

Docking results

Total 115 EPIs were docked successfully and had docking scores from −31.59 to −8.69 kJ/mol. The best pose was recorded and analyzed interacted residues using the *PLIF* tool of MOE. The results are showed in Figure 3. The surface contacts were formed between EPIs and Phe 615 (92%), Phe 178 (88%), Glu 176 (78%). In addition, Glu 176 was also interacted with EPIs by hydrogen bonds (55%).
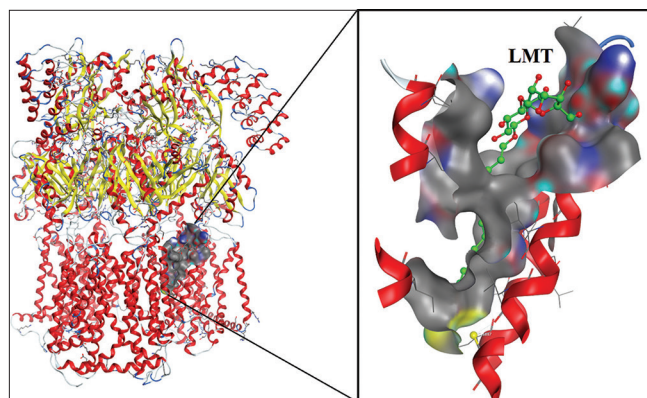

Figure 2: The binding site of AcrB (PDB: 4DX7) is determined within a radius of 6.5 Å from the cocrystallization ligand (LMT: Doxorubicin) at chain B

JMC_2017_60_14_6205_9E had the best docking score (−31.59 kJ/mol). The nitro and ceton groups of this EPI were formed hydrogen bonds with residues Gly 179 and Gln 176; while the amin group was given a donor hydrogen bond with Ile 277. This EPI also shared hydrophobic interactions with surrounding residues, such as Phe 615, Gln 89, Ile 277, Gly 178, and Gln 176 (Figure 4).

The hydrophobic trap was the main binding region at this site. Important residues in this region to form hydrophobic contact were Phe 178 and Phe 615. To be attached to this site, the structure might contain hydrophobic groups. Based on previous analysis, compounds that had low docking scores contain hydrophobic ring systems and nitrogen heterocyclic such as pyrimidine, nitrothiophene carboxamides, pyranopyrimidine, naphthamide, and phenothiazine. Besides that, to increase the binding affinity, it was necessary to have more groups that can create hydrogen bonds.

Important residues forming hydrogen bonds were Gln 176 and Gly 179. The ketones, N polar, primary amine, nitro heterocyclic, methoxy, hydroxyl, quaternary amine salts, and piperazine group [3] also confirmed this hypothesis.

Virtual screening

Pharmacophore model screening application

According to pharmacophore results, three selected models would be used to screen. With TCM database, there were 18,435 compounds met Lipinski's rule of five. The process and summarized results of TCM and DrugBank compounds, which had RMSD value below than 0.5 are displayed in Figure 5.

Docking model screening

The compounds of DrugBank and TCM database that satisfied the previous PH4 models were performed with energy minimization according to the method
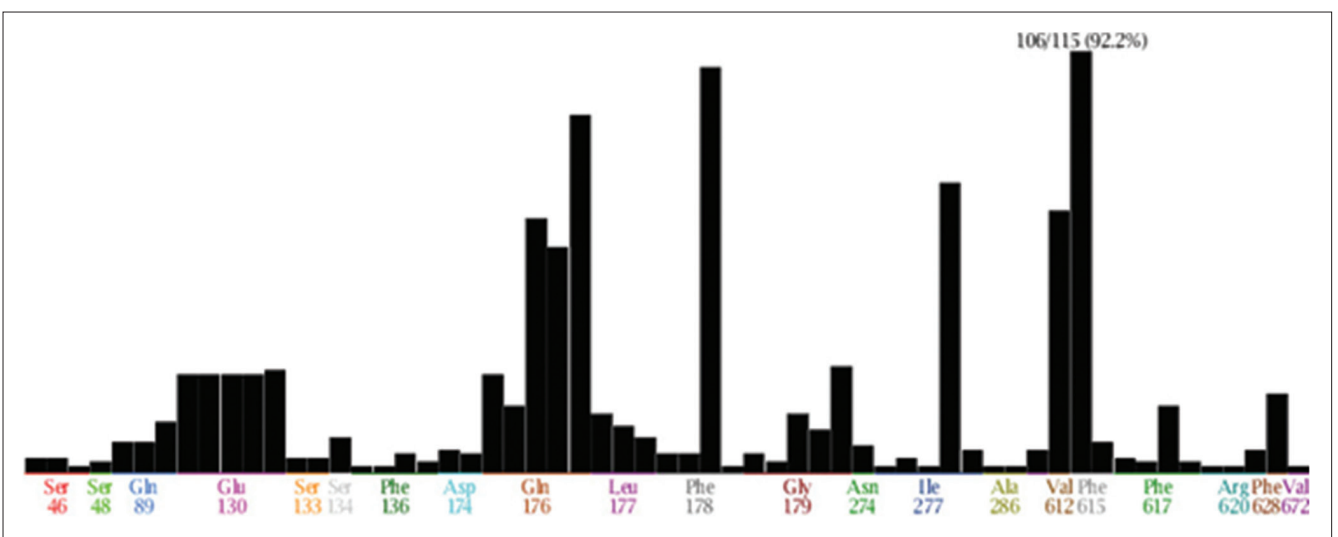
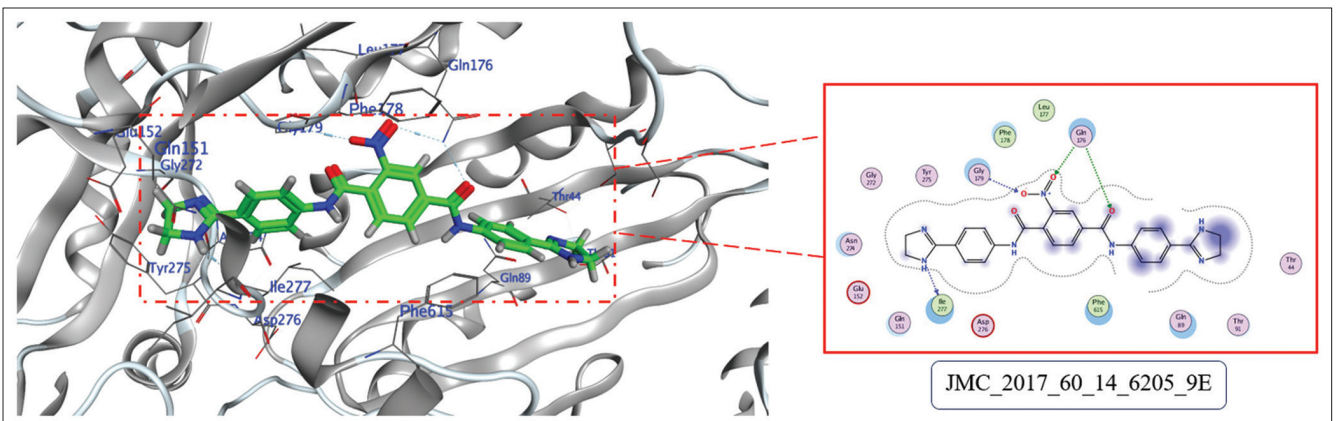Figure 3: Interaction between residues in binding site and EPIs

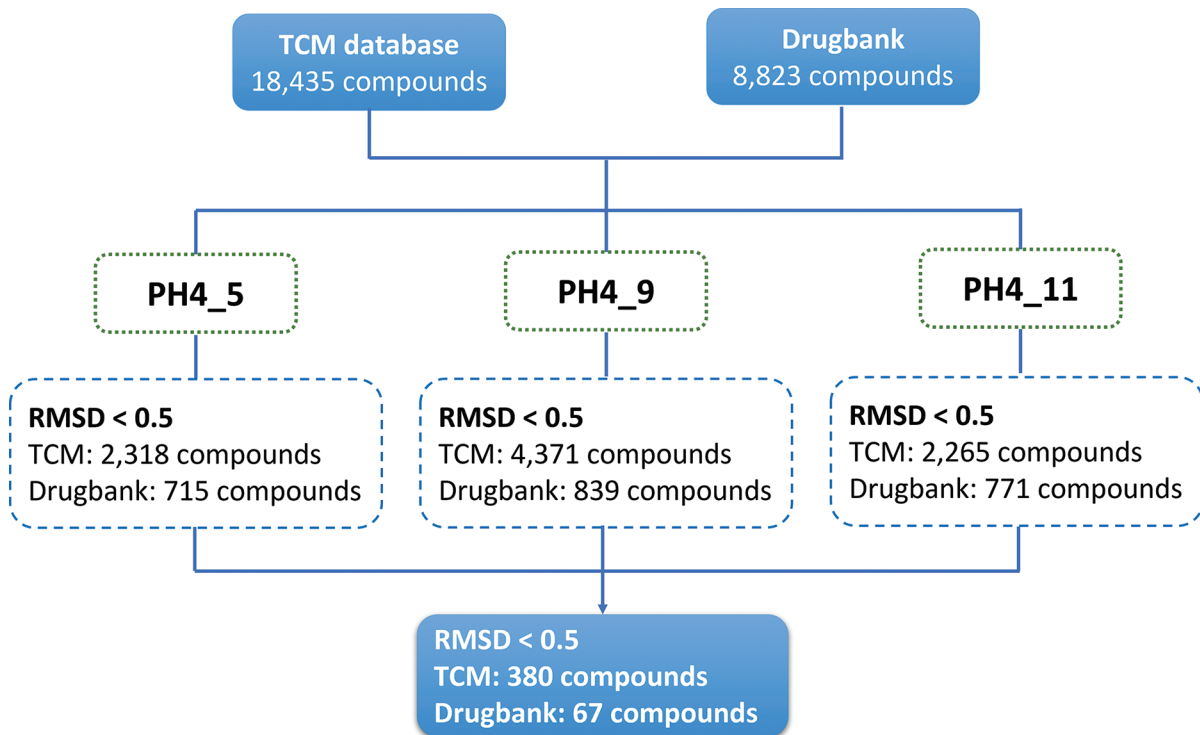Figure 4: JMC_2017_60_14_6205_9E and the residues in AcrB binding site (PDB: 4DX7)

Figure 5: Pharmacophore virtual screening result of TCM and DrugBank database

chapter. Then, the conformations were saved as *.sdf file and docked to the binding site by LeadIT 2.0.2 software.

The results showed that the DrugBank dataset had 63 compounds that successfully docked to the binding site with scores from −26.59 to 9.18 kJ/mol. The seven compounds which had docking score under −20 kJ/mol are DB00303, DB04642, DB08116, DB01184, DB15071, DB02009, and DB04753. Top three compounds were DB00303, DB04642, and DB08116 that had the best docking scores of −26.59, −26.14, and −25.62 kJ/mol, respectively (Figure 6).

Similarly, the docking results with the TCM dataset demonstrated that 368 compounds docked into the binding site with scores from −32.76 to −1.78 kJ/mol. TCM_20290 (−32.76 kJ/mol), TCM_29530 (−24.88 kJ/mol), and 2,5-dimethyl-3-O-D-glucopyranosylnaphthol(−22.82kJ/mol)

were the top three compounds that docked optimally to the binding site (Figure 7). In addition, TCM_4631, Helibisabonol, (+)-3,4-(6",6"-dimethyldihyropyrano)-4',5'-[2'"-(1-hydroxy-1-methylethyl)-dihydrofurano]-2',3'"-dihyroxydihydrochalcone, TCM_32141, Enanderianin_H, TCM_5226, TCM_30642, and TCM_36988 also had good docking scores, below −20 kJ/mol.

Finally, potential compounds which were screened from pharmacophore and molecular docking models formed surface binding to the residues Phe 178, Phe 615, Gln 176, and Gly 179 at the binding site. At the same time, these compounds also formed acceptor and donor hydrogen bonds with residues such as Gln 176 and Gly 179. This was completely consistent with the molecular docking results of previously known EPIs.
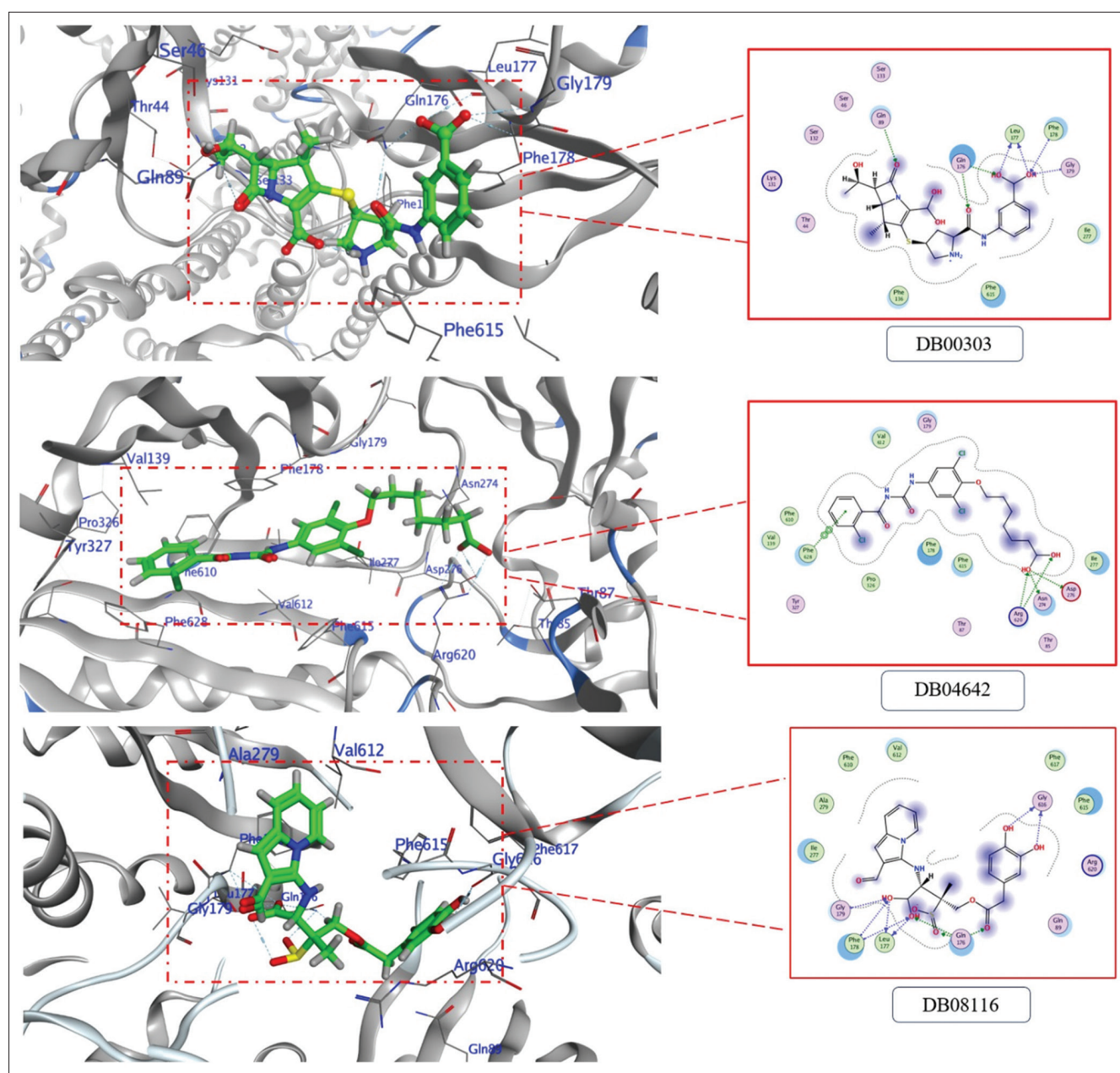

Figure 6: The interaction of DB00303, DB04642, and DB08116 with the residues in binding site AcrB (PDB: 4DX7)
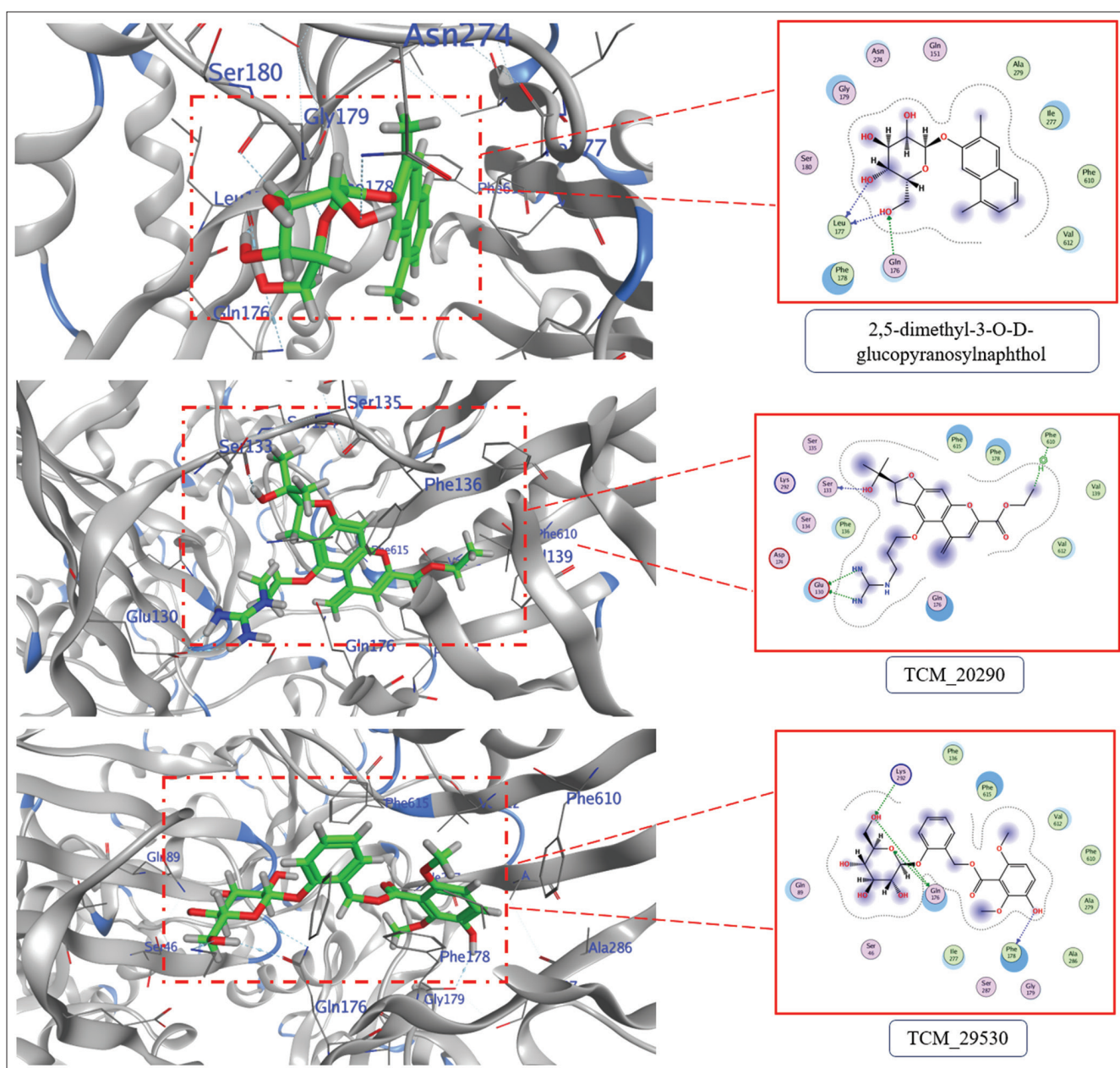
Figure 7: The interaction of TCM_20290, TCM_29530 and 2,5-dimethyl-3-O-D-glucopyranosylnaphthol with the residues in binding site AcrB (PDB: 4DX7)

Discussion

In this study, the collected database from 12 scientific articles was used to develop the new 11 *E. coli* AcraB-TolC inhibitors pharmacophore models. In general, pharmacophore models consisted of features: H-bond acceptor, hydrophobic centroid, and aromatic center | Pi ring center, and these features are suitable for the hydrophobic trap of AcrB. The top three models with the best evaluated result were used for virtual screening.

Based on the structure of X-ray diffraction (4DX7), the binding site was identified, corresponding to the hydrophobic trap in distal pocket [36]. Major inhibitors of AcrB pump were successfully docked into the binding site, and some of them had a good docking score. The docking result was determined Phe 178, Phe 615, Gln 176, and Gly 179 which were the most interacted residues. Phe 178 and Phe 615 generated hydrophobic interaction, meanwhile, Gln 176 and Gly 179 formed hydrogen bonds with ligands. Hydrophobic interactions and hydrogen bonds were the primary interactions in the binding site. Therefore, the potential compounds might contain hydrophobic groups to form hydrophobic interaction and atoms to create hydrogen bonds.

After conducting the virtual screening on DrugBank and TCM database, the result obtained six compounds that were potential EPIs for further studies. In there, DB00303 has generic name as Ertapenem. Ertapenem is a carbapenem antibiotic used for the treatment of moderate-to-severe bacterial infections and

could be a substrate or an inhibitor of the AcrAB-TolC pump. The generic name of DB04642 is 7-[2,6–dichloro–4-[3-(2–chloro-benzoyl)-ureido]-phenoxy]-heptanoic acid which belongs to the class of organic compounds known as n-benzoyl-n'-phenylureas. DB08116 belongs to the class of organic compounds known as d-alpha-amino acids (https://go.drugbank.com/drugs/). Among the potential TCMs, Helibisabonol A belongs to bisabolane sesquiterpenoids class which was found in *Helianthus annuus* [37]. The Enanderianin H is a ent-kauranoid isolated from *Isodon enanderianus* [38].

Conclusions

The efflux pump in bacteria that lead to antibiotic resistance is currently a serious global concern. Finding EPIs are critically necessary to reduce resistance and reinstate the usage of antibiotics. The use of virtual screening in drug design significantly saved time, labor, and financial expenditures. Based on pharmacophore and docking models, six potential inhibitors from DrugBank and TCM database were identified. Further, *in vitro* and *in vivo* research should be required to confirm the inhibitory effects of these compounds.

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