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Structure prediction and identification of potent inhibitors of Ompx Protein employing insilico approaches

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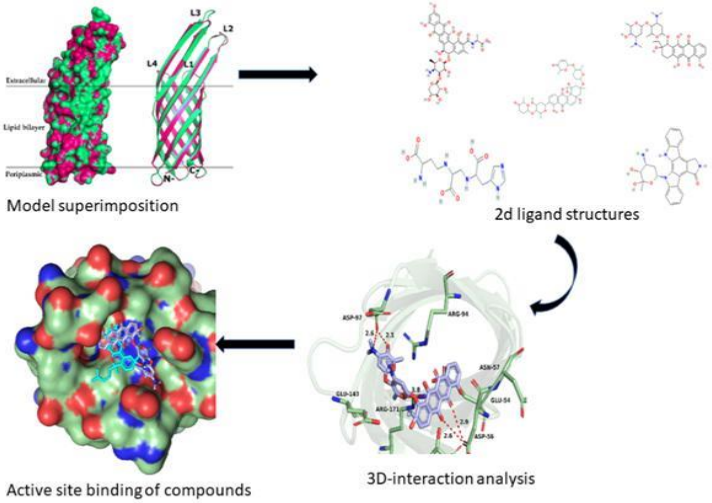
Outer membrane Protein X (OmpX) is naturally occurring in gram negative bacteria *Enterobacter Cloacae* which belong to family *Enterobacteriaceae.*E. Cloacaeis a causative agent in many diseases making its significant drug target in cancer treatment and even beyond. Secondary and three dimensional structures, visualization along with its physiochemical properties, sub-cellular localization, and molecular docking were performed. The SOPMA server predicted 2D structure Random coils detected as the pre-dominant (48.26%) following through extent strand (30.23%) and alpha helix (16.28%). Swiss model server explored three dimensional structure of model which has 83% sequence similarity to template 1QJ8. The Visualization result suggested that model has virtue in term of properties that may use for further study. In our study the novel potent inhibitors were identified from antibacterial compounds demonstrating to inhibit OmpX pathway to molecular docking approaches. The highest binding energies were identified in the range of -10.4792 to -16.4778Kcal/mol. The reported proposed inhibitors strongly bind into the active site which was predicted by site finder tools of MoE. It has been noticed that various residues GLN55,ASP56,ASP 97, and ARG171) essential for the retention of inhibitors in active pocket, and significant confirmation changes happens in the active site region as well as its neighbor following the entry of the ligand inside active pocket. Over all this study will facilitate the process of drug designing against OmpX protein and can be used in the development of potential and therapeutics against several diseases

Keywords: OmpX, EnterobacterCloacae, MoE, Molecular Docking, PyMOL

INTRODUCTION

(*Enterobacter cloacae*) is the member of Enterobacteriaceae family and categorized as a gram negative bacteriaum, with both aerobic as well as anaerobic characteristics. Enterobacter contains rod like shape with circular ends to induce respiratory infection and urinary tract infections (Acharya et al. 2019). It has been reported that positive association was observed among genotypes and species in clinical specimens as very minute reports were available on clinical spread of *E. cloacae*(Izdebski et al. 2015).The pathogenic bacteria *E. cloacae*has achieved from clinical relevance and has involved as either a nosocomial infection from high risk patience notably those on mechanical ventilation. It also causes extended-spectrum of β-lactamase producing (ESBL) ability (Mezzatesta et al. 2012)

Furthermore, E. Cloacaecomplex (ECC) is the major group of disease causing agent responsible for the production of many infections like pneumonia, septicemia and urinary tract infections. Due to their inherent ability of multidrug resistance, like resistant to carbapenemsmeropenem, imipenem, and ertapenem that have developed their potent significance in these organisms (Annavajhala and Gomez-Simmonds 2019).Naturally E. cloacae are resistant to many antibiotics including ampicillin, amoxicillin–clavulanic acid, cephalothin and cefoxitin and hence lower the production of group 1 cephalosporinase.



They have the potential to produce AmpC β-lactamases and as a result chromosomal gene expression is blocked or through carrying of amp C gene on plasmids cause resistant development to third-generation cephalosporins(Davin-Regli 2015).Based on scientific findings of *E.Cloacae* infections various diseases are caused such as bacteremia, skin, urinary tract, lower respiratory tract and intra-abdominal infections. It can also cause CNS and ophthalmic infections, endocarditis, septic arthritis and osteomyelitis as well. Due to the pathogenic nature of Enterobacter various infections that can impose extended hospitalization, diverse tests of imaging and laboratories, surgical events, influential and exclusive antimicrobial mediators (Mariscal and Fontanals 2003).*E Cloacae*infections are also conjoint in burn targets, immune compromised and malignant patients. Major infections are displayed as pulmonary and nosocomial urinary tract infections. Moreover, E. cloacae infection is also reported in polytetrafluoroethylene bypass graft. Occurrence of cloacae infections are also documented in worker of hospital backgrounds. Periodic cases of E. cloacae infections are associated with contaminated intravenous fluids, infant feedings, blood related items and cardioplegic suspension. Other possible way for nosocomial bacteremia is linked with heparin related fluids in intravascular devices (Musilet al. 2010).In Enterobacterclocae, OmpX proteins were identified that contains 172 amino acids residues in their structure. OmpX consist of N terminus structure comprised of 23 signaling amino acids. From sequence analysis of N terminal part of this protein, low molecular weight (from 15 to 18 kDa) having eight fold β-sheet structure domains were projected from the cell surface. Their active roles are reported in physiological activities with binding surface proteins, contributing in signal transduction, invasion, resistant to antibiotic and survival in epithelial cells (Stoorvogel*et al.* 1991). The overproduction of OmpX in bacteria was measured as abiotic stresses adaptive response. In hydrophilic proteins the long hydrophobic sections are deficient. We have illustrated a model having the folding ability in the surface membrane of OmpX protein. Amino acids residues are distributed within OmpX protein (Tommassenet al. 1988).

In this study, we have identified potential receptor protein from *E Cloacae*OmpX as a new target for structure-based Protein antibacterial interactions. The forecast protein model has been adequate in quality and quantity parameters and docked against known antibacterial commonly which were used in various diseases to obtain novel therapeutic agents (among the selected) that might target the OmpX that might be useful for controlling distinct diseases. The ecological toxicity analysis might be useful without toxic effect on non-target organisms. In the current study, in-silico toxicity assessment tool was utilized to extensively analyze antibacterial compounds. The goal of current finding is to assess the efficacy of putative inhibitors that might interact to the key residues of protein and inhibits its pathways and also to protect the organisms from diseases.

MATERIALS AND METHODS

1.2.1 Retrieval of protein sequence

The amino acid sequence of the OmpX of EnterobacterClocae under Accession number P25253 was retrieved from the Uniprot database in fasta format (https://www.uniprot.org/)(Pundir and Magrane 2015).

1.2.2 Analysis of Physiochemical Properties

The physiochemical properties of OmpX protein and its amino acid composition, negatively charged residues,

instability index, atomic composition, positively charged residues, weight, theoretical Pi, and grand average of hydropathicity were examined with use of PortParam tool (http://web .expasy.org/protparam) (Lafarga and O'Connor 2014).

1.2.3 Subcellular localization prediction

SOSUI (http://harrier.nagahama-i-bio.ac.jp/sosui/) server was used to predict subcellular localization of OmpX protein. The term "subcellular localization" refers to determining the precise location of OmpX within a cell (Sahay*et al.* 2020).

1.2.4 Secondary and Tertiary structures Predication

We followed protocol of self-optimized Prediction Method Alignment (SOPMA) (https://npsa-prabi.ibcp.fr/cgibin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) to explored secondary structure of targeted protein along with alpha helix, beta sheet and coils. DistanceP 1.0 was utilized to predict the protein distance constraints. The amino acid sequence of protein was provided to SWISS-MODEL (https://swissmodel.expasy.org/) in fasta format, for Three-dimensional structure prediction (Biasini and Bienert., 2014). Z-score of both model and template were calculated and superimposition were done by using PyMOL software (Wiedersteine*t al.* 2007).

1.2.5 ENERGY MINIMIZATION

To identify a more appropriate and stable structure of target protein, and ligands the energy minimization of three-dimensional structure were conducted using MoE default parameters (Vilar and Cozza2008).

1.2.6 Visualization and Quality assessment of 3D structure

ERRAT, VERIFY 3D tools of structural assessment and confirmation server SAVES (http://nihserver.mbi.ucla.edu/SAVES/) were utilized to analyzed quality of model at primary structure level (Selvamet al. 2017). The three-dimensional structure of model was assigned to Rampage server for Ramanchandran plot. The validation process of plot was successfully completed by PROCHECK server (Rani and Pooja 2018). In this process researchers are ingenious to recognize how much residues of model is locating in most favored, allowed and outlier regions. All the visual presentations were completed by using PyMOL software(Yuan et al.2017).

1.2.7 Retrieval of Antibacterial Compounds

An intensive literature strategy was followed for the selection of anti-bacterial compounds reported against bacterial diseases. Three-dimensional structures of compounds were retrieved from PubChem database in sdf format (Dashti and Wedell 2019). All ligands were optimized in Avogadro software to find out stable structure of compounds.

1.2.8 Molecular docking and interaction analysis

Prior to molecular docking, protein structure was prepared to eliminate all hetero atoms and minimize model structure. Molecular docking of protein for selected compounds was performed by using Molecular Operating Environment (MoE) software. The active site residues of protein structure were confirmed by using prankweb server and site finder tool of MoE. To locate the reported potential binding residues of OmpX protein is based on binding affinity and S-score of the compound were categorized. The best docked poses were chosen for interaction analysis, on the basis of active site binding, hydrogen bonding as well as highest binding affinity. The affinity of drug like molecules within the active site of OmpX protein contribute through 2D graph showing van deer Waals forces, hydrogen bonding, electrostatic interactions and hydrophobic interactions. 3D figure of docking complexes were generated by using PyMOL software

1.2.9 ADME Toxicity/Drug scan.

In the Screening trend two molecular input methods are available, providing a sequence of smiles or submitting an SDF or TXT file. Remember that molecules contain without column headers and indexes, elsewhere the server may declare invalid input type. Once all of the predictions have been finished, each input molecule's findings will be displayed on a distinct row, with the allocated index, SMILES string, 2D molecular structure, and a View button. The forecast information can be viewed by going to the single-molecule evaluation page and selecting the View button of the associated molecule. These findings may also be exported as a CSV file to the user's computer, with actual likely values of classification endpoints supplied to allow users to set their own thresholds to screen out inadequate substances with varying degrees of confidence (Xiong et al. 2021).

RESULTS

1.3.1 Physiochemical properties and Subcellular localization

We employed the ExPASy'sProtParam server to inspect the physiochemical properties of OmpX protein using its amino acid sequence. Most of calculations server displays protein steadiness and stability because the stability is recognized with its suitable function aptitude. Further, we investigated that protein overall comprise of 172 amino acids, having a molecular weight of 18653.67 Daltons while its isoelectric point (PI) is 7.73 representing a positively charged on protein. The instability index of protein was calculated 13.18, and categorized protein is stable. The negative GRAVY index is -0.347 which depicts that protein is hydrophobic and soluble in nature. The

utmost copious amino acid residue was detected to be Gly (22), Ala (17), Asn (14),Tyr (14) and Val (13) tracked by Ser (12) and Thr (36). The lowest was detected as Cys (1). The sequence comprises of 13 negatively charged residues (Aspartic acid + Glutamic acid) and 14 positively charged residues (Arginine + Lysine). The molecular formula of the protein was investigated as C826H1251N225O260S5 while the total number of atoms in the protein is 2567. Protein subcellular localization predictions comprise the computational expectancy of where a protein exists inside a cell. Envisaging unidentified protein shows the subcellular localization can give the evidence about their cellular purposes. In better comprehension of drugs designing and disease mechanism this evidence could be employed for better comprehension(Rabbi *et al.* 2021).The OmpX protein having one transmembraneous helix, that confirmed by using the PSORTb v3.2.0.

The subcellular location of the OmpX protein was determined using the SOSUI server, which revealed that the protein is membranous and has one transmembrane helix, which was confirmed using the PSORTb v3.2.0 and Predict Protein servers. The subcellular localization shown by OmpXproteinwas evaluated by SOSUI server and identified protein has one transmembrane helix and validated via PSORTb v3.2.0 and Predict Protein severs

1.3.2 StructureAnalysis, Quality assessment and validation of protein

The SOPMA server was utilized to explore the secondary structure of protein. The random coils were revealed to becomes the most frequent (48.26%) in structure precede by prolonged strand (30.23%) and alpha helix (16.28%) shown in fig 1a , additionally a beta turn of 5.23% was found. The Protein Distance Constraints Matrix was generated by employing DistanceP1.0 shown in Fig 1b. EasyPred 1.0 was employed for binding motif predication.

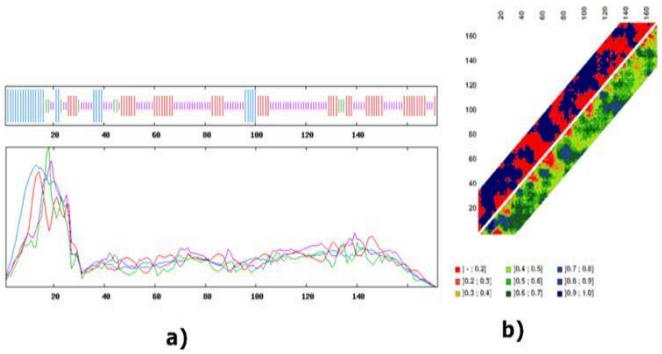


Figure 1: (a) represented the secondary structure of protein while (b) shown residues location which predicted by Distance P server

Swiss model performed Homology model of a protein structure based on its sequence alignment to one or more relevant protein structure. Whenever protein sequence provided to Swiss model server it can run BlastP hunt automatically to identified best templates for prediction. The quality of every recognized template has been assessed on the basis of topographies of target templates alignment. Afterwards the highest quality templates were chosen for model development. In this case PDB ID 1qj8 was adapted to the homology model because it having 83% sequence similarities. The ERRAT result examines the characteristics of non-bonded relationships among various atom groups, depending on typical atomic associations, which was used to validate the model's reliability. The total quality factor was calculated to be 76.8293, indicating that using this model is beneficial. Ramachandran plot analysis revealed that 91.8% of the protein's model structure's residues were in the favored area, with just 6.2 percent and 2.1% in the allowed and outlier regions, indicating that the model was more accurate and higher quality which is mention in figure 2b.

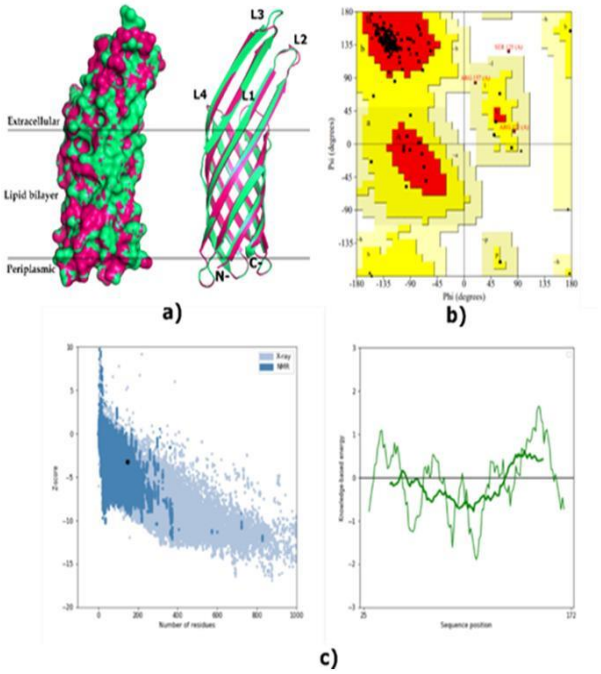


Figure 2: a superimpositions of model (green color) to template iqj8 (hot pink) color by using Pymol software, b Quality assessment of the model Ramachandran plot of model structure validated by PROCHECK program, and c represent z score of predicted model was validate by prosa web -3.02. and -3.25 model score

The Superimposition of predicted model onto template (PDB ID: iqj8) was done by using PyMOL which is shown in Figure 2a, RMSD value 0.068Å evidence that model has a superior quality. The Z-score analysis based on entire quality of model which can be used to assess structure fall in the range typically observed for native protein. The Z-score were founds from Prosa Web server,

OmpX model (-3.25) and template -3.2 suggesting similarities between template and model.

1.3.3 Binding interaction

A computer analytical approach called as molecular docking, that used to identify the binding pattern of various compounds to proteins. The ligands, which may acts as a chemical, docked into the protein that generally operate as a receptor. Molecular docking analysis revealed structural insight into the binding mechanisms of OmpX proteins and their inhibitors (Ferreira et al. 2015). Active sites residues of protein were identified by employing site finder tools of MoE, these residues were GLN55, ASP56, ARG171, ASP97, ASN58, ARG94 fells in the active region of OmpX structure. Docking of 40 antibacterial compounds into the active pocket of protein and its binding energies were computed. Only highly effective compounds were registered in (Table 1). Interaction of best compounds were performed and additionally subjected to Admet analysis. The binding energies of compounds were in the ranged from -16.4778 to -9.9029 kcal/mol. Antibacterial compounds Benanomicin B (-16.4778 Kcal/mol) tends to be at the top of list with respect to binding affinity measured using dock score approaches followed by Saquayamycin F (-15.7654 Kcal/mol) and Roseorubicin B (-14.3154 Kcal/mol) with a slightly lower binding affinity compared to Benanomicin B. It is one of the extrinsic compounds identified in the present study that binds efficiently to active pocket with high binding affinity. The Orientation of Benanomicin B made five hydrogen bonds interaction with site residues ASP97 and Arg171 at their respective bond lengths 2.34 and 3.4. it has been also involved in Van der Waals interactions with its nearest neighbors residues ARG94, ASN96, ASN140, ILE142 and GLU143. The H-bonds pattern were identified to be similar in these compounds Benanomicin B, saquayamycin F, Holyrine B and Dihydroxy-4-Hydroxymethyl-Acetanilide but varied in some feature such as binding energies. Saquayamycin F formed six hydrogen bonds with site residues ASP97 and ARG171 at distances. Furthermore, Holyrine B in turn to connect with three hydrogen bonds with residues ASP97 and Arg171 with their bond lengths while Dihydroxy-4-Hydroxymethyl-Acetanilide was observed to construct three hydrogen bonds with ASP97 and Arg171. Saquayamycin F makes maximum possible contacts with active site residues GLU54, ASP56, ASN58, PRO59, ARG94, TRP98, ASN140, PRO141 and GLU143 encapsulated both hydrophobic and vdw interactions. Other residues around Holyrine B facilitated by hydrophobic and vdw interactions were ARG94, TRP98 ASN140, ILE142 and GLU143 while ARG94 and GLU143 are the key residues which show vdw interaction around Dihydroxy-4-Hydroxymethyl-Acetanilide.

Subsequently, residues GLN55, ASP56, ASP97 and ARG171 formed hydrogen bonds with compounds Roseorubicin B (-14.3154) and Methoxydebromomarinone (-12.3488).

Roseorubicin B engaged with six hydrogen bonds while Methoxydebromomarin one was observed with three hydrogen bonds due to missing of residue ASP97 in the interaction pattern. The retention of both compounds inside the active pocket is strengthened by the residues THR25, GLU54, ARG94, ASP97 and GLU143 involved in strong hydrophobic and Van der Waals interactions. Furthermore, Amphistin compound observed with the maximum number of residues (GLN55, ASP56, ASN58, ARG94 and ARG171) which shows strong hydrogen bonds interactions with their respective distances. Apart from making strong hydrogen bonds, this compound are also involved in hydrophobic as well as Van der Waal interactions. The key residues that come in contact are GLU54, ASN57, ASP97 and GLU143.

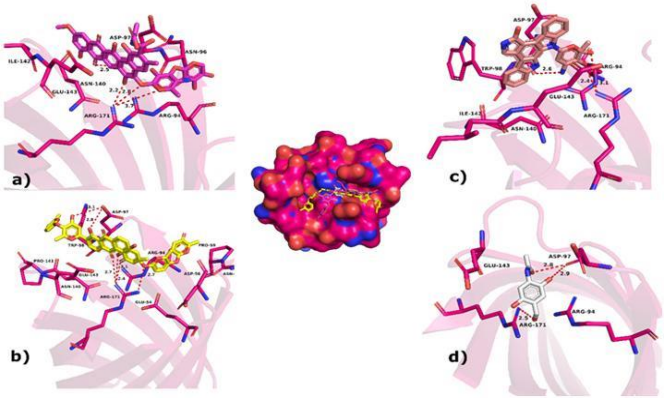


Figure 3:Three-dimensional structure of complex were illustrate in PyMOL software.

Hot pink dotted line represent hydrogen bonds, distances were also explored in figure. whereas the (fig a) represented Benanomicin B, (b) Saquayamycin F and (c) Holyrine B and (d) 2,5-Dihydroxy-4-Hydroxymethyl-Acetanilide

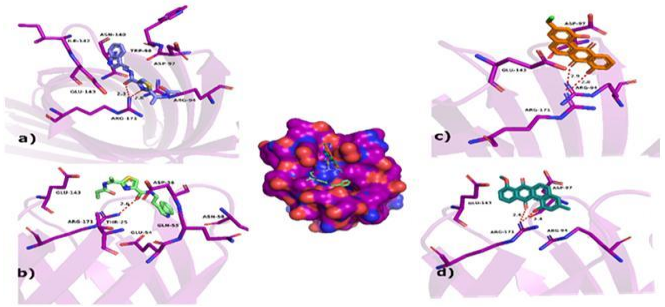


Figure 4: 3D interaction of the top ranked compounds in the binding site of OmpX.

The compounds and residues shown as sticks. The red dashed lines indicate the hydrogen bonds which are labeled with their respective bond lengths. (a) 2-(1-acetylamino-ethyl)-thiazole-4-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-amide (b) 2-(1-Propionylaminoethyl)Thiazole-4-Carboxylic Acid [2-(1h-Indol-3-Yl)Ethyl]Amide (c) hlorotetrangulol and (d) X-14881 E

Table 1: Top ranked antimicrobial compounds screened against OmpX. Active sites residueswith their respective docking score, interacting residues.

S.No	Compounds Name	s-score	H-Bond	Hydrophobic van deer waals interaction
1	Benanomicin B	-16.4778	ARG171, ASP97	ARG94, ASN96, ASN140, ILE142, GLU143
2	Saquayamycin F	-15.7654	ASP97, ARG171	GLU54, ASP56, ASN58 , PRO59, ARG94, TRP98, ASN140, PRO141, GLU143
3	Roseorubicin B	-14.3154	GLN55, ASP56 , ASP97, ARG171	THR25, GLU54, ARG94, GLU143
4	Holyrine B	-13.5458	ASP97, ARG171	ARG94, TRP98 ASN140, ILE142, GLU143
5	Amphistin	-13.4093	GLN55, ASP56, ASN58 , ARG94, ARG171	GLU54, ASN57, ASP97, GLU143
6	2-(1-acetylamino-ethyl)-thiazole-4-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-amide	-12.3830	ARG171	ARG94, ASP97, TRP98, ASN140, ILE142, GLU143
7	Methoxydebromomarinone	-12.3488	GLN55, ASP56, ARG171	THR25, GLU54, ARG94, ASP97, GLU143
8	2-(1-Propionylaminoethyl) Thiazole-4-Carboxylic Acid [2-(1h-Indol-3-Yl)Ethyl]Amide	-12.2880	ARG171	THR25, GLU54, GLN55, ASP56, ASN58, PRO59, ARG94, GLU143
9	hlorotetrangulol	-12.0948	ARG171	ARG94, GLU143
10	X-14881 E	-11.5791	ARG171	ARG94, ASP97, GLU143
11	2,5-Dihydroxy-4-Hydroxymethyl-Acetanilide	-10.4792	ASP97, ARG171	ARG94, GLU143

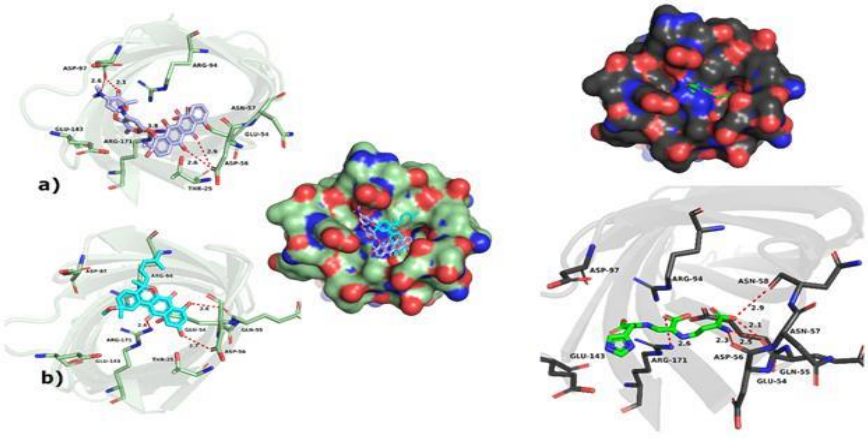


Figure 5: 3d interaction of docked complexes. Compounds and residues are shown in sticks whereas the dash dotted line represents hydrogen bonds. (a) Roseorubicin B (b) Methoxydebromomarinone and (c) Amphistin. The distances of hydrogen bonds were shown in every figure the cuttoff values of hydrogen bonds were 4 Angstrom

Table 2: AdmetSar properties of the Actinomyces Compounds

Model	Holyrine B	Amphistin	2-(1-acetylamino-ethyl)-thiazole-4-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-amide	Methoxydebromomarinone	2-(1Propionylaminoethyl) Thiazole-4-Carboxylic Acid [2-(1h-Indol-3-Yl)Ethyl]Amide	X-14881 E	2,5-Dihydroxy-4-Hydroxymethyl-Acetanilide
Absorption							
Caco-2 Permeability	-5.733	-6.512	-5.047	-4.931	-5.061	-4.990	-4.798
MDCK Permeability	3.8e-06	1.2e-05	1.4e-05	1.9e-05	1.6e-05	2.3e-05	3.1e-06
Pgp-inhibitor	- - -	- - -	---	+++	--	---	- - -
Pgp-substrate	+++	- - -	+++	- - -	+++	---	++
HIA	++	- -	- - -	- - -	- - -	---	- - -
F20%	-	- - -	---	-	- - -	---	+++
F30%	+++	- - -	+++	- -	+++	+++	+++
Distribution							
PPB	93.028%	11.556%	88.011%	99.086%	95.168%	98.999%	16.559%
VD	1.151	0.674	1.015	1.065	1.225	0.385	0.660
BBB Penetration	- - -	-	+	- - -	-	--	- - -
Fu	2.562%	89.994%	17.614%	2.430%	**5.266%**	1.040%	65.321%
Metabolism							
CYP1A2 inhibitor	++	---	+++	+++	+++	+++	--
CYP1A2 substrate	+++	- - -	-	+	-	++	--
CYP2C19 inhibitor	- -	---	+++	+ + +	+++	+	- - -
CYP2C19 substrate	- -	---	- - -	- - -	---	---	- - -
CYP2C9 inhibitor	++	---	+	++	++	+	- - -
CYP2C9 substrate	+	- -	++	-	+	++	+
CYP2D6 inhibitor	-	- - -	- -	+++	-	-	- - -
CYP2D6 substrate	-	- - -	+	- - -	-	+	- -
CYP3A4 inhibitor	+	- - -	++	+++	++	-	- - -
CYP3A4 substrate	--	- - -	- -	- -	-	- -	- -
Excretion							
CL	9.603	3.294	2.440	11.894	4.488	7.440	6.690
T1/2	0.221	0.825	0.391	0.107	0.375	0.090	0.949
Toxicity							
AMES Toxicity	+	---	---	-	- - -	++	+
Carcinogencity	- - -	---	---	+	- - -	+++	- - -

Interestingly, arg171 is observed in the interaction pattern of four different compounds 2-(1-acetylamino-ethyl)-thiazole-4-carboxylic acid [2-(1H-indol-3-yl)-ethyl]-amide (-12.3830), 2-(1-Propionylaminoethyl)Thiazole-4-Carboxylic Acid [2-(1h-Indol-3-Yl)Ethyl]Amide (-12.2880), hlorotetrangulol (-12.0948) and X-14881 E (-11.5791) by making hydrogen bonds in the activation segment of OmpXprotein.These compounds slightly reduces the binding affinity with target OmpX. These compounds comes into contact with THR25, GLU54, GLN55, ASP56, ASN58, PRO59, ARG94 and GLU143 through hydrophobicand Vander Waals interaction

1 .3.4. ADMET Lab 2.0 server

In the assesment pattern, two methods are offered searching the SMILES of compounds or designing the molecular structure with JMSE molecule editor. When a user submit a task by any methods the web server specifies the input data and calculate all of the output immediately. The web-server primarily displays prediction outcomes in tabular form along with 2D molecular graph characterizing the physio-chemical quality of compounds. Concrete prediction values are presented for endpoints predicted by regression models, such as Caco-2 permeability, plasma protein binding, and so on. The prediction possible value are turned into six symbols for outcome indicated by classification of models for instance Pgp-inhibitor, hERG Blocker so on. These symbols having distinct meaning in ADMET properties, 0-0.1 assigned (−−−) symbols, 0.1-0.3(−−), 0.3-0.5(−), 0.5-0.7(+), 0.7-0.9(++), and 0.9-1.0(+++).

2.3.4. ADMET Lab 2.0 server

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Generally the token '+++' or '++' indicating that compounds is much more likely to be toxic or defective, whereas '−−−' or '−−' signifies molecules is nontoxic or acceptable. we would not encouraged trusting on forecasts symbolled by '+' or '−' which are most likely values in the range of 0.3-0.7, and their related molecules needs to further investigation (Pires, Blundell et al. 2015). The ADMET properties of compounds, were determined using ADMETLAB 2.0 server, out of eleven 8 compounds satisfied Admet parameters whereas 3 compounds does not fulfill the ADMET criteria, which having higher molecular weight from others compounds.

DISCUSSION

In current study OmpX structure of *E.cloacae* was predicted having 83 % sequence similarity to template (ijq8). Furthermore predicted mode was visualized accordingly by using Ramachandran plot. Mostly residues lies in most favored region of plot which suggested our model is superior in quality. Similar to our findings other researchers have also investigated sequence similarity 86% of OmpX protein of E. cloacae with OmpK17 of K. pneumonia. OmpX Sequence similarity with other templates were 45%, 39%, 38% and 30% in Ail of c. enterocolitica while PagC and Rck from S. typhimurium and Lom from bacteriophage λ(Vogt and Schulz 1999)(Stoorvogelet al. 1991) find out that similar eight-stranded β barrel having elongated extracellular loops and tinyperiplasmicmembrane was reported in OmpX from E. cloacae.OmpA model fall in the range of limited information content (McClean 2012). In the present work physiochemical property of predicted model along with isoelectric point, instability and Gravy index were calculated these all analysis described that our predicted model was better in all aspects of parameters. Protein comprised of 172 amino acids in its structure and having 18656.67dalton weights. The isoelectric points (PI) of proteins was 7.73, instability index was measured (13.18) and its negative gravity index was calculated -0.347 these all parameters indicating that predicted model has a positive charged, stable, hydrophobic and soluble in nature. Parallel to our results, (Santhoshkumar and Yusuf 2020) reported physicochemical aspects of the CURS proteins. Based on molecular weight of CURS1, PI value was 4.93 and their aliphatic index was 99.19. Similarly molecular weight of other proteins i.e. CURS2 and CURS3 are 20266.13Da and 20629.52Da having PI 5.28 and 4.96 values. Their aliphatic index was reported as 89.30 and 86.37. Furthermore they have mentioned the predicted phosphorylation sites in CURS1, CURS2 and CURS3 proteinswith slight variation in their Ser, Thr, and Tyr residues (Santhoshkumar and Yusuf 2020). The subcellular localization showed that OmpX protein has present within the cell and consists of one trans-membrane helix. In correlation with our findings of subcellular localization of the AEG74552.1 protein gave information about their survival inside a cell by performing relevant functions. This protein might be expected to involve in cellular and enzymatic functions (Saad Ur Rehman 2019). In our research antibacterial compounds were docked to OmpX protein, all of compounds were binds to the active site residues of protein structure. The various residues were involved in multiple interaction for instances hydrogen bonds, hydrophobic as well as van

deer waals interaction. Various anti-microbial drugs are routinely used to control or inhibit the growth of infectious agents by blocking nucleic acid, protein and membranes synthesis (Liu and Sheng 2018). Various tests were performed for in vitro and in silico study of Penicillium setosumto find out their potent role through docking with antibacterial compounds of Penicilliumsetosum). The ADMETweb 2.0 protocol was utilized to examine ADMET property of compounds. This results revealed that 8 compounds were followed the lipenskiruke of five while 3 compounds Benanomicin B, Saquayamycin F, Roseorubicin B having higher molecular weight. For the determination of pharmacokinetic behavior of chalcone, various physicochemical properties like ionization, partition, solubility and distribution coefficients were carried out for ADMET. For their bioavailability as drug candidates, lipophilic compounds need to be evaluated rather than physiological medium (de Lange *et al.* 2005;Veber*et al.* 2002).

CONCLUSION

The current research addresses the critical suppression of the OmpX protein, because protein activity leading to human diseases. In addition, a molecular study approach was employed to inhibit OmpX activity and prevent wide range of various diseases. Therefore the present study the role of OmpX protein as a target for identification of possible therapeutic inhibitors of pharmacological interest. Similar inhibitors are identified for structurally distant OmpX protein focusing on multi-target drug approach. Out of 11 compounds Benanomicin, Bsaquayamycin F and Roseorubicin B compounds have not satisfy the lipenski's rule of 5, while rest of 7 inhibitors have been identified to perform functions by competing with OmpX active site residues of catalytic site binding. The formation of hydrogen bonds inside the active site of the protein is used as the major benchmark for assessing strong inhibitor binding. In contrast to the hydrogen bonds, hydrophobic as well as Van Der Waals interactions are the significant player in this system. It has been discovered that particular residues of protein structure (ASP 97, ARG171) and regions are essential for the stability of inhibitors in active pocket and major confirmation changes occurs in the active site region including its surrounding residues following the entry of the ligand inside active pockets. We may inferred, that 7 inhibitors have the potential as therapeutic agents against several diseases.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

BA, AA, and AS designed the study AG and AZ perform the experiments and also wrote the manuscript. AS,HN and TM run the tools and data analysis. ZA and HA reviewed the manuscript. All authors read and approved the final version.

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