Tropical Journal of Pharmaceutical Research November 2021; 20 (11): 2363-2370
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i11.19

Original Research Article

Exploring the action of new FimH inhibitors against CTX–15 enzyme by enzoinformatics approach: A plausible arsenal against drug-resistant uropathogenic bacterial strains

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Sent for review: 24 September 2020 *Revised accepted: 6 October 2021*

Abstract

Purpose: *To explore the potency of FimH inhibitors against CTX-M β-lactamase enzyme type 15, in view of the increasing prevalence of CTX-M 15 in uropathogenic strains which has reduced the treatment options to minimal.*
Method: *FimH inhibitors were targeted against CTXM-15 by a molecular docking approach. Thereafter, the best ligand-target confirmation was selected and analyzed using LIGPLOT+ Version v.2.1. The hydrophobic and hydrogen bonding among the catalytic site amino acids of CTXM-15 and the FimH inhibitors were analyzed and 3-D structures were converted into 2-D images by LIGPLOT algorithm.*
Results: *Out of all the FimH inhibitors tested, 3'-chloro-4'- (α-D-mannopyranosyloxy) biphenyl-4-carbonitrile, para-biphenyl-2-methyl-3'-methylamidemannoside, para-biphenyl-2-methyl-3',5'di-methylamide-α-D-mannoside, and thiazolylamino mannoside exhibited better interaction with the CTX-M 15 active site than the positive control avibactam. Moreover, in CTX-M 15, the amino acid residues, Ser70, Tyr105, Ser130, Asn132, Thr216, Thr235, Gly236, and Ser237 were commonly interacting with these FimH inhibitors as well as avibactam.*
Conclusion: *The predicted findings suggest that these FimH inhibitors could be explored as potential CTX-M 15 inhibitors to cope-up with resistance issues of uropathogenic bacteria in the form of an alternate strategy.*

Keywords: *Antibiotic resistance, CTX-M 15 enzyme, Extended-spectrum β-lactamases, FimH, Urinary tract infections, Uropathogenic bacteria*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Globally, around 150 million individuals suffer from urinary tract infections (UTI) each year, and its occurrence is neither gender-specific nor age-dependent [1,2]. The causal bacteria in most UTI cases belong to *the Enterobacteriaceae* family [3], and the prevalence of extended-spectrum β lactamases (ESBLs) in this family has hindered the treatment options [4-6]. ESBLs of the

(Cefotaximase-Munich) CTX-M type, particularly CTX-M 15 enzyme, has become more common in clinical samples [5,7]. Thus, CTX-M 15 enzyme was selected as a target for performing the docking analysis in the present study.

On the other hand, in any infection process, adherence of bacteria to the mucosal surface of the host is usually the first crucial step, and this statement holds true in the case of UTI caused by *Enterobacteriaceae* species [8]. The uropathogenic strains of the Enterobacteriaceae family have type-1 fimbriae as an adhesive organelle. The FimH protein Hung is present at the end of type-1 fimbriae that is utilized by the bacteria for host cell attachment [9,10]. Various studies have suggested that type-1 fimbriae or FimH play a crucial role in the UTI caused by *Escherichia coli* and *Klebsiella pneumoniae* [11-14]. Thus, the advantages of using FimH inhibitors over the currently used antibiotics are their specificity for some bacterial species' particular adherence process, no effect on host microflora, and, most importantly, no resistance issues as it interferes with a bacterial attachment without bactericidal action [9]. The majority of FimH inhibitors structures were formulated using x-ray crystallographic findings such as 8-(methoxycarbonyl)octyl-α-D-mannoside, heptyl α-D-mannopyranoside, para-biphenyl-2-methyl-3′,5′di-methylamide-α-D-mannoside, para-biphenyl- 2-methyl-3′-methylamide mannoside, 3′-chloro-4′-(α-D-mannopyranosyloxy)biphenyl-4-carbonitrile, and thiazolylamino mannoside [9, 15-17]. In the present study, all these FimH inhibitors were selected to target the CTX-M 15-type ESBLs by applying the enzoinformatics approach. The findings suggested that FimH inhibitors might also be used against uropathogenic CTX-M 15 (ESBLs)-producing resistant bacteria.

They serve two purposes: (1) Hinder the attachment of uropathogenic strains, and (2) Avoid resistance due to CTX-M 15. However, wet-lab experimental analysis is needed to establish the findings of the present study. Nonetheless, these FimH inhibitors could be explored further to transform the status of the antibiotic therapy regimen for UTI treatment.

METHODS

FimH inhibitors, aviabactam and target protein structure retrieval

FimH inhibitors three-dimensional structure were obtained from the 'FimH-inhibitors' complex present in protein data bank. The FimH inhibitors used for the study were 8-(Methoxycarbonyl)octyl-α-D-mannoside [PDB ID: 4AVI], Heptyl α-D-mannopyranoside [PDB ID: 4BUQ], 3'-Chloro-4'- (α-D-mannopyranosyloxy) biphenyl-4-carbonitrile [PDB ID: 4CST], para-Biphenyl- 2-methyl-3'-methylamidemannoside [PDB ID: 5F3F], para-Biphenyl-2-methyl-3',5'di-methylamide-α-D-mannoside [PDB ID: 5F2F], and Thiazolylaminomannoside [PDB ID: 5MTS]. Target protein structure of 'Cefotaximase-Munich 15 (CTX-M 15)' [PDB ID: 4S2I] was also obtained from a Protein Data Bank. However, positive control avibactam [CID: 9835049] 3D structure was retrieved from PubChem database.

Physicochemical properties and toxicity potential prediction

The physicochemical properties and toxicity potential of FimH inhibitors and positive control were estimated by using the Osiris Datawarrior property explorer tool.

Initially, different physicochemical parameters such as no. of hydrogen bond acceptors and donors, cLogP value, molecular weight, topological polar surface area, number of rotatable bonds, and the Lipinski's rule violation [18] were calculated. Thereafter, absorption was estimated as in Eq 1 [19].

Absorption % = 109 – (0.345 × TPSA)……(1)

Prediction of toxicity was also evaluated by the orisis datawarrior tool, in which predictions are based on comparative analysis of our tested compounds with the pre-estimated set of known structural molecules. Mutagenicity, tumorigenicity, reproductive effects and irritability features of our tested compounds were predicted for toxicity assessment.

Molecular docking

'FimH inhibitors and avibactam' were considered as ligands and docked to target protein 'CTX-M 15' by following Rizvi *et al* protocol [20]. Each ligand energy was minimized by applying the MMFF94 force field, followed by gasteiger charges addition. Rotatable bonds were specified after adding non-polar hydrogen atoms. Kollman united atom type charges, solvation parameters, and hydrogen atoms were added using AutoDock 4.2. Autogrid was used to keep 60 x 60 x 60 Å as a grid dimension, with points separated by 0.375 Å. For explicitly targeting the 'CTX-M 15 catalytic site', the values of x, y, and z coordinates were kept as 6.930, 14.060, 9.920. Electrostatic and Van der Waals parameters

were estimated by applying dielectric functions and default parameters of AutoDock 4.2. 'Lamarckian and Solis and Wets local' algorithm was used to perform molecular docking experiments. One hundred different runs were applied for each docking experiment that was further set to end after 2,500,000 energy evaluations, and population size was kept as 150. At the end, final AutoDock 4.2 figures were studied using Discovery Studio 2.5 (Accelrys).

LIGPLOT+ analysis of docked results

After performing the docking, the best ligand-target confirmation was selected and analyzed using LIGPLOT+ Version v.2.1. The hydrophobic and hydrogen bonding among the catalytic site amino acids of CTXM-15 and the FimH inhibitors were analyzed and 3-D structures were converted into 2-D images by LIGPLOT algorithm.

RESULTS

In the present study, six FimH inhibitors, namely 8-(methoxycarbonyl)octyl-α-D-mannoside; heptyl α-D-mannopyranoside; para-biphenyl-2-methyl-3′,5′di-methyl amide-α-D-mannoside; 3′-chloro-4′-(α-D-mannopyranosyloxy)biphenyl-4-carbonitrile; para-biphenyl-2-methyl-3′-methyl amide mannoside and thiazolylamino mannoside were chosen to target CTX-M 15 using molecular docking approach. Prior to molecular interaction study, the physicochemical properties and toxicity potential of these inhibitors were evaluated (Table 1 and Table 2). In the physicochemical property assessment, 8-(methoxycarbonyl)octyl-α-D-mannoside; thiazolylamino mannoside and para-biphenyl-2-methyl-3′,5′di-methylamide-α-D-mannoside did not adhere to one of the "Lipinski's rule of five" in terms of the number of rotatable bonds, hydrogen bond acceptors and donors, respectively (Table 1). On the other hand, out of all the FimH inhibitors tested, only 3′-chloro-4′-(α-D-mannopyranosyloxy)biphenyl-4-carbonitrile exhibited high toxicity with mutagenic, tumorigenic, reproductive, and irritant effects (Table 2), whereas the positive control avibactam exhibited mutagenic and irritant effects.

Molecular docking study revealed Gibbs free energy change (ΔG) and inhibition constant (Ki) of 'FimH inhibitors' interaction with 'CTX-M 15 enzyme' (Table 3). The ΔG and K_i values for the interaction between 3′-chloro-4′-(α-D-mannopyranosyloxy) etbiphenyl-4-carbonitrile and CTX-M 15 were -7.46 kcal/mol and 3.38 mM, respectively (Table 3). Amino acid residues of CTX-M 15 interacted with 3′-chloro-4′-(α-D-mannopyranosyloxy)biphenyl-4-carbonitrile were Ser70, Asn104, Tyr105, Tyr129, Ser130, Asn132, Pro167, Asn170, Thr171, Thr216, Lys234, Thr235, Gly236, Ser237, Gly238, and Gly240. LIGPLOT showed that Ser130, Lys234, and Thr235 amino acids of CTX-M 15 were involved in hydrogen bonding, whereas Ser70, Asn104, Tyr105, Tyr129, Asn132, Pro167, Asn170, Thr171, Thr216, Gly236, Ser237, Gly238, and Gly240 were involved in hydrophobic interactions (Figure 1). Furthermore, the total intermolecular energy for the interaction was -8.66 kcal/mol, while, 'Van der Waals + Hydrogen bond + Desolvation energy' were -8.52 kcal/mol with electrostatic energy of -0.14 kcal/mol.

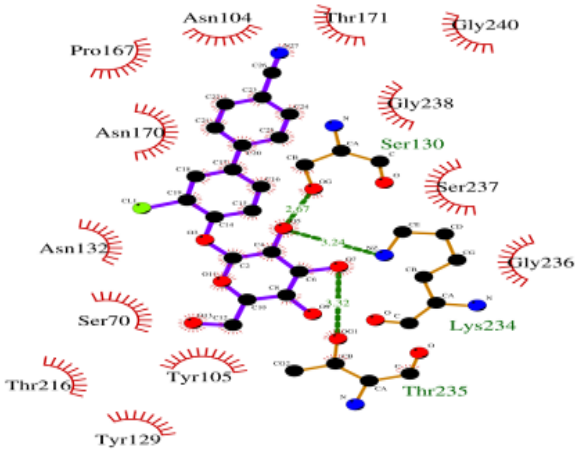

Figure 1: Ligplot analysis of '3'-chloro-4'- (α-D-mannopyranosyloxy) biphenyl-4-carbonitrile' - 'CTX-M 15' interaction. Red arcs represents the amino acid involved in hydrophobic interactions, whereas, green dashed lines represents the hydrogen bonds along with bond lengths

CTX-M 15 interaction results with para-biphenyl-2-methyl-3′,5′di-methylamide-α-D-mannoside showed K_i and ΔG values of 2.54 mM and -7.63 kcal/mol, respectively (Table 3). Total intermolecular, electrostatic and 'Hydrogen bond, Van der Waals and desolvation' energy values for this interaction were -9.42 kcal/mol, +0.12 kcal/mol and -9.54 kcal/mol, respectively. Thirteen amino acids (Ser70, Lys73, Tyr105, Pro107, Tyr129, Ser130, Asn132, Thr215, Thr216, Thr235, Gly236, Ser237, and Arg276) of CTX-M 15 enzyme interacted with para-biphenyl-2-methyl-3′,5′di-methylamide-α-D-mannoside.

Out of these 13 residues, Ser130, Ser237, and Arg276 were involved in hydrogen bonding while Ser70, Lys73, Tyr105, Pro107, Tyr129, Asn132, Thr215, Thr216, Thr235, and Gly236 were involved in hydrophobic interactions (Figure 2).

Table 1: Physicochemical properties of natural FimH inhibitors and control compound

Compound	Physiochemical parameter							
	Abs (%)	***Topological polar surface area (Å)²***	***Mol wt***	***cLogP***	***Hydrogen bond donors***	***Hydrogen bond acceptors***	***No. of rotatable bonds***	***Lipinski's violation***
Rule	**-**	**-**	**<500**	**≤5**	**<5**	**<10**	**≤10**	**≤1**
8-(Methoxycarbonyl)octyl-α-D-mannoside	65.64	125.68	350.40	0.545	4	8	12	1
Heptyl α-D-mannopyranoside	74.71	99.38	278.34	0.485	4	6	8	0
3′-Chloro-4′- (α-D-mannopyranosyloxy) biphenyl-4-carbonitrile	66.50	123.17	393.82	0.398	4	7	3	0
para-Biphenyl-2-methyl-3′,5′di-methyl amide-α-D-mannoside	54.63	157.58	460.48	0.205	6	10	6	1
para-Biphenyl- 2-methyl-3′-methyl amide mannoside	64.67	128.48	403.43	0.766	5	8	5	0
Thiazolylaminomannoside	31.78	223.82	465.51	-1.879	5	11	5	1
Aviabactam*	61.17	138.62	265.245	-2.688	2	9	3	0

*Control CTX-M 15 inhibitor; **Percentage of Absorption (% of Absorption) was calculated by: % of Absorption= 109 – [0.345 × Topological Polar Surface Area]; ***Logarithm of compound partition coefficient between *n*-octanol and water

Table 2: Toxicity potential of natural FimH inhibitors and control compound

Compound	Toxicity risk			
	Mutagenic	***Tumorigenic***	***Reproductive effect***	***Irritant***
8-(Methoxycarbonyl)octyl-α-D-mannoside	None	None	None	None
Heptyl α-D-mannopyranoside	None	None	None	None
3′-Chloro-4′- (α-D-mannopyranosyloxy)biphenyl-4-carbonitrile	High	High	High	High
para-Biphenyl-2-methyl-3′,5′di-methylamide-α-D-mannoside	None	None	None	None
para-Biphenyl- 2-methyl-3′-methylamidemannoside	None	None	None	None
Thiazolylaminomannoside	None	None	None	None
Aviabactam*	High	None	High	None

*Control CTX-M 15 inhibitor

Table 3: Molecular docking results of 'Cefotaximase-Munich 15 (CTX-M 15)' interaction with FimH inhibitor and control

Compound	Binding energy (ΔG)	Inhibition constant (Ki)
8-(Methoxycarbonyl)octyl-α-D-mannoside	-4.21kcal/mol	817.22mM
Heptyl α-D-mannopyranoside	-5.09kcal/mol	184.75mM
3′-Chloro-4′- (α-D-mannopyranosyloxy)biphenyl-4-carbonitrile	-7.46kcal/mol	3.38mM
para-Biphenyl-2-methyl-3′,5′di-methylamide-α-D-mannoside	-7.63kcal/mol	2.54mM
para-Biphenyl- 2-methyl-3′-methylamidemannoside	-7.47kcal/mol	3.35mM
Thiazolylaminomannoside	-7.63kcal/mol	2.57mM
Aviabactam*	-6.23kcal/mol	26.99mM

*Control CTX-M 15 inhibitor

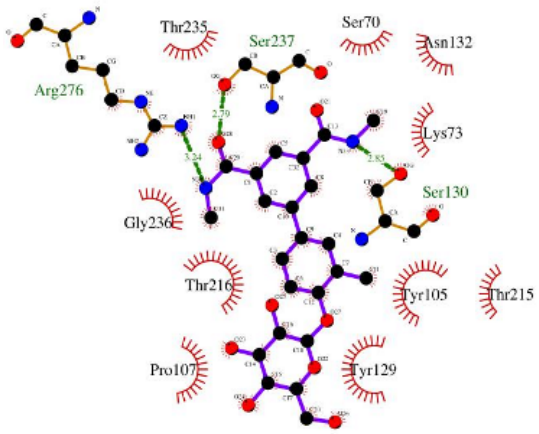

Figure 2: Ligplot analysis of 'para-biphenyl-2-methyl-3',5'di-methylamide-α-D-mannoside' - 'CTX-M 15' interaction. Red arcs represents the amino acid involved in hydrophobic interactions, whereas, green dashed lines represents the hydrogen bonds along with bond lengths

The ΔG and K_i values of para-biphenyl-2-methyl-3′-methylamidemannoside interaction with CTX-M 15 were -7.47 kcal/mol and 3.35 mM, respectively (Table 3). The intermolecular energy for this interaction was -8.96 kcal/mol. The total energy of Hydrogen bond, Van der Waals and Desolvation was -9.04 kcal/mol, while the energy for electrostatic bonding was +0.08 kcal/mol. CTX-M 15 amino acids Ser70, Asn104, Tyr105, Ser130, Asn132, Pro167, Thr168, Asn170, Thr171, Thr216, Lys234, Thr235, Gly236, Ser237, Gly238, and Gly240 interacted with para-biphenyl-2-methyl-3′-methylamidemanno-side. Among these amino acid residues, Ser130, Lys234, and Thr235 were involved in hydrogen bonding, while the others were involved in hydrophobic interactions (Figure 3).

The ΔG and Ki values for the interaction between thiazolylamino mannoside and CTX-M 15 were -7.63 kcal/mol and 2.57 mM, respectively (Table 3). The total energy of intermolecular was -9.42 kcal/mol, whereas, energy for electrostatic bonding was -0.14 kcal/mol. The collective energy for hydrogen bond, Van der Waals interaction and desolvation was -9.28 kcal/mol. Seventeen amino acids (Ser70, Asn104, Tyr105, Ser130, Asn132, Pro167, Asn170, Thr171, Thr216, Ser220, Lys234, Thr235, Gly236, Ser237, Gly238, Gly240, and Arg276) of CTX-M 15 catalytic site interacted with thiazolylamino mannoside. Out of these amino acids, Ser70, Ser130, and Ser237 engaged in hydrogen bonding with thiazolylamino mannoside, while Asn104, Tyr105, Asn132, Pro167, Asn170, Thr216, Thr235, Gly236, Gly238, Gly240, and Arg276 were involved in hydrophobic interactions (Figure 4).

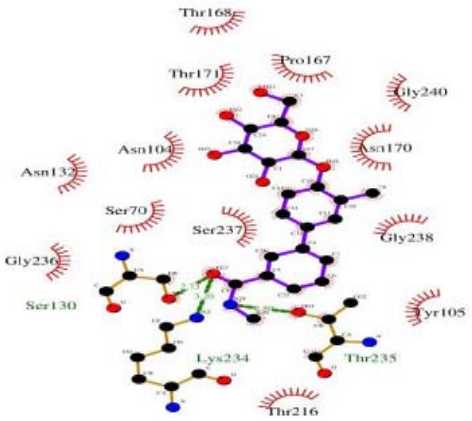

Figure 3: Ligplot analysis of 'para-biphenyl- 2-methyl-3'-methylamidemannoside' - 'CTX-M 15' interaction. Red arcs denote the amino acid involved in hydrophobic interactions, whereas, green dashed lines represents the hydrogen bonds along with bond lengths

For the avibactam (positive control)-CTX-M 15 interaction, ΔG and K_i values were -6.23 kcal/mol and 26.99 mM, respectively (Table 3). Ser70, Lys73, Asn104, Tyr105, Ser130, Asn132, Thr216, Lys234, Thr235, Gly236, Ser237, and Arg276 were the twelve amino acid residues of the active site of CTX-M 15 that were involved in the avibactam-CTX-M 15 interaction. Most

importantly, six amino acid residues of CTX-M 15, i.e., Ser130, Asn132, Thr216, Thr235, Ser237, and Arg276 were involved in hydrogen bonding, while Ser70, Tyr105, and Gly236 were engaged in hydrophobic interactions (Figure 5).

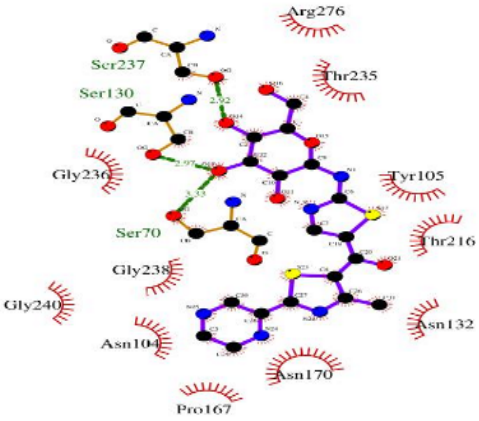

Figure 4: Ligplot analysis of 'thiazolylamino mannoside' - 'CTX-M 15' interaction. Red arcs denote the amino acid involved in hydrophobic interactions, whereas, green dashed lines represents the hydrogen bonds along with bond lengths

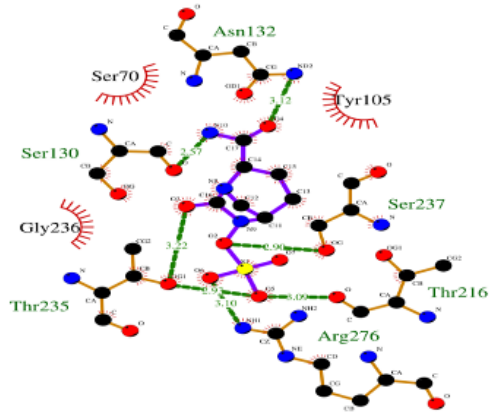

Figure 5: Ligplot analysis of 'Avibactam' - 'CTX-M 15' interaction. Red arcs denote the amino acid involved in hydrophobic interactions, whereas, green dashed lines represents the hydrogen bonds along with bond lengths

DISCUSSION

Antibiotic resistance in uropathogenic bacteria due to ESBLs, especially CTX-M 15, has become a significant issue globally [4-6]. Thus, finding an alternative solution to this issue is a matter of great concern. Several studies have reported that uropathogenic bacteria belonging to Enterobacteriaceae family have been regarded as the primary causative agents for most UTI cases [3]. In addition, bacterial adherence to the host mucosal surface is a crucial step in any UTI case, and the FimH produced by the Enterobacteriaceae family pathogens helps them attach to the mannosylated glycoproteins present on the epithelial cell surface of the urinary tract [8-10,21,22]. Therefore, FimH inhibitors might play an essential role in coping with the issues of antibiotic resistance by targeting cell adhesion without exhibiting bactericidal action. However, in the present study, the new potential of these FimH inhibitors was evaluated by assessing their CTX-M 15 inhibition potency. The present study outcomes might provide an add-on boost to over-the-counter antibiotics available for UTI treatment, by targeting both cell adhesion and ESBLs (CTX-M 15).

Among the FimH inhibitors, four inhibitors namely, 3′-chloro-4′-(α-D-mannopyranosyloxy) biphenyl-4-carbonitrile, para-biphenyl-2-methyl-3′,5′di-methylamide-α-D-mannoside, para-biphenyl-2-methyl-3′-methylamidemannoside, and thiazolylamino mannoside showed better interaction with CTX-M 15 than positive control avibactam when Gibbs free energy change (ΔG) were compared (Table 3). It is an entrenched fact that “ligand-target protein” interaction occurs only if the value of ΔG is negative; in addition, high negative ΔG also reflects enhanced affinity. Comparative analysis of interacting amino acids of all the molecular interactions showed that Ser70, Tyr105, Ser130, Asn132, Thr216, Thr235, Gly236, and Ser237 were the commonly interacting amino acids with both FimH inhibitors and avibactam. CTX-M 15 uses an acylation and deacylation process to hydrolyze b-lactam antibiotics. In fact, after the interaction between b-lactam antibiotics and CTX-M 15, the deacylation rate increases many-fold, leading to rapid CTX-M 15 regeneration. In addition, CTX-M 15 inhibitors forms strong acyl-enzyme complex bonding, thus reducing the deacylation or regeneration efficacy of CTX-M 15. Hence, CTX-M 15 inhibitor efficiency is measured by low deacylation and high acylation rates of CTX-M 15 [23].

Structure-activity relationship studies have shown the importance of Ser70 and Ser130 during the acylation process of CTX-M 15 by their inhibitors [7,24]. In the present work, two amino acid residues, Ser70 and Ser130, found in the CTX-M 15 active site commonly interacted with the FimH inhibitors. Thus, the FimH inhibitors tested in this study could be further explored as CTX-M 15 inhibitors as well. Further experimental validations are needed to ascertain the CTX-M 15 inhibition potential of FimH inhibitors and to convert them as dual inhibitors of FimH and CTX-M 15 enzyme. However, *in silico* findings have been reported to often

correlate well with wet-lab outcomes. The preliminary findings of the present study suggest a plausible alternate strategy for combating the current resistance issues caused by CTX-M 15-producing uropathogenic bacterial strains.

CONCLUSION

FimH inhibitors are currently under development for their use against adherence of uropathogenic bacterial strains to host cells. In the present study, FimH inhibitors were docked to CTX-M 15 enzyme to predict their dual targeting potential. Interestingly, they have shown better interaction with the catalytic site of CTX-M 15 than positive control. This would provide an add-on advantage to the ongoing research on FimH inhibitors, in order to develop them as alternative antibacterial therapy candidates. In addition, the findings of this study would help researchers to design more versatile and potent CTX-M 15 inhibitors to cope with the antibiotic resistance issues of uropathogenic bacteria more effectively.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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