Hierarchical-Clustering, Scaffold-Mining Exercises and Dynamics Simulations for Effectual Inhibitors Against Lipid-A Biosynthesis of *Helicobacter pylori*

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Abstract

Introduction—Treatment failures of standard regimens and new strains egression are due to the augmented drug resistance conundrum. These confounding factors now became the drug designers spotlight to implement therapeutics against *Helicobacter pylori* strains and to safeguard infected victims with devoid of adverse drug reactions. Thereby, to navigate the chemical space for medicine, paramount vital drug target opting considerations should be imperative. The study is therefore aimed to develop potent therapeutic variants against an insightful extrapolative, common target LpxC as a follow-up to previous studies.

Methods—We explored the relationships between existing inhibitors and novel leads at the scaffold level in an appropriate conformational plasticity for lead-optimization campaign. Hierarchical-clustering and shape-based screening against an in-house library of > 21 million compounds resulted in panel of 11,000 compounds. Rigid-receptor docking through virtual screening cascade, quantum-polarized-ligand, induced-fit dockings, post-docking processes and system stability assessments were performed.

Results—After docking experiments, an enrichment performance unveiled seven ranked actives better binding efficiencies with Zinc-binding potency than substrate and in-actives (decoy-set) with ROC (1.0) and area under accumulation curve (0.90) metrics. Physics-based membrane permeability accompanied ADME/T predictions and long-range dynamic simulations of 250 ns chemical time have depicted good passive diffusion with no toxicity of leads and sustained consistency of lead1-LpxC in the physiological milieu respectively.

Conclusions—In the study, as these static outcomes obtained from this approach competed with the substrate and existing ligands in binding affinity estimations as well as positively correlated from different aspects of predictions, which could

facilitate promiscuous new chemical entities against *H. pylori*.

Keywords—LpxC, Energy minimization, Quantum-mechanics, Induced-fit docking, Virtual-screening, Decoy-set, Enrichment-factor, Radius of gyration.

ABBREVIATIONS

DOPE	Discrete optimized protein energy
OPLS_AA	Optimized potential for liquid simulations for all atoms
VSW	Virtual screening workflow
RRD	Rigid receptor docking
QPLD	Quantum polarized ligand docking
IFD	Induced fit docking
ROC	Receiver operating characteristic (ROC)
AUC	Area under the curve
MM/GBSA	Molecular mechanics/generalized born surface area
RMSD	Root-mean-square deviation
RMSF	Root mean square fluctuation
PE	Potential energy
LBFGS	Limited memory Broyden-Fletcher-Goldfarb-Shanno

INTRODUCTION

Infections caused by Gram negative pathogenic bacterium, *Helicobacter pylori* is a major health concern throughout the world. Peptic ulcers, chronic gastritis, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma are various ailments.³⁰ Pro-

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gressive *H. pylori* infection leads to cascade of complications such as pan-gastric atrophy to intestinal metaplasia, and ultimately to gastric cancer (GC).³⁰ GC imposes a considerable global health burden accounted for an estimated two-thirds of all cases, the fifth most common cancer, and became a salient cause of cancer death globally.^{8,30,47} The surveillance research is needed for the eradication of *H. pylori* which reduces gastric cancer risk (WHO).²⁹

The frequent indication for anti-*H. pylori* therapy, together with the limited choice of drugs, has resulted in the development of antibiotic resistance in *H. pylori*, which substantially impairs the treatment of *H. pylori*-associated disorders and the treatment remains a challenge for physicians.^{14,16,45,47} Besides, the adverse effects of anti-*H. pylori* drugs vary from regimen to the regimen. Diarrhea, nausea, or vomiting and altered tastes are commonly associated side effects of the treatment. Drug resistance rates are increasing by fostering the development of more resistant strains and new antibacterial agents to treat these infections are few in number.^{7,16,30} Over the years different solutions to these problems have been proposed and some applied but have no effect on emerging drug resistant new genetically diverse strains due to gene mutations.¹⁶ These potential problems must be reckoned with the triaging of hits require a consideration of chemical tractability for success of novel proof-of-concept leads and breakthrough medicines through common target-based drug discovery. Thus, there is an immense need to discover novel anti-*H. pylori* agents with new mechanisms of action against common targets of diverse strains. But, in drug discovery of novel inhibitors, other requirements are selectivity and safety of a useful antibacterial spectrum; low propensity for rapid resistance selection, and pharmacological properties that allow effective systemic dosing. Broad spectrum target analyses are of paramount importance to curtail the emergence and spread of resistance and devise innovative therapeutic approaches against multidrug-resistant organisms. Choosing molecular targets for new antibiotics does seem a good basis for achieving these criteria.³⁷ Therefore, for conserved molecular targets, we explored complete proteomes of 53 *H. pylori* strains in our previous common drug target identification study.³² Consequently, we obtained UDP-3-O-[(R)-3-hydroxymyristoyl]-*N*-acetyl glucosamine deacetylase (LpxC) enzyme as putative common target involved in lipopolysaccharide (LPS) biosynthesis. The synthesized lipopolysaccharide (LPS) is the main component of the outer membrane serves as a permeability barrier and so protects *H. pylori* from many antibiotics.²⁷

Several earlier studies demonstrate that UDP-3-O-[(R)-3-hydroxymyristoyl]-*N*-acetyl glucosamine deacetylase (LpxC) as an essential enzyme in virtually all Gram-negative bacteria and it is a paramount significant target for treatment of multidrug-resistant Gram-negative infections.^{6,11,20,46} LpxC is Zn^{2+} -dependent metalloamidase uses Zn^{2+} to activate a nucleophilic attack by water on the amide of the *N*-acetylglucosamine (GlcNAc) moiety of the substrate in the hydrophobic tunnel of active site³; it catalyzes the second committed, irreversible step of biosynthesis of lipid-A required for cell wall formation.

Therefore, the intervention of LpxC enzyme with proper drug treatment leads to inhibition of lipid-A production and LPS biosynthesis; consequently, further growth and cell viability of *H. pylori* pathogen could be arrested. Currently, administration and prolonged utilization of selective LpxC inhibitors towards moderating the LpxC has been associated with different side effects. For instance, the literature reports that previously described LpxC inhibitors contain a hydroxamic acid which lead to unwanted side effects.⁶ Thereby, to overcome the challenges of adverse chemical reactions of developing drugs, common LpxC should intensively be investigated to permit the development of *H. pylori* selective therapeutic agents.²⁰ An adept therapeutic design has become an attractive choice in the development of the novel antibiotic therapy over the present conventions for deconvoluting the complexities of molecular pharmacology.

In this study, therefore, the appropriate common target (LpxC) of *H. pylori* has no sequence homology with any mammalian protein was preferred through data mining in order to investigate and expedite the computer-aided molecular design of novel potent scaffolds.²⁰ Herein, we report a scaffold-exploring approach to identify new scaffolds for inhibiting substrate-binding hydrophobic passage of LpxC enzyme to overcome *H. pylori* resistance conundrum and its ailments.

MATERIAL AND METHODS

Structure Prediction, Optimization and Minimization

The UDP-3-O-[(R)-3-hydroxymyristoyl]-*N*-acetyl glucosamine deacetylase (LpxC) of 53 *H. pylori* strains was selected from the pool of common drug targets identified in our previous study.³² Protein sequence of LpxC was retrieved from the UniProt (G2MBM9). As unavailability of crystal structure, comparative structure modeling technique was implemented to build tertiary structure. The X-ray structure of LpxC of *Pseudomonas aeruginosa* in complex with inhibitor

(2R)-N-hydroxy-3-naphthalen-2-yl-2-[(naphthalen-2-ylsulfonyl)amino]propanamide (GVR ligand) or BB-78485 (PDB: 2VES) was chosen as a structural template.^{1,28,33} Modeller (9v17) python script was defined to include two Zn ions and co-crystal inhibitor (BB-48785) during homology modeling and run to generate one hundred models.^{40,42} The best model was selected based on the lowest discrete optimized protein energy (DOPE) score and Genetic Algorithm 341 (GA341). The overall quality factor of protein model was evaluated by ProSA,⁴⁴ ProQ^{40,41} and PROCHECK through PDBsum.²³ Further, 3D-model structure was evaluated by Verify 3D server.¹¹ The model reliability was judged by root-mean-square deviation (RMSD) calculation and protein packing through target-template's superimposition process using superposition and protein reliability report panels respectively of Schrödinger LLC (2017-1). The validated LpxC model was submitted to the protein model database (PMDb).⁴

The stable protein structure is essential for the development of therapeutic lead molecules, so the amelioration of the model quality was still enhanced by absolved steric clashes of atoms (Lys⁶⁵: O—Asp⁶⁶: C and Ile¹⁶²:O—Ala¹⁶³: C with distances 2.71 and 2.72 respectively) and preparation. Herein, preprocessing of LpxC model was carried out by protein preparation wizard in Maestro v11.1 (Schrödinger LLC, 2017-1). All hydrogens were added which were subsequently minimized with optimized potentials for liquid simulations (OPLS_3) force field parameters using the molecular mechanics engine Impact v7.4 (Schrodinger LLC, 2011).⁴⁰ The geometry of all the hetero groups was corrected by assigning bond orders using chemical components dictionary (CCD) database.⁴³ The energy minimization was carried out restraining the heavy atoms with the hydrogen torsion parameters turned off, to allow free rotation of the hydrogens by utilizing in-built constraint of max root-mean-square deviation (RMSD) of 0.30Å.^{33,40} Final improved model structure was utilized as a receptor for scaffold screening studies.

Receptor Grid Generation

Active site residues were obtained from the optimized LpxC (receptor) model in complex with BB-78485 of *H. pylori*.^{9,33} In the study, an enclosing box of actual dimensions with XYZ coordinates (57.21, 37.38, 15.98 (Å³)) was generated on the centroid of the active site residues of the receptor so that the docked ligand is confined to the binding region within the box. Each docked ligand is required to remain within smaller, nested box, therefore, the required ligand diameter midpoint box of 21 Å × 21 Å × 21 Å was further set by

applying rotatable (hydroxyl and thiol) groups, constraints, Van der Waals (VdW) radius scaling factor 1.00, charge cutoff 0.25 and OPLS_3 force field by using Glide v7.4 for docking experiment.^{13,33}

Ligands Selection and Energy Minimization

About thirty six published inhibitors (ligands) including phase-1 clinical trial inhibitor (ACNH-975) were obtained through the literature survey (Table 1). All the ligands were prepared for docking using Lig-Prep node (Schrödinger, LLC 2017-1). Where, the optimized ligand minimization algorithm is for speed; LigPrep returned more conformers for ligands with larger numbers of rotatable bonds, improved efficiency and robustness (Schrodinger, LLC 2017-1). Thereby, we generated different possible protonation states, tautomers and ring conformations with charge states and as far as minimizing the ligand structures in the range of pH values of 7.0 and +/- 2.0 according to the pKa values of the different chemical groups on the ligand by using the OPLS_3 force feild, Premin, Truncated Newton Conjugate Gradient (TNCG) and Epik v3.9 nodes. The computations of stereochemistry were performed by Stereoizer to generate multiple output structures for each input ligand structure based upon the properties of the chiral atoms in the structure and to eliminate unfavorable combinations of chiralities.

Then, equipped ligands with preparation were docked into the grid box generated on active site residues of LpxC using Glide (extra-precision, XP in rigid receptor docking) with satisfactory cutoffs (ligand VdW scaling: 0.8 Å, partial charge cutoff: 0.15, RMSD: < 0.5 Å, maximum atomic displacement: < 1.3 Å and threshold for strain correction: 4.0 kcal mol⁻¹). Docking (extra-precision glide, XPG) scores were calculated and ligands were ranked based on Gscore (XP). XPGLide score is an empirical scoring function that approximates the ligand binding energy and the parameters such as force fields, penalties for the interactions that have the influence of ligand binding with the receptor.⁵ As a scoring function, it is comprised of terms that account for the physics of the binding process, including a lipophilic-lipophilic term, hydrogen bond terms, a rotatable bond penalty, and contributions of protein-ligand coulomb-vdW energies. The XPG Score is given by:

$$\text{GScore} = a * \text{vdW} + b * \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

whereas, VdW denotes van der Waals energy, Coul denotes Coulomb energy, Lipo denotes lipophilic contacts, HBond indicates hydrogen-bonding, Metal indicates metal-binding, BuryP indicates penalty for buried polar groups, RotB indicates penalty for

TABLE 1. Published inhibitors of LpxC from experimental and non-experimental studies.

S. No.	Published inhibitor	Docking (XPG) score (kcal/mol)	S. No.	Published inhibitor	Docking (XPG) score (kcal/mol)
1.	1-68A(UDP)	– 10.061	19.	CHIR_90	– 4.945
2.	ACHN-975	– 8.541	20.	81V	– 4.841
3.	PBF	– 7.663	21.	LPC-012	– 4.834
4.	L63	– 7.215	22.	LPC-014	– 4.789
5.	1-68A (PMT)	– 7.145	23.	LPC-009	– 4.678
6.	TU-514	– 7.135	24.	C13H12N2O4	– 4.563
7.	O3I	– 6.774	25.	8Q8	– 4.284
8.	7TD	– 6.630	26.	P76	– 4.176
9.	BB78485	– 6.369	27.	LPC-138	– 4.171
10.	L-161-240	– 5.987	28.	BB-7848	– 4[12].5
11.	23J	– 5.9	29.	LPC -050	– 4.008
12.	LPC-011	– 5.874	30.	SCH1379777	– 3.644
13.	L59	– 5.774	31.	D1D	– 3.457
14.	EPE	– 5.766	32.	LPC-040	– 3.371
15.	LPC-012	– 5.349	33.	EDO	– 3.288
16.	1JS	– 5.341	34.	LPC-053	– 3.226
17.	LPC-053	– 5.298	35.	L-161-240	– 2.244
18.	LPC-051	– 5.231	36.	DMS	– 1.621

freezing the rotatable bonds, Site denotes polar interactions with the residues in the active site and the $a = 0.065$ and $b = 0.130$ are coefficient constants of Van der Waals energy and Coulomb energy respectively.⁵

Hierarchical Clustering

The prepared existing-ligands dataset was assigned to Hierarchical clustering (HC) method using Canvas v3.1, the cheminformatics platform for structural diversity analysis based on the correlation matrix of either similarity or distance. Euclidean distance between inter-cluster centroids and Tanimoto similarity were chosen as the cluster linkage method and metric respectively. After the initial clustering step, clusters were selected at the Kelley level. The Kelley measure balances the normalized “spread” of the clusters at a particular level with the number of clusters at that level.²² This method does a very good job of separating compounds into different chemical classes (Schrodinger LLC, 2017-1).²² Moreover, in the diversity based selection, Correlation Matrix of original properties of ligands set was calculated which relied on molecular descriptor predictions and Soergel distance metric. In another excerpt, we computed the maximum common substructure (MCS) facility in order to find common scaffolds in a dataset using CanvasMCS.

Probing Structural Analogs from In-House Library

Structure-based drug design approach was applied to propose novel chemical scaffolds. Of the beforehand described 36 existing LpxC inhibitors, the best diverse molecules were selected by HC and preparatory docking simulations for maturity of seed structures

against LpxC. Multiple databases were used in a single Phase screening calculation. This library comprises small molecules of eMolecules®, ChemBank, ChemPDB, Drug likeliness NCI, KEGG Ligand, Anti-HIV NCI, AKos GmbH, Unannotated NCI, Asinex Ltd., and TimTec subsets. In these aspects, the eleven compounds including the nine outcomes of HC, co-crystal ligand (BB-78485) and the MCS ligand (LPC-050) were subjected to shape-based screening cogitations by opting pharmacophore types volume score against a prepared in-house library of > 21 million compounds (lead-like, and drug-like compounds) using Phase v5.1 module to obtain the best docked leads. Consequently, a dataset of screened small molecules was appended for docking strategies.

Glide Docking Procedures

Rigid Receptor Docking

The dataset of small molecules was allowed to rigid receptor docking (RRD) passing through twelve-stage virtual screening workflow (VSW) using Glide. Herein, the conflicts of ligands were reduced through lead optimization approach by applying OPLS_3 force fields with a defined pH range 7.0 ± 2.0 . ADME/T, Lipinski's filter and reactive filter by utilizing QikProp, LigFilter nodes for initial pragmatic screening of an original molecular set and identified prospective optimized hits.^{5,25,26,39} Then, VSW pursued a three-tier subsequent docking configurations for enhanced refinement such as high throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) docking with respective constraining about 10, 10 and 100% of compounds ex-

pected to be kept as the final best binding molecules.^{22,33} HTVS can dock compounds and trades sampling breath for higher speeds. SP performs exhaustive sampling and is the recommended balance between speed and accuracy. Glide XP employs an anchor-and-grow sampling approach and a different functional form for Glide score (Schrödinger LLC, 2017-1).

Quantum Mechanics and Molecular Mechanics (QM/MM) in Docking Quest

To improve the docking accuracy, the polarization effect in the binding process should also be taken into consideration apart from receptor flexibility.^{5,10} The correlation with experimental binding affinities was considerably improved with QPLD compared to Glide SP/XP.³¹ Hence, the obtained better posed seed structures in RRD were further investigated through quantum-polarized ligand docking (QPLD) using Q-Site module at the B3LYP/6-31G* level, which yield excellent results for atomization energies and transition states in a wide range of chemical systems.³¹ Afterwards, we ranked when comparing their binding affinities (kcal mol⁻¹) of QPLD leads against pre-existing inhibitors (positive controls) as the best decisions to adaptable docking.^{5,10} At initial stage of QPLD (SP), polarizable ligand charges were induced by the active site of the receptor with semiempirical method and QM/MM were calculated in inaccurate mode using Jaguar v9.5, a rapid *ab initio* electronic structure and quantum chemistry package (Schrödinger, LLC, 2017-1).⁵ The best outcomes were further assigned to redocking experiment and final selection (10 poses per ligand to save) accomplished by previously described procedures (XP mode) and Coulomb-van der Waals (CvdW) respectively.

Induced-Fit Docking Protocol

The active site geometry of a protein complex depends heavily upon conformational changes induced by the bound ligand and hence the drug discovery needs to find the exact conformational binding measures.^{5,12,36} So, in order to consider the flexibility of both ligand and active site of receptor, induced fit docking (IFD) was carried out with formerly described and additional Glide docking parameters (penalizing non-planar amide torsions, buried polar penalty: 0.0, CvdW < 200.0, Hbond: 0.0, Metal-ligand cutoff: 10.0) for calculations.^{5,10,39} The flexible docking simulations of LpxC-lead1 were consequently generated for twenty replications and analyzed reproducible conformers. The Glide score (Gscore) was used for all the prescribed docking calculations. The correlation was already discovered between the docking score or cal-

culated free energy (kcal mol⁻¹) and the experimental pIC50 values of inhibitors.¹⁰ Therefore, in the study, the best compounds were selected based on glide docking score along with binding mode through the screening a multitude of chemical moieties in the lead optimization campaign.

Evaluation of Leads Using Weighted Metrics

Lead optimization efforts were succeeded for the obtained the best lead compounds when compared against the substrate (UDP-3-O-(3-hydroxymyristoyl)-N-acetylglucosamine) on RRD and QPLD docking platforms after consecutive hierarchical filters. Moreover, lead1 and substrate in IFD were aligned to measure executive RMSD while bound to the active site of LpxC. The docking protocol was validated through the enrichment performance for true positive rate (TPR, or specificity) against the false positive rate (FPR, or 1- sensitivity) in post-docking process. Herein, the enrichment of the best lead molecules in a virtual screening context using the DUD (directory of useful decoys) dataset (1000 drug-like compounds as inactives) and the best published inhibitors from each cluster was carried out with receiver operating characteristic (ROC) curves, robust initial enhancement (RIE) and area under accumulation curve (AUAC) metrics (Schrodinger LLC, 2017-1).^{17,38}

Prediction of Pharmacological Properties

Pharmacokinetic profile (absorption, distribution, metabolism, excretion, and toxicity (ADME/T)) of the existing inhibitors and new leads of the dataset were predicted using QikProp v5.1 to remove the inefficient molecules, to significant savings in research and development costs.¹⁹ It predicts both physically significant descriptors and pharmaceutically relevant properties.²⁵ Although the presence of the target is necessary to insure the desired spectrum, it is not sufficient, as the permeability features of Gram-negatives, are critical determinants of antibacterial activity.³⁷ Thus, we performed physics-based membrane permeability analysis to investigate the passive membrane diffusion characteristics of a series of designing scaffolds using Schrödinger Membrane Permeability Predictor.³⁵

Dynamic Events Analysis: Stability Determination

Molecular systems are dynamic in nature; therefore analyzing their motions at the molecular and atomistic level is essential to understanding key physicochemical phenomena. To conquer drug discovery, the stability assessment at the atomistic level by measuring the effect of compounds (new scaffolds against existing in-

hibitors) on the stability of the receptor becomes an indispensable component as dynamic nature of the protein and ligand at the physiological milieu. Static structure-based approaches coupled with MD simulations have made important strides in advancing drug discovery (Schrodinger LLC., 2017). We therefore captured dynamic events of a molecular system LpxC-lead1 by performing molecular dynamics (MD) simulations for 250 nanoseconds using Desmond v4.9 with exceptional scalability.³⁹ The observed phenomena of reliable consistency of LpxC-lead1 complex was equated with the same simulations constraints for 50ns against LpxC-co-crystal ligand GVR (BB-78485), the second molecular system as control.

In this arena, the system was built with TIP4 water model, wherein water molecules were embedded with OPLS_3 force field parameters to depict a solvated model system. The system was neutralized by adding minimum amount of sodium and chloride ions in order to balance, the net charge in the solvated system and to mimic the osmotic effect of water. Electrostatic interactions were applied using particle mesh Ewald (PME).³³ The system was minimized before simulating and was carried out with the periodic boundary conditions in the Isothermal-isobaric (NPT) ensemble class. The temperature and pressure were kept at 300 K and 1.01325 bar pressure using Nose-Hoover temperature coupling and isotropic scaling. The minimized system was passed through Canonical ensemble (NVT) for short 12 ps (picoseconds) simulations at 10 K temperature; additionally non-hydrogen solute atoms were restrained for 24 ps at 300 K temperature.^{33,39} Further, the system was simulated for 24 ps in NPT ensemble at 300 K temperature without restrains so as to attain an equilibrium state. The minimized system without restrains was subjected to 50 ns NPT simulation production. Trajectories were recorded for every 4.8 ps time interval.

MD simulation of the Lpxc-lead1 molecular system was further extended up to 50 ns using Desmond v5.3 (Schrödinger, 2018-1) to check the stability of the complex over a longer time scale. Herein, trajectories were recorded for every 1000 ps time interval. To study the conformational variations in the structures of the two molecular systems, the root-mean-square deviation (RMSD) of the atomic positions with respect to their starting reference structures was calculated. The convergence of MD simulations was analyzed through monitoring the potential energy, RMSD, and root-mean-square fluctuation (RMSF). Simulation quality analysis was performed for the kinetic, potential, and total energies estimate the variations in the energetics of the system. The deviations relative to the C α , backbone and hetero atoms were calculated; RMSF of C- α , backbone and side-chain atoms beside interaction patterns with the aim to evaluate the stabilization of

the systems throughout the MD simulations. in entire course of dynamic simulations.

RESULTS AND DISCUSSIONS

Discovery of novel antimicrobial compounds appears to be a good solution to overcome therapeutic problems resulting from increasing resistance of *H. pylori* strains to antibiotics and chemotherapeutics. Ghotaslou *et al.*, 2015 and De Francesco *et al.*, 2010 reported that *H. pylori* antibiotic resistance rates (worldwide) were 47.22% for metronidazole, 19.74% for clarithromycin, 18.94% for levofloxacin, 14.67% for amoxicillin, 11.70% for tetracycline, 11.5% for furazolidon and 6.75% for rifabutin.^{7,15} Advances in genomics, proteomics and innovative strategies such as data mining and appropriate selection of common target for numerous drug resistant *H. pylori* strains provide a novel route to investigate and expedite the design of novel potent inhibitor scaffolds. Enzymes remain prime targets for drug design because the altering enzyme activity imposes immediate and defined effects.³⁴ In this way, UDP-3-*O*-[(R)-3-hydroxymyristoyl]-N-acetyl glucosamine deacetylase (LpxC) was identified as a common, highly conserved drug target in selected 53 *H. pylori* strains,³² sharing 99.9 % sequence identity in active site. It is involved in LPS biosynthesis with no alternative way for UDP-3-*O*-(3-hydroxy-tetradeconyl)-D-glucosamine synthesis.

Protein Model Generation, Preparation, Optimization and Minimization

The generated tertiary structure of drug target is the initial requirement for structure-based drug design. Incorporating ligands into the homology model from the structural template enhances overall accuracy of the predicted models which also helps in determining ligand binding pocket.³³ LpxC uses Zn²⁺ to activate a nucleophilic attack by water on the amide of the N-acetylglucosamine (GlcNAc) moiety of the substrate.³ Hence, inhibitor BB-78485 (GVR or (2R)-*N*-hydroxy-3-naphthalen-2-yl-2-[(naphthalen-2-ylsulfonyl)amino]propanamide) and two Zn²⁺ were incorporated into the generated 100 LpxC structure models. The sixth model of LpxC with the lowest DOPE score of -31101.178 kcal mol⁻¹ and GA341 score of 1.0 was selected for structure-based drug discovery. Stereochemistry assessment of the selected model showed 99.6% residues in allowed regions of Ramachandran plot (Figure S1(A)). Z-score value of -6.25 from ProSA revealed that the model is well within the range of native conformations of X-ray crystal structures and overall residue energies remain largely negative (Fig-

FIGURE 1. Verify 3D plot of common target LpxC model. 3D-model was qualified with 88.47% of the residues have averaged 3D-1D score ≥ 0.2 .

FIGURE 2. Model structure of LpxC of *H. pylori* (PMDb ID: PM0080940) superimposed (RMSD = 0.271 Å) with template 2VES in the presence of GVR co-crystal ligand (BB-78485). The color code representations for the picture as magenta: template; cyan: target. Zn ion represented as sphere. Conserved active site residues of target and template in the presence of GVR were labeled. The amino acids represented in lines and GVR were in sticks.

ure S1 (B, C)). ProQ tool assessment showed LG score of 4.708 represents the model is of extremely good quality.⁴¹ Further, Verify 3D revealed that the LpxC model was successfully passed with 88.47% of the residues have averaged 3D-1D score ≥ 0.2 (Fig. 1). As per the program, at least 80% of the amino acids should score ≥ 0.2 in the 3D/1D profile.¹¹ The superposition of the overall atoms of the target—template

reflects the close resemblance with experimental one and the interpreted lower RMSD of 0.271 Å and model's protein packing score of -1.15 is more or less equal to the template (-1.2) show higher structural conservation (Fig. 2).^{33,39,40} So, the stereo-chemistry of the residues in this LpxC model structure was appropriately well alike as refined and good resolution structures.⁴¹ The validated model was considerably

accepted by the PMDB with less than 3% stereochemical check failure and deposited (ID: PM0080940).⁴

The protein preparation amended the LpxC protein structure with the improved refinements, optimization and minimization for protein-ligand binding evaluations. Consequently, the model quality was still enhanced by absolved steric clashes of atoms (Lys65: O—Asp66: C and Ile162: O—Ala163: C with distances 2.71 and 2.72 respectively). Receptor rotatable groups such as R—OH groups of Ser71 (2906 H-atom), Thr72 (2910 H-atom), Tyr107 (3174 H-atom), Thr185 (3813 H-atom), Ser258 (4379 H-atom) and R—SH of Cys209 (3997 H-atom) were found to be involved in rotatable bonds. The defined constraints such as occupied spherical regions or shells of centre of an atom of particular ligand, receptor atoms that could participate in H-bond/ metal ligand interactions, excluded volumes and metal coordination (the ideal coordination geometry for that metal given its environment) were chosen during docking.

Ligand Preparation and Diversity Analysis

The ligand preparation of 36 published inhibitors resulted ten various conformations, however, the conformer with the lowest in conformational energy, contain probable protonation, tautomerization state and obvious ring conformations preferred for binding to an active site. As a result of docking of 36 published inhibitors, the XPG scores were calculated and the consecutive order of ranked ligands including LpxC co-crystal ligand (BB-78485) was obtained (Table 1).

ADME/Tox predicts both physically significant descriptors and pharmaceutically relevant properties.^{25,26} The ADME/T properties of selected inhibitors are shown in Table S1-S2. Inefficient molecules having poor ADME/T lineaments were removed from the drug design pipeline.¹⁹ The Kelley index is a criterion to select an optimal number of clusters in the hierarchical-clustering has resulted nine clusters with structural diversity (Figs. 3a and 3b). The clustering level with the lowest penalty represents a state where the clusters are as highly populated as possible, while simultaneously maintaining the smallest spread.¹³ Among nine clusters, structures of third cluster are deployed to smaller spread with higher population size of 25 and the rest of them found with a higher spread refers more diversity (Figs. 3a and 3b). The smaller the spread of the clusters, the more similar are the conformations of its members; the greater the population of a cluster, the smaller is the chance of excluding a member of similar conformation.²² The correlation matrix of more than twenty molecular descriptors of the ligand dataset afforded

(average $R^2 = 0.4242$ and clustering strain = 1.01) beaucoup diversity in ligands.

In the study, the best XP Gscored (kcal mol^{-1}) existing inhibitors such as 1-68A (− 10.061), L-161-240 (− 5.987), ACHN-975 (− 8.541), LPC-053 (− 5.298), 23J (− 5.900), TU514 (− 7.135), EPE (− 5.766), and BB-7848 (− 4.5) were successively observed from nine clusters respectively while docking with LpxC (Table 1 and Fig. 3(a)). These nine ligands were selected. Supportingly, some of which were experimentally reported for LpxC as better inhibitors such as 1-68Aa (IC₅₀: 50 μM) by Barb *et al.*, 2009, ACHN-975 (IC₅₀: 0.02 nM) by Kalinin *et al.*, 2017 and so on^{2,21,47}; however, surveillance studies toward novel scaffold design are now still concerned with growing drug resistance. Thus, implementing the current approach could probably be useful to discover new drugs for combat resistance strains. Moreover, LPC-050 was determined as common substructure among the 36 ligands with current MCS size (34 atoms and 36 bonds) after computation (Fig. 4). So, in addition to aforementioned nine ligands, the LPC-050 and substrate (Udp-3-hmaglc) were also selected, consequently, a total of eleven ligands were henceforth considered as positive controls for advanced screening procedures.

Library Screening Strategy

In the shape screening procedures, an each one of eleven ligands ensued one thousand similar contoured small moles. Consequently, a dataset of 11000 chemical compounds was produced for advanced three-tier docking strategies.

Predicting Binding Accuracy and Scoring Functions

We curated the dataset of 11000 small molecules using screening filters commenced with QikProp processed 8507 compounds with physicochemical descriptors and pharmaceutically relevant properties (ADME/T).⁵ In which, 8094 compounds have been passed through Lipinski's filter and reactive filters. Three-tier docking protocol of RRD provides an array of options in the balance of speed vs. accuracy for most situations and thereby always improved the resultants through docking funnel (HTVS (10%) resulted 809; SP (10%) resulted 80; XP (10%) resulted 8 compounds). Consequently, eight optimized hits were yielded with predicted significant protein-ligand complex geometries. All these compounds have a good binding affinity over the eleven existing diverse ligands and it was reported in the Glide Score (Table 2).

In QPLD process, the betterment of docking accuracy with charge calculations of seven out of eight contours was observed with the lowest XPG score and

FIGURE 3. (a) Clusters of existing published inhibitors, (b) Graphic of clusters size (order) of published inhibitors deployed higher population (cluster-3, size-25 ligands) in central portion of smaller spread with low penalty shown in yellow to dark-red color; The smaller the spread of the clusters, the more similar are the conformations of its members; the greater the population of a cluster, the smaller is the chance of excluding a member of similar conformation.²²

enhanced binding strength over the eleven diverse existing inhibitors; and substantially ascertained outcomes as novel lead scaffolds (Table 2). In the assessment, Glide XP visualizer showed the specific interactions of ranked seven leads such as protein-li-

gand interaction energies, H-bonds, hydrophobic interactions, internal energy, π - π stacking interactions. Moreover, all the seven leads are still exhibited more capable Zn-binding tendency with electrostatic and other associated metal co-ordinations after practical

FIGURE 4. Selected LPC_050 as common maximum substructure (executive RMSD = 0.00132) among the published inhibitors for screening inhouse-library.

QM/MM calculations. Electrostatic interactions determined computationally are more significant for protein-ligand binding.¹⁰ The experimental data demonstrate that LpxC is a metalloenzyme that requires bound Zn^{2+} for its optimal catalytic activity [46].¹⁸ Thereby, this opportunistic metal binding forces expedient the new leads in view of the enhanced contribution of chemical attraction towards LpxC binding cleft than previous existing ones.

As observed in Table 2, all the 7 lead scaffolds have the higher binding affinity than the positive controls (published inhibitors) with including phase-I clinical trial compound ACHN-975 in terms of molecular, Zn-binding interactions, XP Gscoring functions and toxic filters. So, these seven compounds comparatively analyzed and ranked to propose as potential leads in the strategy of lead discovery. However, lead1 is determined as the best scaffold among the eight with XP Gscore of -11.992 kcal mol⁻¹ with bonded (H-bonds: Phe164, Lys233(2); electrostatic interactions: Zn296(3)) and non-bonded molecular interactions (Leu17, Lys58, Met59, Ala60, His75, Ile99, Thr185, Gly187, Glu191, Val192, Leu195, Ala201, Gly204, Ser205, Asn208, Cys209, Ser258, and His259).

In biological context, due to dynamic nature of protein undergoes side chain or backbone movements, or both, upon ligand binding which make over the receptor to alter its binding cleft, so that it more closely conforms to the shape and binding mode of the ligand (induced fit).³⁹ Although Glide can almost reproduce the binding mode of the native ligand, the protein should adopt corresponding conformation to the new lead molecule so it is necessary to consider the flexibility of protein and becomes a complication factor in docking challenges. Thereby, the induced fit docking protocol was utilized to predict the effect of ligand docking on protein structure with facilitating flexibility of lead1 and side chain or backbone of the active site residues.

As a result of IFD, the docked complex pose was further improved dramatically as induced fit accurately predicted the active site geometry LpxC with lead1 (Figs. 5, 6); this docked pose almost overlapped with native model structure with co-crystal ligand (GVR). It also showed the binding site of the best-ranking IFD model for lead-1 compound with partial charges and molecular weights of the entire LpxC-lead1 system are -2.0 and 27893.1Da respectively. However, docking (IFD) score of -11.890 kcal mol⁻¹ is almost equiva-

TABLE 2. Analysis of leads and previous ligands against decoys.

Compounds	Actives ranking against Decoys/inactives	XPG (docking) score (kcal/mol)			Physics-based membrane permeability (ADME_Membrane dG_Insert)		Salient chemistry annotations
		RRD	QPLD	IFD			
Lead1	1	– 10.883	– 11.992	– 11.890	39.297	Contains an acetal/ aminal-like group (X-CH(R)-Y where X, Y are N, S, or O) that may be acid/ base labile, releasing an aldehyde	
Lead2	2	– 11.631	– 11.267	–	62.552	Possesses an imino group and may undergo hydrolysis.	
Lead3	3	– 10.644	– 11.215	–	63.638	Contains an acetal/ aminal-like group (X-CH(R)-Y where X, Y are N, S, or O) that may be acid/ base labile, releasing an aldehyde	
Lead4	4	– 10.722	– 11.002	–	27.442	Has an imine and may undergo hydrolysis. This compound contains an acetal/ aminal-like group (X-CH(R)-Y where X, Y are N, S, or O) that may be acid/ base labile, releasing an aldehyde	
						Contains an acetal/ aminal-like group (X-CH(R)-Y where X, Y are N, S, or O) that may be acid/ base labile, releasing an aldehyde	
Lead5	5	– 10.463	– 10.689	–	52.015		
Lead6	6	– 11.064	– 10.259	–	53.366	Contains an acetal/ aminal-like group (X-CH(R)-Y where X, Y are N, S, or O) that may be acid/ base labile, releasing an aldehyde	
Lead7	7	– 10.448	– 10.258	–	46.210	Contains an acetal/ aminal-like group (X-CH(R)-Y where X, Y are N, S, or O) that may be acid/ base labile, releasing an aldehyde	
Substrate	–	– 10.869	– 10.866	–	29.768	–	
Ranked previous inhibitors among inactives / decoys							
1-68A (UDP)	9	– 10.061	– 10.142	–	42.731	Contains an acetal/ aminal-like group (X-CH(R)-Y where X, Y are N, S, or O) that may be acid/ base labile, releasing an aldehyde.	
ACHN-975	18	– 8.541	– 9.689	–	12.621	Has a N-S or N-O group and may undergo hydrolysis at high or low pH	
L63	19	– 7.215	– 7.193	–	20.936	This compound has a N-S or N-O group and may undergo hydrolysis at high or low pH	
TU-514	23	– 7.135	– 8.440	–	3.693	Possesses an ester and may undergo hydrolysis at high or low pH. This compound has a N-S or N-O group and may undergo hydrolysis at high or low pH

FIGURE 5. (a) Lead 1 molecule in-depth occupied into the binding cleft (red in color) with good enough binding orientation and interactions formed by the Zn and active site residues (lateral view). (b) Schematic represents the precisely buried lead1 and the unbound exterior segment of substrate molecule lying out of the binding cleft (front view).

lent to QPLD outcome.³⁹ While focusing on interactions, the bonded (H-bonds: Thr185, Glu191(2), Lys233; electrostatic: Lys233, Zn296(2)) and non-bonded (Leu195, Val192, Phe188, Gly187, Phe186, Cys209, Ser205, Gly204, Leu17, Ile99, Pro98, Ile97, Thr72, Glu74, His75, Ala60, Met59, His259, Asp236 and His232) molecular contacts were observed which are also correlated with previous existing ligands. At all docking stages the non-covalent interactions were found to contribute entropically favorable conformations for the stable binding provision of LpxC-lead1 (Fig. 6(a–c)). Herein, even at dynamic flexible state, Zn-binding of active site was observed with lead1, it is essential for the activity of LpxC enzyme. Moreover, all the proposed lead molecules offer better binding efficacy with active site in perspective of charges, orientation and binding interactions than the substrate which may competitively inhibit the catalytic activity of the enzyme so that in turn lipid-A synthesis of the cell wall could be inhibited. The scoring outcomes of the lead molecules are better than that of the substrate at the RRD and even QPLD (Table 2). The executive RMS score of 0.213 was resulted from the substrate-LpxC complex and lead1-LpxC complex (Fig. 5). The

comparison of lead1 with cocrystal ligand (GVR) and substrate in terms of XPG scoring functions and interactions demonstrated that the lead1 has higher binding effect with the binding cleft of LpxC (Fig. 6).

Validation of Docking Protocol: Enrichment Performance

Post-docking analysis is about practical heuristics for the best selection of the number of actives and decoys using weighted metrics. Receiver operating characteristic curves are a popular way to visualize the tradeoffs between sensitivity and specificity in a binary classifier. As shown in Fig. 7, the resulted step sizes are inversely proportional to the number of actual positives (in the Y-direction, false positive rate (FPR, or 1—sensitivity)) or negatives (in the X-direction, true positive rate (TPR, or specificity)), so the path always ends at coordinates (1, 1).

Enrichment factor calculations derived ROC plot and % screen plot wherein the number of ranked actives under the accumulation curve are merely seven compounds of sixteen (7 new leads and 9 existing best inhibitors from clusters) total actives and the rest of

FIGURE 6. Binding efficiency of Lead1, cocrystal-ligand (GVR) and substrate from docking phenomena. Herein, (a), (b) and (c) of lead1 retrieved from RRD, QPLD and IFD docking experiments respectively.

FIGURE 7. (a) ROC plot, (b) Percent screen plot.

FIGURE 8. Proposed potential inhibitors of common target LpxC of *H. pylori* strains.

FIGURE 9. Molecular dynamic simulations of GVR-LpxC complex (50 ns runtime) and Lead1-LpxC (250 ns runtime). (a), (b) Potential energy plot, (c), (d) RMSD, (e), (f) RMSF (protein residues that interact with the ligand are marked with green-colored vertical bars), and (g), (h) Ligand fit on binding site (shows the ligand fluctuations, with respect to the protein).

them outranked with ROC: 1.0, AUAC: 0.90 and RIE: 12.51 (Figs. 7a and 7b). Ninety-nine average outranking decoys are also found in between outranked actives (Fig. 7). Thereby, which led to practical recommendations for the selection of seven leads as top ranked actives positioned in consecutive order (Table 2, Fig. 8). Followed by four previous inhibitors included 1-68A (UDP), L63, ACHN-975, TU-514 were lower ranked 8, 17, 18 and 22 respectively out of 16 actives from overall inactive decoys.

ADME/Tox Analysis

ADME/Tox creates a safer drug-discovery pipeline, and hence analyzing pharmacological properties of the drug is an imperative part of the drug discovery process

in order to elude adverse biochemical reactions in human. ADMET investigation of small molecules inferred that the seven leads have good pharmacological features with no toxicity, fall within the range of ~ 95% of FDA approved drugs, as well as better than that of preexisting inhibitors and even cocrystal ligand (GVR) (Table 2, Table S1-S2). The experimental evidence suggests that intramolecular hydrogen bonding (IntraHB) of a ligand may play a role in the passive membrane diffusion characteristics by reducing the energetic cost of desolvating hydrogen bond donors, especially amide N–H groups and showed that membrane diffusion rates correlated with the degree of IntraHB.³⁵ In the study, physics-based membrane permeability predictions determined that all the lead compounds were attributed with > 2 IntraHB (Lead1 showed in Fig. 6a) and better

FIGURE 10. Involvement of active site residues in the bonded and non-bonded contacts. (a) GVR ligand—LpxC, (b) Lead1—LpxC.

passive diffusion features above the thresholds (Log₋Perm_{RRCK}: − 6.63; Membrane_{dG_Insert}: 31.13; Membrane_{Penalty}:10.04; Membrane_{HDLD}: 21.09) when compared to previous ligands (Schrödinger LLC) (Table 2, Table S3). Thereby, regarding all these views the resulted outcomes suggested to propose as the best lead scaffolds against targeted LpxC.

Analysis of Molecular Dynamics Simulations

Conformational dynamics plays a major role in enzyme catalysis, allosteric regulation of protein functions and assembly of macromolecular complexes.²⁴ Besides, a number of marvelous biological functions in proteins with their profound dynamics mechanisms, such as switching between active and inactive states, cooperative effects and so on, can be revealed by studying their internal motions.²² Thus, in the study, the dynamic aspects of complex and ligand-

induced conformational changes of active site were monitored through 1000 trajectories of 250ns (lead1) and 10416 trajectories of 50 ns (GVR) chemical time. The final systems of LpxC-lead1 and LpxC-GVR (co-crystal ligand) contained 31,728 and 32,997 atoms respectively; however, the difference with lower molecular weight of the lead than that of co-crystal inhibitor can be optimistic for experimental analyses. In simulation quality analysis, all the trajectory data showed that total energies (kcal mol^{−1}) of both systems − 85450.70 (Std. Dev.:703.55) and − 83981.74 (734.95) respectively. Whereas average potential energies of both complexes (kcal mol^{−1}) are − 137150.78 (Std. Dev.:158.74) and − 106726.02 (Std. Dev.:156.64) respectively (Figs. 9a and 9b). In Figs. 9c and 9d, all protein frames are first aligned on the reference frame backbone, and then the RMSD is calculated based on the atom selection. Monitoring the RMSD of the protein can give insights into its structural conforma-

FIGURE 11. Interactions of the active site residues from MD simulations. (a) GVR-LpxC, (b). Lead1-LpxC.

tion throughout the simulation. RMSD analysis can indicate if the simulation has equilibrated and its fluctuations towards the end of the simulation are around some thermal average structure. The average RMSD value of LpxC protein while bound with GVR ligand is 0.56 Å and lead1 is 0.98 Å; observed with C α (1.95, 2.23), backbone (2.17, 2.41), side-chain (3.16, 3.10) and heavy atoms (2.82, 2.89) (Figs. 9c and 9d). Both the complexes were stabilized after a small rearrangement from the initial conformations (10–20 ns). Protein RMSF determines fluctuations of each residue in both complexes (Fig. 9). The protein-ligand complex is first aligned on the protein backbone and then the ligand RMSF is measured on the ligand heavy atoms. ‘Ligand’ line shows fluctuations where the ligand in each frame is aligned on the ligand in the reference frame, and its fluctuations are measured for the ligand heavy atoms (Figs. 9e and 9f). The ligand RMSF may give you insights on how ligand fragments interact with the protein and their entropic role in the binding event. The ‘fit ligand on protein’ plot shows the ligand fluctuations, with respect to the protein, wherein, the lead1 was found well with consistency throughout 250 ns whereas, GVR ligand showed fluctuations on LpxC (Figs. 9g and 9h). These RMSF values reflect the internal atom fluctuations of the li-

gand. However, LpxC-GVR ligand complex is on an average observed with higher amino acid fluctuations of backbone (1.27Å) and side chain atoms (1.86 Å) when compared with LpxC-lead1 complex of backbone (0.98 Å) and side chain (1.38Å) fluctuations thereby leads can obviously be suggested towards clinical perspective studies. Protein secondary structure elements (SSE) of and lead1-LpxC and GVR ligand-LpxC complexes like alpha-helices and beta-strands are monitored throughout the 250 and 50 ns MD simulations respectively. The generated plots reported percentages of SSE distribution of lead1-LpxC (helix: 17.47 %, strand: 27.50 % and total SSE: 44.98%) and GVR-LpxC (helix: 18.28 %, strand: 27.16 and total SSE: 45.44%) by residue index throughout the simulations (Figure S2). Transient variations of a set of interactions were also observed in LpxC binding cleft domain while bound with ligand/ substrate and lead1 individually, however, better persistent contacts can be seen in lead1-LpxC complex than. During the period of 250 ns simulations, lead1 interacted consistently with LpxC. In which, five water-bridges by Glu74 (100%), Phe186 (44%), Thr185 (25%), Val192 (50%) and His259 (40%); H-bond interactions of Asn208 (45%), Ser205 (2) (63%, 82%), Met100 (63%), His18 (20%), Phe186 (44%), Lys233 (65%) and Met59 (24%).

Moreover, His75 (100%), Asp230(2) (100%, 48%) and His 232 (100%) are found in metal coordination for Zn-binding.

In contrast, cocrystal ligand GVR made few interactions by LpxC such as H-bonds with Met59(2) (91%, 61%), Glu74 (49%); Van der Waals with Leu17, Lys209 and zinc-metal coordinations with His75 (100%), His232 (100%), and Asp236(2) (100%, 99%) in dynamic simulations. Glu74, His232, Asp236, Thr185 and Lys233 were found to be correlated in docking and simulations persistently involved in interaction with more rigidity while binding substrate/ligand/lead1 suggesting that these interactions as vital hotspots could contribute to the strong activity (Figs. 10 and 11). However, the radius of gyration (rGyr) measures the 'extendedness' of a ligand (lead1 or GVR cocrystal ligand) or compactness of the complex, and is equivalent to its principal moment of inertia. The dynamic simulations revealed that the compactness of lead1-LpxC (3.79 Å) was found better than that of GVR-LpxC complex (3.95 Å). Therefore, the esteeming interactional fractions of both complexes and the capability of Zn-binding with an ester of phosphoric acid (the conjugate base of the hydrogen phosphate ion) of lead1 ascertained the higher interaction stability or binding strength in LpxC-lead1 complex than LpxC-substrate or existing ligand complex. In Gram-negative *H. pylori*, LpxC metalloenzyme significantly is involved in LPS biosynthesis essential for cell wall formation. Therefore, the intervention of catalytic activity this enzyme with the designed and proposed lead compounds (Fig. 8) could have great impact on inhibition of cell wall formation, consequently which led to the growth inhibition of *H. pylori*.

CONCLUSION

In the study, the drug design process exploiting for better inhibitors in the lead breakthrough campaigns with exploring the relationships between existing published inhibitors and novel leads at the scaffold level to binding free energy levels. Better XPG scoring functions, molecular contacts, ADME/T and good binding affinity of proposed 7 leads of LpxC than GVR, co-crystal ligand and substrate (UDP-3-O-(3-hydroxymyristoyl)-*N*-acetylglucosamine). All the aspects of ADME are reflected in the pharmacokinetic (PK) profile of the leads. Besides, long-length essential dynamics simulations have led to yield improved therapeutic scaffolds with elucidating the impact of distal residues binding on structure than previous inhibitors having inhibition constants and persistent complex stability even at biological milieu. Therefore,

the results provide new insights into the design of next-generation LpxC inhibitors for *H. pylori* strains with occupying the drug-design space. In the context of receptor-lead interactions, all of the proposed leads encompassed good binding patterns with binding cleft residues and a Zn²⁺-binding moiety attached to a structural element addressing the hydrophobic tunnel or the UDP binding site of LpxC, thereby, the seven leads can show plausible competitive inhibition over the substrate (Udp-3-hmaglc) by binding to LpxC at the receptor binding domain. Moreover, since the target is non-homology to human, these lead scaffolds conduce to the inhibition of functional activity of LpxC without affecting the human physiology. Therefore, the resulted seven firm-binding anti-*H. pylori* cocktails could be recommended as potential inhibitors against LpxC of LPS pathway essential for cell wall could effectively circumvent the further chance of development of *H. pylori* resistance mechanisms.

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CONFLICT OF INTEREST

All authors declared that they have no conflicts of interest.

ETHICAL APPROVAL

Neither human studies, nor animal studies were carried out by the authors for this article.

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