In Silico Design and Molecular Docking Studies of Carbapenem Analogues Targeting Acinetobacter baumannii PBP1A Receptor

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Abstract:

Carbapenems are considered as the most effective antibiotic against Acinetobacter baumannii infections, as the pathogen has a resistance to the most of the other beta-lactam antibiotics; however, recent studies proved that this pathogen has developed resistance to carbapenems, as well. Therefore, development of novel therapeutics targeting A. baumannii resistant strains is an urgent global requirement. One of the causes responsible for this bacterial resistance against beta-lactam antibiotics is the decreased strength of interactions between A. baumannii Penicillin-Binding Proteins 1A (PBP1A) and carbapenems. Therefore, the aim of this study is to design a novel analogue of imipenem with significantly higher binding affinity and improved drug-likeness properties to overcome resistance of the pathogen and optimize bioavailability, respectively. De novo drug design was performed using virtual screening to predict the ligand(s) with the highest binding affinity. The two-dimensional and three-dimensional structure of the designed molecules were sketched using Chemdraw professional and MarvinSketch, respectively. After separating the targeted protein from A. baumannii PBP1A-imipenem complex structure (3UDX) and retaining a monomer (chain A) from a dimer of the protein structure using Text Editor (ConTEXT v0.98.6), docking was achieved using virtual screening AutoDock Vina program. Finally, drug-likeness properties were assessed. The results could find the selected compounds with significantly higher binding affinity and improved physicochemical properties compared with imipenem.

Key words: Acinetobacter baumannii, Penicillin-Binding Proteins, Binding affinity, Drug-likeness properties.

تصميم السيليكو ودراسات الارساء الجزيئي لمشتقات الكاربابينيم التي تستهدف بروتين اسينيتوباكتر باومانيى

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الخلاصة:

يعتبر الكاربابينيمس من أكثر المضادات الحيوية فعالية لعلاج الامراض التي تسببها بكتيريا اسينيتوباكتر باومانيى, ولكن البكتيريا تمتلك المقاومة لمعظم المضادات الحيوية الاخرى من مجموعة البيتا لاكتام, حيث اثبتت الدراسات الحديثة ان البكتيريا طور مقاومة لفعالية الكاربابينيمس ايضا. لذلك فأن تطوير ادوية جديدة تستهدف البكتيريا المقاومة للأدوية مطلبا عالميا عاجلا. وقد تبين ان أحد الاسباب المسؤولة عن هذه المقاومة ضد فعالية المضادات الحيوية من مجموعة البيتا لاكتام هي انخفاض قوة ارتباط الكاربابينيمس وبروتينات الاسينيتوباكتر باومانيى. الهدف من هذه الدراسة هو تصميم مشتق جديد من دواء الايميبينيم ذو قابلية ارتباط عالية مع بروتينات الاسينيتوباكتر باومانيى, وذلك للتغلب على مقاومة البكتيريا لفعالية الدواء, وتحسين الخصائص الدوائية لزيادة التوافر البيولوجي عن طريق الفم. في هذه الدراسة تم استخلاص البروتين المستهدف من البنية البلورية والاحتفاظ بمونومير (chain A) من بنية البروتين باستخدام محرر النصوص (ConTEXT v0.98.6) , ثم تم تحقيق الارساء باستخدام الجزيئات المصممة بتطبيق برنامج أوتدوك فينا, بالإضافة لتقييم الخصائص

الفيزيائية والكيمياوية للجزيئات المصممة. وقد تم الاستنتاج الى ان بعض المركبات المصممة لديها قدرة ارتباط اعلى بكثير للبروتين المستهدف مع تحسين الخصائص الفيزيائية والكيمياوية مقارنة مع دواء الايميبينيم.

الكلمات المفتاحية: سينيتوباكتر باومانيي, البروتينات المستهدفة من قبل البنسيلين, قابلية الارتباط, الخصائص الدوائية.

Introduction:

Generally, pathogenic bacteria can be stopped through using miracle drugs (antibiotics), as these drugs cured diseases related to bacterial infections and saved millions lives (1). However, bacterial resistance to various antibiotics through penicillinase production is the main obstacle and it has a long history, which has started from the discovery of penicillin's era (2) and in a growing trend. Recently, human community is facing a burgeoning threat of antimicrobial resistance, particularly, a serious concern regarding resistance against organisms responsible on nosocomial infections, which are the following six pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. (ESKAPE) (3). *A. baumannii* is one of the ESKAPE pathogens; it's a Gram-negative aerobic bacterium causes various diseases, such as urinary tract infections, secondary meningitis, bacteraemia, wound infection, and pneumonia (4). It can cause hospital acquired infections (nosocomial) (5), specifically in the critically ill patients have trauma, invasive procedures, mechanical ventilation, indwelling catheters, and burns; also, this infection could happen significantly in the immunocompromised patients and elderly (6, 7). Estimated mortality rate of this pathogen is around 26-68% (8); however, its challenging to identify the exact mortality rate because of the fact that most of the patients infected with this bacterium are seriously ill patients (9). In brief, *A. baumannii* is considered as one of the most perilous pathogens responsible on the severe contagions in hospitals and cause life-threatening infection because of the capability of the bacteria to resist multiple antibiotics, such as penicillin's, aminoglycosides, cephalosporins, tetracyclines, and quinolones (10).

The most popular medicine for the treatment of these infections was the beta-lactam antibiotics owing to their minimal side effects, patient compliance, affordable price, and effectivity. They are classified into four groups; the first and the earliest group is penicillins, followed by cephalosporins, monobactams, and lastly carbapenems group (11). Reports in all over the world reveal that resistance of *A. baumannii* isolates has been recorded against beta-lactam antibiotics (12-14). The prevalence of their resistance is increasing significantly (15). Nevertheless, carbapenems still consider as the most effective, safe, and justifiable class of antibiotics to cure *A. baumannii* infections. Although recently, appearing resistance to this class makes this therapeutic option under threat, as well (16). For example, according to the researches from southern Europe, the resistance rate of the invasive *A.* species isolates more than 50% against carbapenems (17, 18). Numerous research recognized the causes of this pathogen resistance. One of the factors related to the bacterial resistance against beta-lactam antibiotics is reducing binding affinity between Penicillin-Binding Proteins (PBP) and the beta-lactam antibiotics (19). And more specifically, according to the publication by Gehrlein *et al.* (1991), modifications in PBP1A of *A. baumannii* can cause resistance to imipenem due to the inhibition of binding affinity between the ligand and the protein (20). Another explanation of this resistance could be due to beta-lactamase enzyme (OXA-51), which could strongly bind with penicillins and cephalosporins, while it has significantly less binding affinity to carbapenems (11). However, specific

studies have reported that *A. baumannii* could develop resistance to all discovered antibacterial drugs rapidly through unpredicted mechanisms (21-25). Eventually, development of novel therapeutics targeting carbapenem resistant *A. baumannii* is the need of the hour by the World Health Organization, as its dangerous nosocomial pathogen (26, 27).

The carbapenems can be defined as the latest generation of beta-lactam antibiotics with the broadest spectrum of activity. The history of this lactam antibiotic class belonged to 1985 after discovery of the lead compound imipenem as a derivative of thienamycin (Figure 1A). It could treat millions of serious infections with broad-spectrum and highly potent characteristics. It has a strong safety profile and prevent the emergence resistance (28). After that, the newer analogues of the lead approved at the end of twentieth century and the beginning of this century, such as meropenem in 1993, ertapenem in 2001, and doripenem in 2007 (29). The same as other lactam antibiotics, carbapenem blocks cell wall transpeptidation of the peptidoglycan through binding to the PBPs of the bacterium, which leads to the cell death. As presented in Figure 1B, the fundamental chemical groups in the structure of carbapenems are beta-lactam ring, thiazolidine ring, and variable side chains to achieve biological activity (30). In addition, the availability of trans-1-hydroxyethyl group at the position 6 of carbapenems makes them significantly more stable and prevents hydrolysis of the bond between C4 and C7 compared with penicillins and other lactam antibiotics (31). Most of the current carbapenem antibiotics, except the natural thienamycin, imipenem, and panipenem have 1 beta methyl group because it can protect the carbapenems from hydrolysis by renal dehydropeptidase 1 enzyme (32). Regarding the structure of the designed molecules in this study, nucleus of the carbapenems has preserved. All of the compounds have a methyl group on C4 to prevent hydrolysis and trans-hydroxyethyl group on C6 to protect their stability. All of the modifications have been performed on R1 (C3) and R2 (C2) side chains (Figure 1B). The structures of R1 and R1 for each of the designed molecule are illustrated in the supplementary material Table S1.

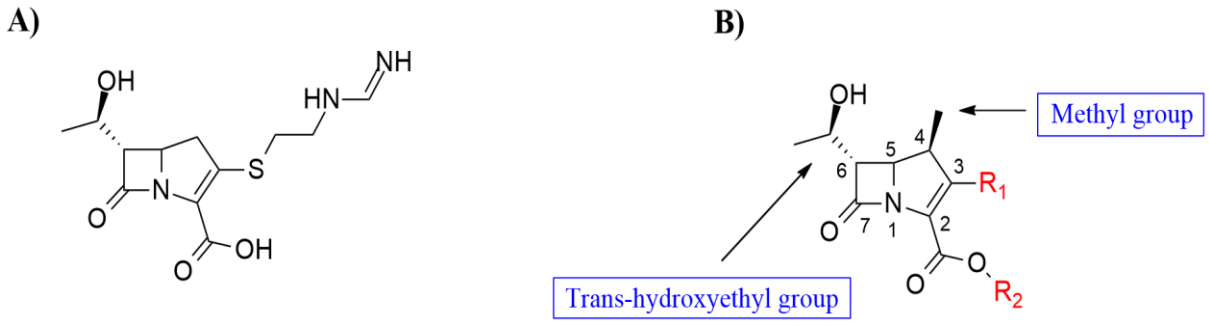


Figure (1): The 2D structures of carbapenem. (A) Imipenem structure, (B) Basic structure of the designed imipenem analogues.

Currently, sophisticated approaches of rational drug design, such as computational approaches could apply in the drug discovery projects to accelerate the drug discovery process, reduce costs, and increase success rate (33). One of the essential uses of computational modeling is their capability to predict binding affinity between small molecules and biological targets, which can speed-up the process to find hit compounds. The accurate estimation and calculation of binding affinity is the key point of success in the drug discovery process, while still in the continuous optimization (34). One of the programs to predict binding affinity is

known as molecular docking program, which is a part of structure-based drug design. This approach of molecular modeling can predict the interactions between two molecules and, particularly the favored conformation of ligand against the targeted protein or receptor to provide a stable adduct (35). Ligand-protein binding affinities are calculated through scoring functions, which is the preferred conformation of the ligand (36). Docking program is implemented through virtual screening process, when numerous small compounds can be screened and docked virtually against the pocket of the target proteins to realize which compound has the most shape and chemical complementarity to the hot spot of the protein (37).

The aim of this study is to design a novel, sensitive, and potential ligand derivative of carbapenems against the target *A. baumannii* PBP1A protein. Along the way, a subsidiary goal is to improve the drug-likeness properties of the selected ligand analogues compared with imipenem, using molecular docking program to reduce time and cost of the work. It starts with analyzing the crystal structure of PBP1A protein treated with imipenem, identifying the protein's active site (pocket) and investigating interactions with imipenem. The next step is to design a library of new ligand molecules based on the structure of carbapenems and PBP1A hot spot; after that, calculations of binding free energy and physicochemical properties of each designed molecule will achieve. Finally, analysis of the binding interactions between the selected analogues and the receptor will perform to realize the causes of changing potency.

Materials and Methods

Ligand Preparation

The two-dimensional structures of the designed molecules were sketched using Chemdraw professional 16.0. The 2D structures may not denote the exact atom's position in the actual molecule and acquires high energy. Therefore, the three-dimensional models of the compounds were generated applying MarvinSketch from a suite of applications called Marvin (38), which can use a molecular dynamics (MD) and energy minimization algorithm through calculating a new position of each atom in the molecules. PDBQT files of these designed compounds were generated.

Accession of Target Protein

Protein Data Bank (PDB) is a structural store house of macromolecules like proteins and their complexes (www.rcsb.org/pdb) (39, 40). The starting coordinates of the *A. baumannii* PBP1A-imipenem complex (solved at 2.5 Å resolution) was downloaded from the PDB website (https://www.rcsb.org, access code 3UDX (41). After downloading the three-dimensional structure file of the ligand-protein, Text Editor (ConTEXT v0.98.6) was used to differentiate the protein from imipenem and retain only a monomer (chain A) from a dimer of the protein structure. Finally, the pure protein structure was saved as PDBQT file for molecular docking. However, before starting the docking process, all of the water molecules were removed from PBP1A protein.

Lipinski's Rule of 5 Screening

Lipinski's rule of 5 or Pfizer's rule of 5 is an essential parameter to determine the drug-likeness properties of small molecules. Any proposed ligand follows this rule means it could be absorbed orally, while violating the rule leads to absorption and permeation problems. Designing small lead molecules in most current drug discovery projects should follow this rule to proceed as a drug candidate molecule (42, 43). The calculations were achieved using Chemaxon's Marvin Software (38).

Structure-Based Drug Design and Virtual Screening

After preparing the 3D-structure of the drug target (*A. baumannii* PBP1A protein) and designing novel ligands as a complementary (in shape and physicochemical properties) of the ligand-binding site, virtual screening was achieved, which is automated computational algorithms. This algorithm

tries to select potential ligands through matching the chemical complementarity and shape of the target protein and each of the ligands, recursively. This process is known as 'Docking'. In this process, ligands are ranked according to their potentiality. This ranking is known as scoring functions, which depends on the binding poses. In this study, the AutoDock Vina (http://vina.scripps.edu/) program was used as a virtual screening program (44) and PyRX 0.8 application was applied to recognize the binding modes of the designed molecules (45). To determine the scoring function in this method, specification of search space inside the coordination system of the protein is necessary, in which different positions of the ligand should be examined. The magnitude of the search space was determined with grid centre of X: -22, Y: -58, Z: -4.8, and the number of points in each magnitude was X:45, Y:45, Z:45 in Å. Each output file has a variety of binding mode ranked in downward manner according to their binding energy. The calculated binding affinity is measured in kcal/mole. Ultimately, the best mode (with highest binding affinity) is selected for further analysis (46, 47).

Visualization and Plotting Software

UCSF Chimera 1.6 package from the Resource for Biocomputing, Visualization, and Informatics at the University of California San Francisco was used to visualize interactions between various ligands and PBP1A protein, measuring distances between selected atoms, and conformational changes of the protein-ligand complex systems (48). The protein-ligand interactions, such as hydrophobic and H-bond interactions were investigated through using Ligplot Plus software version 1.4.5, which can generate and plot 2D representation of the interactions (49).

Results and Discussion

This project was carried out because despite serious intervention required to treat *A. baumannii* infection, most of the research to develop new treatments is focused on Gram-positive bacteria (50, 51) and continue to abandon research on this field owing to the challenging results and high risk of the investment (50). However, efforts from several organizations are continuing without a definite solution yet (52). This project focused on improving binding free energy of ligand-receptor to overcome the bacterial resistance because one of the causes of *A. baumannii* resistance against beta-lactam antibiotics is decreasing binding affinity between *A. baumannii* PBP1A and carbapenems. It was achieved through using molecular docking because it is a vital aspect in the drug discovery process to predict protein-ligand binding affinity (53). Various docking programs available worldwide; however, each docking tool has a different characteristic, such as license conditions, algorithms, endorsed platforms, and scoring functions. We used AutoDock Vina, which is considered as one of the most accurate and speed programs of molecular docking and virtual screening; it can apply a sophisticated gradient optimization method and has a relatively accurate binding mode estimation (44).

Binding Free Energy

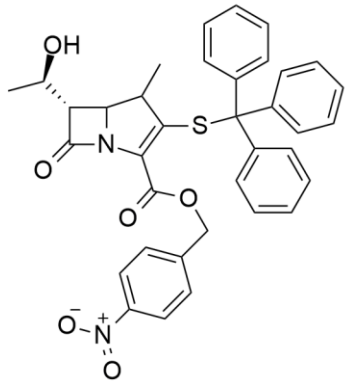
Prediction of binding free energy of imipenem and their derivatives with *A. baumannii* PBPA1 were performed using PyRx application. During the process, the program can sample as much conformational space of the ligands as possible, but only the top 10 conformations with the highest binding free energy were selected. The binding poses for each compound into the receptor protein were determined and different poses were produced based on the dock score. The binding affinity of each ligand-protein complex was identified through total score. The binding conformation for each imipenem analogue into the PBP1A protein was assessed; the highest and the lowest docking score were produced. The

highest scores express the better ligand-protein binding affinity values. The docking program provides binding free energy results in terms of $\Delta G_{binding}$. The results of screening 120 designed molecules and imipenem to predict comparative $\Delta G_{binding}$ are displayed in Table 1. It is clear that all of the designed molecules have higher binding free energy compared with imipenem. Among the designed molecule, the highest calculated binding score value is for the compounds H011, H045, and H106 (-9.9 kcal/mol) and the lowest docking energy is for the compound H095 (-7.4 kcal/mol).

ClogP

This value is a calculation of LopP, which can accurately predict octanol/water partition coefficient (54). It is an essential determinant of molecules physical properties and one of the parameters to evaluate absorption and distribution of the drug substances depending on the lipid solubility and understand drugs behavior *in vivo*. ClogP were calculated for all of the 120 designed molecules and imipenem using chemaxon cheminformatics and bioinformatics software (https:// chemicalize.com). Both of the compounds H076 and H080 have ClogP >5, which are 5.29 and 6.38, respectively. The high lipophilicity of these 2 molecules results in low solubility, poor oral bioavailability, and high metabolic turnover. Furthermore, these compounds may lead to off-target issues and toxicity owing to their tendency to interact with hydrophobic proteins. As shown in Figure 2A, the cause behind lipophilicity of the compound H080 that has the highest ClogP value is the presence of many aromatic rings, which is directly correlates with increasing attrition rate during the drug development process. On the other hand, 62 of the designed molecules and imipenem are highly hydrophilic because of their ClogP <1. The most hydrophilic molecule is H009 with a ClogP value -7.81. The causes of this compound's hydrophilicity are the presence of free carboxylic group at R2 position, moreover, 2 carboxylic group, heterocyclic group, amino group a hydroxyl group at R1 position (Figure 2B). Like highly lipophilic compound, the highly hydrophilic molecules have a negative impact on the drug-likeness properties; it leads to decrease permeability, efficacy, and bioavailability of the molecules. Therefore, to maintain optimum physicochemical and pharmacokinetic properties, ClogP should be in the range of 1-5 (55). Present findings confirm that the designed compounds fall their ClogP in the range of 1-5 are 56 compounds.

A)



B)

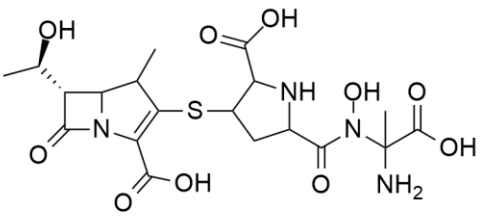


Figure (2): The designed molecules with the highest lipophilicity and hydrophilicity. (A) Compound H080. (B) Compound H009.

Size of the Molecules

Small molecule drugs are those compounds with a molecular weight around 500 Dalton (Da) to be orally bioavailable (56). According to our results, the smallest molecule in the Table 1 is imipenem (299.35 g/mol). Regarding the designed molecules, the smallest compound is H005 (415.1 g/mol) and the largest molecule is H106 (839.19 g/mol). Thus, even the smallest designed compound has higher MW than imipenem. From these results, it's clear that 86 of the designed compounds have a MW > 500 Da and the remaining 34 compounds have a MW < 500 Da (Table 1).

Drug-likeness and Lipinski's Rule of Five

Drug likeness is a balance of structural features and diverse properties of a ligand. Lipinski's rule of five can be applied to evaluate molecular properties related to pharmacokinetic behaviors of the molecule, as it can predict physicochemical properties of ligand, such as ClogP, molecular weight, hydrogen bond (H-bond) donors and acceptors (42). However, some research shows that these properties may not be restricted by this rule (57). According to this rule, an organic molecule is presumably orally active when H-bond donor < 5, H-bond acceptor < 10, partition coefficient (logP) < 5, and MW < 500 Da. In this study, drug likeness results are shown in Table 1. The results showed that out of 121 compounds, imipenem and 32 of the designed compounds passed this rule. The basic structure of the designed molecules and the R1 and R2 modifications are illustrated in the supplementary material (Table S1).

Table (1): The chemical formula, $\Delta G_{binding}$ values, ClogP, MW, and Lipinski's rule of five calculation of imipenem and the designed analogous.

Compound	Chemical formula	Binding free energy (kcal/mol)	CLogP	Rule of 5 criteria	Molar mass (g/mol)
H001	$C_{24}H_{30}N_6O_7S$	-8.8	-5.81	no	546.1
H002	$C_{24}H_{28}N_4O_9S$	-9	-3.97	no	548.15
H003	$C_{28}H_{36}N_6O_9S$	-9.1	-6.79	no	632.22
H004	$C_{30}H_{33}N_5O_{10}S$	-8.5	-2.95	no	655.19
H005	$C_{16}H_{21}N_3O_8S$	-8.4	-4.55	yes	415.1
Compound	**Chemical formula**	**Binding free energy (kcal/mol)**	**CLogP**	**Rule of 5 criteria**	**Molar mass (g/mol)**
H006	$C_{17}H_{21}N_3O_{10}S$	-8	-4.97	no	459.09
H007	$C_{17}H_{22}N_4O_9S$	-8.2	-5.67	no	458.11
H008	$C_{19}H_{24}N_4O_{10}S$	-7.7	-7.77	no	500.12
H009	$C_{19}H_{26}N_4O_{10}S$	-8.2	-7.81	no	502.13
H010	$C_{33}H_{38}N_4O_{10}S$	-8.2	-2.2	no	682.23
H011	$C_{33}H_{38}N_4O_{10}S$	-9.9	-2.2	no	682.23
H012	$C_{34}H_{39}N_5O_{11}S$	-9.7	-3.24	no	725.23
H013	$C_{25}H_{32}N_4O_7S$	-9.5	-3.63	no	532.19
H014	$C_{33}H_{39}N_5O_9S$	-9.3	-2.89	no	681.24
H015	$C_{29}H_{37}N_5O_8S$	-9.8	-4.3	no	615.23
H016	$C_{31}H_{36}N_4O_8S$	-9.2	-2.28	no	624.22
H017	$C_{25}H_{31}N_5O_7S$	-9.2	-3.9	no	545.19
H018	$C_{29}H_{31}N_3O_7S$	-9.8	-0.83	no	565.8
H019	$C_{22}H_{24}N_5O_6S$	-8.9	-3.6	yes	486.14
H020	$C_{33}H_{40}N_6O_{11}S_2$	-9.6	1.71	no	760.21
H021	$C_{22}H_{25}F_3N_4O_6S$	-9.5	-1.27	no	530.14
H022	$C_{35}H_{43}N_5O_9S$	-8.7	-0.38	no	709.27
H023	$C_{33}H_{37}N_3O_8S$	-9.2	2.05	no	635.23
H024	$C_{23}H_{26}BrN_5O_9S$	-9.3	0.09	no	627.06
H025	$C_{26}H_{31}N_3O_8S$	-8.7	-0.81	no	545.18
H026	$C_{24}H_{29}N_3O_7S$	-8.9	-0.07	no	503.17
H027	$C_{18}H_{25}N_3O_7S$	-8	-1.8	yes	427.14
H028	$C_{24}H_{29}N_3O_6S$	-8.7	-0.42	yes	487.17
H029	$C_{24}H_{29}N_3O_6S$	-7.8	-0.42	yes	487.17
H030	$C_{20}H_{27}N_5O_6S_2$	-8.1	-2.93	yes	497.14
H031	$C_{22}H_{27}N_3O_6S_2$	-8.2	-0.5	yes	493.13
H032	$C_{24}H_{30}N_4O_6S$	-8.9	-1.81	no	502.18
H033	$C_{33}H_{38}N_6O_{10}S$	-9.4	1.01	no	710.23
H034	$C_{33}H_{39}N_5O_9S$	-9.3	0.63	no	681.24
H035	$C_{35}H_{40}N_6O_8S$	-9.4	1.17	no	704.26
H036	$C_{33}H_{39}N_5O_8S$	-9.1	1.07	no	665.25
H037	$C_{30}H_{37}N_7O_8S$	-8.9	-1.35	no	655.24
H038	$C_{29}H_{37}N_5O_9S$	-8.7	-1.25	no	631.23
H039	$C_{29}H_{39}N_5O_8S$	-8.6	0.3	no	617.25
H040	$C_{27}H_{35}N_5O_9S$	-8.4	-1.63	no	605.21
H041	$C_{29}H_{39}N_5O_8S_2$	-8.1	0.07	no	649.22
H042	$C_{27}H_{35}N_5O_8S_2$	-8.4	-0.53	no	621.19
H043	$C_{18}H_{25}N_3O_7S$	-8.1	-1.8	yes	427.14
H044	$C_{18}H_{25}N_3O_7S$	-8.2	-1.8	yes	427.14
H045	$C_{36}H_{33}N_3O_{10}S$	-9.9	3.95	no	699.18
H046	$C_{29}H_{29}N_3O_9S$	-9	1.99	no	595.16

H047	$C_{23}H_{22}N_2O_6S$	-8.3	2.91	yes	454.11
H048	$C_{22}H_{34}N_4O_6S_2$	-7.9	-4.24	no	514.19
H049	$C_{20}H_{30}N_4O_6S_2$	-7.9	-4.84	yes	486.14
H050	$C_{31}H_{35}N_5O_8S$	-9.3	1.68	no	637.2
Compound	**Chemical formula**	**Binding free energy (kcal/mol)**	**CLogP**	**Rule of 5 criteria**	**Molar mass (g/mol)**
H051	$C_{32}H_{34}N_4O_{10}S$	-9	1.76	no	666.19
H052	$C_{31}H_{34}N_4O_{10}S$	-8.5	2.4	no	654.19
H053	$C_{32}H_{34}N_4O_{11}S$	-8.6	2.56	no	682.19
H054	$C_{19}H_{19}N_5O_7S$	-8.9	0.9	yes	461.1
H055	$C_{39}H_{38}N_4O_7S$	-7.8	4.95	no	706.24
H056	$C_{22}H_{23}N_5O_6S$	-8.8	1.21	yes	485.13
H057	$C_{22}H_{26}N_2O_6S$	-8.6	2.68	yes	446.15
H058	$C_{23}H_{18}F_4N_2O_6S$	-8.5	3.48	no	526.08
H059	$C_{23}H_{20}Cl_2N_2O_6S$	-8.2	4.12	no	522.04
H060	$C_{23}H_{21}BrN_2O_6S$	-8.6	3.68	no	532.03
H061	$C_{23}H_{21}BrN_2O_6S$	-8.5	3.68	no	532.03
H062	$C_{23}H_{21}N_3O_8S$	-9.1	2.85	yes	499.1
H063	$C_{23}H_{22}ClN_3O_6S$	-8.6	2.69	no	503.09
H064	$C_{23}H_{22}N_2O_7S$	-8.8	2.61	yes	470.11
H065	$C_{23}H_{22}N_2O_7S$	-8.3	2.61	yes	470.11
H066	$C_{23}H_{22}N_2O_6S_2$	-7.9	3	yes	486.09
H067	$C_{24}H_{20}FN_3O_6S_2$	-8.2	3.88	no	529.07
H068	$C_{24}H_{21}F_3N_2O_7S$	-9.1	4.34	no	538.1
H069	$C_{24}H_{22}F_2N_2O_6S$	-8.3	3.44	no	504.11
H070	$C_{24}H_{22}N_2O_8S$	-8.7	2.57	yes	498.1
H071	$C_{24}H_{23}ClN_2O_6S$	-9	3.76	no	502.09
H072	$C_{24}H_{23}FN_2O_7S$	-8.9	2.29	no	502.12
H073	$C_{25}H_{26}N_2O_7S$	-9	3	yes	498.14
H074	$C_{25}H_{24}N_2O_8S$	-8.9	2.81	no	512.12
H075	$C_{25}H_{24}N_2O_8S$	-9.1	2.55	no	512.12
H076	$C_{31}H_{30}N_2O_6S_2$	-9.3	5.29	no	590.15
H077	$C_{27}H_{24}N_2O_6S$	-7.9	3.9	no	504.13
H078	$C_{27}H_{30}N_2O_6S$	-9.1	4.46	no	510.28
H079	$C_{29}H_{26}N_2O_6S_2$	-9.1	4.65	no	562.12
H080	$C_{36}H_{32}N_2O_6S$	-7.6	6.38	no	620.19
H081	$C_{23}H_{21}FN_2O_6S$	-8.3	3.05	yes	472.11
H082	$C_{23}H_{23}N_3O_6S$	-8.4	2.08	yes	469.13
H083	$C_{24}H_{21}FN_2O_8S$	-8.7	2.71	no	516.1
H084	$C_{25}H_{23}F_3N_2O_7S$	-8.5	4.59	no	552.11
H085	$C_{25}H_{23}N_5O_7S$	-9.7	2.24	no	537.13
H086	$C_{25}H_{26}N_2O_8S$	-8.5	2.6	no	514.14
H087	$C_{25}H_{27}N_3O_6S$	-8.9	3.02	yes	497.16
H088	$C_{23}H_{22}N_6O_{10}$	-9.2	2.74	no	542.13
H089	$C_{24}H_{22}N_2O_8$	-8.8	2.15	yes	466.13
H090	$C_{24}H_{22}N_2O_9$	-9.4	2.94	yes	482.13
H091	$C_{24}H_{24}N_4O_6S$	-9	2.61	yes	496.14
H092	$C_{25}H_{30}N_4O_9S$	-7.6	0.25	no	562.17
H093	$C_{25}H_{24}N_2O_8$	-7.9	1.99	yes	480.15
H094	$C_{26}H_{26}N_2O_8S$	-8.9	3.16	no	526.14
H095	$C_{19}H_{18}N_4O_6S_3$	-7.4	2.15	yes	494.03
H096	$C_{25}H_{23}N_5O_7S$	-7.8	2.16	no	537.13

H097	$C_{22}H_{34}N_4O_6S$	-8	-4	yes	482.21
H098	$C_{24}H_{22}N_2O_9$	-8.2	2.09	yes	482.13
H099	$C_{33}H_{39}N_5O_9S$	-8.5	-1.01	no	681.24
H100	$C_{33}H_{39}N_5O_{10}S$	-9.2	-1.09	no	697.24
H101	$C_{35}H_{41}N_5O_{11}S$	-8.9	-3.03	no	739.25
H102	$C_{25}H_{32}N_4O_9S$	-8.5	-4.6	no	564.18
H103	$C_{26}H_{32}N_4O_{10}S$	-8.5	-6.42	no	592.18
H104	$C_{25}H_{32}N_4O_8S$	-9.8	-4.48	no	548.19
H105	$C_{25}H_{32}N_4O_7S$	-9.7	-3.63	no	532.19
H106	$C_{38}H_{35}F_2N_5O_{13}S$	-9.9	4.22	no	839.19
H107	$C_{32}H_{35}N_5O_{11}S$	-8.7	2.06	no	697.2
H108	$C_{25}H_{30}N_4O_9S$	-9.1	0.01	no	562.17
H109	$C_{26}H_{34}N_4O_7S$	-9.1	-3.54	no	546.21
H110	$C_{31}H_{34}N_4O_9S$	-9.3	1.97	no	638.2
H111	$C_{31}H_{34}N_4O_8S$	-9.1	1.63	no	622.2
H112	$C_{27}H_{32}N_6O_8S_2$	-9.6	-0.5	no	632.17
H113	$C_{23}H_{27}N_7O_6S_3$	-8.8	-0.87	no	593.11
H114	$C_{26}H_{34}N_4O_7S$	-9.1	-3.54	no	546.21
H115	$C_{28}H_{35}N_5O_6S$	-9	-3.14	no	569.23
H116	$C_{26}H_{34}N_4O_6S$	-8.9	-3.24	no	530.21
H117	$C_{23}H_{32}N_6O_6S$	-8.7	-5.27	no	520.21
H118	$C_{29}H_{37}N_5O_8S$	-9.2	-0.1	no	615.23
H119	$C_{22}H_{32}N_4O_6S$	-8	-4.62	yes	480.2
H120	$C_{22}H_{32}N_4O_7S$	-7.6	-5.77	yes	496.19
Imipenem	$C_{12}H_{17}N_3O_4S$	-6.6	-3.85	yes	299.35

Imipenem-PBP1A Binding Interactions

In this study, imipenem was used as a positive control. As shown in Figure 3, various bonding interactions exist between the protein's active site and imipenem. One of the essential types of interactions is H-bond, since this ligand forms 6 H-bonds with the receptor. Trans-hydroxyethyl group is essential for the activity of imipenem, as its hydroxyl group produces H-bond with the side-chain hydroxyl group of Ser434. The next binding is between the carbonyl oxygen of beta lactam and the backbone N-H group of Thr672. Moreover, carboxylic acid group of imipenem interacts with both of the side-chain amino group of Lys669 and the hydroxyl group of Thr670, bond distances are 2.5 Å and 2.9 Å, respectively. As realized in Figure 3A, the C2 side chain has a flexible structure that produces significant interactions with the side-chains hydroxyl group of Thr670 and Thr672. In addition, the carboxylate anion of imipenem produces salt-bridge with the positive ammonium group of Lys669. Despite the hydrophilicity of imipenem molecule, hydrophobic contacts are formed with 7 residues of the protein's active site (Figure 3B).

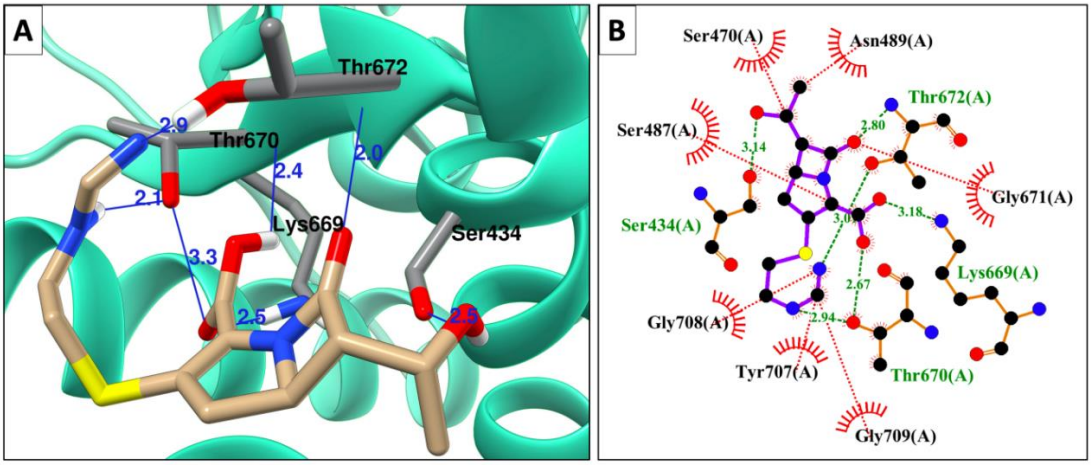


Figure (3): The binding interactions between imipenem and the A. baumannii PBP1a active site. A) Intermolecular H-bonds of the ligand-receptor. Blue lines represent H-bond distances (by angstrom). B) 2D shape of the binding modes produced through Ligplot plus. H-bonds, green dotted lines; non-ligand residues involved in hydrophobic interactions, red curved spikes; the ligand-receptor hydrophobic contacts; red dotted lines.

H045-PBP1A Binding Interactions

Investigations of the docked conformations were achieved to observe the binding mode of compound H045 into PBP1A target protein. Despite three of the designed compounds (H011, H045, and H106) have the same and the highest binding affinity (-9.9 kcal/mol), compound H045 was selected to investigate the binding interactions, since it has an acceptable ClogP value (3.95); however, it could not pass the rule of five criteria's due to the molecule's size (699.18 g/mol) (Table 1). As explained in Figure 4, the structure of H045 binds with the *A. baumannii* PBP1A pocket through H-bond, hydrophobic contacts, and pi-pi interactions. Three crucial H-bonds are shown between R1 group of H045 and side chains of Ser489, Lys669, and Thr670 residues, with a bond distance 1.9 Å, 2.3 Å, and 2.4 Å, respectively. The compound H045 produces hydrophobic contacts with various residues of the receptor due to the lipophilicity of the molecule; residues contact with the ligand are Ser434, Ser470, Tyr485, Leu486, Asn489, Leu526, Gly671, Thr672, Asn674, Asp675, Ala676, Gly703, Arg704, and Tyr707 (Figure 4B). Then, intra and inter molecular pi-pi interactions are realized; the intramolecular sandwich pi-pi interaction can be demonstrated between the two benzyl rings of the ligand. In addition, two ligand-receptor pi-pi interactions are recognized; the first is between the receptor's Tyr485 side-chain and the ligand's R1 benzoic acid group. The second is between the receptor's Tyr707 side-chain and the ligand's R1 benzoyl group. Furthermore, T-shaped interaction is formed between the side-chain of Tyr707 amino acid and *para*-nitrobenzyl group (PNB) of the ligand's R2 (Figure 4A). Overall, this designed molecule is nested in the pocket of PBP1A protein and surrounded by various residues of the protein to form H-bonds, various hydrophobic contacts and pi-stackings (Figure 4C).

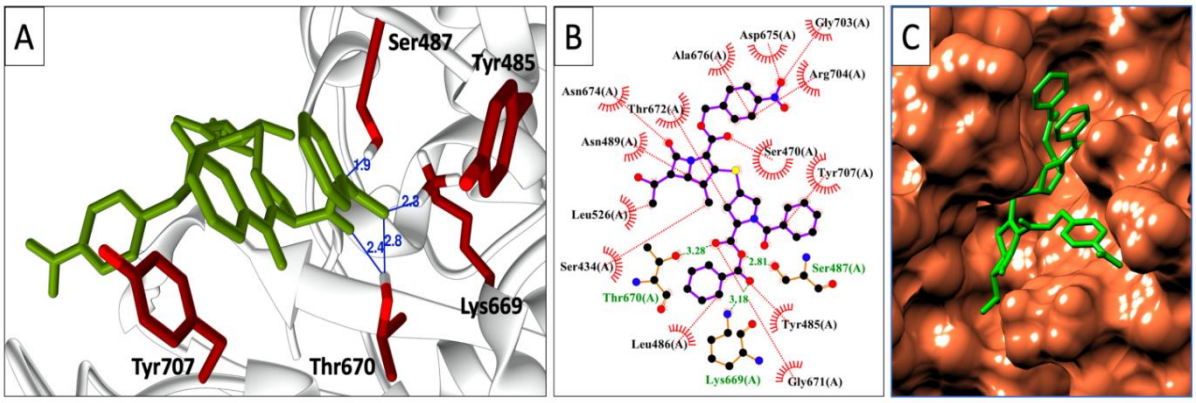


Figure (4): The H045-A. baumannii PBP1A protein complex interactions. (A) The 3D model showing H-bond interactions of the ligand-receptor complex. H-bonds, blue lines. (B) 2D plot of the binding modes created through Ligplot plus. H-bonds, green dotted lines; non-ligand residues involved in hydrophobic interactions, red curved spikes; the ligand-receptor hydrophobic contacts; red dotted lines. (C) The ligand nested in the active pocket of A. baumannii PBP1A protein. H045 is green and the protein is a brown Surface.

H090-PBP1A Binding Interactions

Our results confirm that the compound H090 can be selected as the molecule of choice, as it passed Lipinski's rule of 5, ClogP is in the range of oral bioavailability (2.94), and MW < 500 Da (Table 1). Furthermore, it has the highest binding affinity ($\Delta G_{binding}$= -9.4 kcal/mol) compared with the designed molecules that passed the rule. From the post docking analysis, we observed that H090 reveals high binding free energy with *A. baumannii* PBP1A. Upon study of the interactions of the designed compound in the active site of the target protein, various H-bonds, hydrophobic interactions and pi-pi interactions were revealed. The H-bond interactions are illustrated in Figure 5; the side-chain hydroxyl group of Ser434 interacts with the ligand's beta-lactam carbonyl group. The amide side-chain of Asn489 binds with the carbonyl oxygen atom of the ligand's R2 group (distance 2.4 Å). Next, the backbone carbonyl of Leu526 interacts with the hydroxyl group of trans-hydroxyethyl group of the ligand. Both of Asp675 and Ala676 are interacted through the backbone N-H group and are positioned about 2.5 Å and 2 Å from the ligand's R1 phenolic hydroxyl group, respectively, while Thr673 is interacted through the main-chain carbonyl group with the same hydroxyl group (distance 2.8 Å). As a result of the lipophilicity of H090, it participates in diverse hydrophobic contacts with the active site of the protein. Residues Gly433, Ser470, Thr670, Gly671, Thr672, Asn674, and Tyr707 of the protein is contributed in the hydrophobic interactions with the ligand molecule (Figure 5B). Moreover, two pi effect investigated. The first is the T-shaped pi-pi interaction occurred between the Tyr707 side-chain and the ligand's R1 phenolic group. The second is the sandwich shaped pi-pi interaction between the Tyr707 side-chain and the ligand's PNB group (Figure 5A).

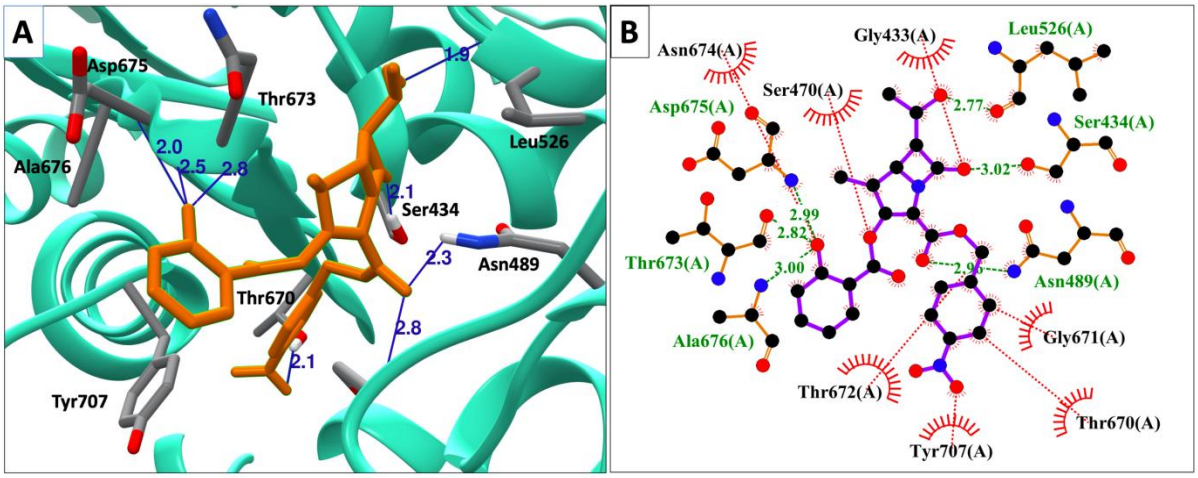


Figure (5): The H090-PBP1A interactions. A) The ligand-receptor 3D molecular interactions. Blue lines are denoted H-bond distances between the ligand and receptor. B) 2D plot of the binding modes created through Ligplot plus. H-bonds, green dotted lines; non-ligand residues involved in hydrophobic interactions, red curved spikes; the ligand-receptor hydrophobic contacts; red dotted lines.

Conclusion

Improving the potency of beta-lactam antibiotics could be a prerequisite factor to overwhelm antibiotic resistance; therefore, designing novel carbapenem analogues against *A. baumannii* PBP1A protein with significantly increased relative binding free energy is crucial. The results of virtual screening and drug-likeness properties testing for a library of the designed molecules concluded that the compound H090 is predicted to be a hit molecule owing to a significantly higher binding affinity (-9.4 kcal/mol) compared to imipenem and passing the Lipinski's rule of five criteria; it has an optimum ClogP value (2.94) and the MW is less than 500 Da (482.13). The results realize how *in silico* drug design could assist designing carbapenem analogues against the target protein. This promising analogue has not only higher binding affinity, but also improved physicochemical properties. Although, all of the results conclude unequivocally that the designed molecules require further studies through additional investigations to confirm computational outcomes and experimental assays, such as biophysical assays and minimum inhibitory concentration analysis.

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