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DETERMINATION OF THE INSILICO ANTIBACTERIAL ACTIVITY OF AMLODIPINE

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ABSTRACT

The dihydropyridine amlodipine was proven to be very effective bactericidal. Here we attempted to propose a possible mechanism of action of amlodipine as antibacterial by insilico docking at the catalytic domain (CA) of various bacterial histidine kinases of six bacterial strains and by comparing the binding energies and types of interactions between amlodipine and the different enzymes. Results revealed that amlodipine could be antibacterial by inhibiting the phosphorylation at the catalytic domain of bacterial histidine kinases.

KEYWORDS: Amlodipine, histidine kinase, insilico docking, non-antibiotics antimicrobials.

INTRODUCTION

A variety of compounds which are used in the management of diseases of non-infectious etiology have shown some in vitro antimicrobial activity against bacteria and other microorganisms, such compounds are called non-antibiotics antimicrobials.[1]

The emergence of antibiotic-resistance which had resulted in decreased efficacy and withdrawal of the antibiotic from widespread usage, led to the need of an alternative therapeutic approach that could interrupt host pathways used by bacteria in various stages of their life cycle, namely, adhesion, entry and growth,[2,3,4] there for new broad spectrum

therapeutics that are safe, effective and not vulnerable to the development of bacterial resistance are highly desirable.

Histidine kinase (HK) receptors are elements of the two-component signal transduction systems(TCST) commonly found in bacteria and lower eukaryotes, where they are crucial for environmental adaptation through the coupling of extracellular changes to intracellular responses.[5] Signal-transductions mediated by histidine kinases play a central role in information processing and they regulate large variety of cellular responses, including bacterial chemotaxis, osmoregulation, photosensitivity, sporulation, plant response to ethylene, microbial pathogenesis, nutrient acquisition, biofilm formation, and antibiotic resistance.[6,7]

The common feature of signal propagation among this family of proteins is that a dimeric histidine kinase undergoes ATP-dependent autophosphorylation on a specific histidine residue and subsequently transfers the phosphoryl group to an aspartate residue on a cognate DNA binding response regulator, altering the latter's transcriptional, enzymatic or mechanistic properties.[8,9]

In general the nature of histidine kinase sensor proteins is such that the catalytic domain and other cytoplasmic elements are conserved whereas the sensor domains are modular and highly variable in sequence.[5,10] According to their vital role in bacterial physiology, many evidences suggest that bacterial histidine kinases could represent a new and interesting antibacterial targets.

Many efforts have targeted variable regions of TCS proteins to exclusively inhibit a single pathway. Instead, targeting a conserved domain will result in a single molecule that might inhibit multiple TCSs per organism and perhaps organisms. Many compounds have been shown to inhibit different types of histidine kinases in various bacterial strains, typical examples are the thiazolidinone derivatives against the YycG histidine kinase of *Staphylococcus epidermidis*,[11] which also showed in addition to a few benzamide, furan and pyrimidinone derivatives to inhibit the auto-phosphorylation by binding to the ATP binding domains.[12] Vick histidine kinase of *Streptococcus pneumonia* was also shown to be inhibited by various compounds that prevent its phosphorylation.[13] Four compounds had been proven to be drug candidates as inhibitors of the phosphorylation of the Pho HK of *Shigella flexeneri* and *Salmonella sp.*[14]

The organized investigation of non-antibiotics has shown that many antihistamines tranquilizers, antihypertensives, antispasmodics and anti-inflammatory agents possess moderate to powerful *in vitro* and *in vivo* antibacterial activity.[15] Other drugs that were found to have antibacterial effect are dihydropyridines (DHPs) such as amlodipine and lacidipine[16] and statins such as simvastatin.[17]

Beside the cardiovascular effects in humans - by antagonizing the calcium mediated effects chiefly at the L- type calcium channels[18,19,20] - amlodipine had been proved to possess powerful antibacterial activity both through in vitro and in vivo tests. While sensitive bacterial strains occurred among *S. aureus, V. cholera, Bacillus spp.* and some enterobacteria, the drug was much less active with respect to *shigellae, salmonellae, E. coli, klebsiellae* and *pseudomonads*. Amlodipine is bactericidal in nature when tested *in vitro* against gram-positive and gram-negative bacteria.[21] Also the effect of its combination with antibiotics such as Streptomycin by disc diffusion technique revealed synergism.[22] which also had been proved for imipenem and amlodipine combination in some bacterial isolates.[23]

Many pyridine containing compounds were shown to be HK inhibitors[12] and Amlodipine is a pyridine derivative also sequence alignment between the DHP binding sequences of Calcium channels and the CA domain of HKs showed sequence homology. These indicate that AML may act as phosphorlytion inhibitor by binding to the conserved CA domain in bacterial HKs.

METHODOLOGY

Searching the database

Amlodipine (AML)

The 3D structure of AML was obtained from Pubchem database (CID 2162) in sdf format and converted by Bable tool for format conversion in to PDB format.

Calcium channel isoforms:

The amino acid sequences (in fasta format) of the four isoforms of the L-type calcium channels $Ca_v1.1$, α1s (Q13698), $Ca_v1.2$, α1C (Q13936), $Ca_v1.3$, α1D (Q01668) and $Ca_v1.4$, α1F (O60840) were retrieved from UNIPROT database.

Bacterial histidine kinases (HK)

The 3D structure and the amino acid (a.a) sequence of the catalytic (ATP binding) domain of *Bacillius subtilis* Yycg HK (3SL2) and the cytoplasmic domain of CpxA HK of *Escherichia coli* (4BIV) were retrieved from PDB database.

The fasta sequences of four HKs from *Staphylococcus aureus* accession number (wp:000871604.1), *Salmonella enterica* (wp:061381612.1), *Vibrio cholerae* (wp:057558169.1) and *Pseudomonas aeruginosa* (wp:051638076.1) were obtained from the ref-seq database.

Sequence alignment

Alignment of the ATP binding domain with Calcium channel isoforms

Sequence alignment was achieved between the DHP binding regions of each isoform with the CA domain of the HK (Yycg) of *Bacillus subtilis* using BLAST (Basic Local Alignment Sequence Tool) $Ca_v1.1$: α1S (Q13698) we found to be 1,873 a.a length with the DHP binding sequences of amino acids at (988-1077) and (1337-1403).

$Ca_v1.2$: α1C (Q13936) is 2,221 a.a length with DHP binding sites of amino acids at (1109-1199) and (1478-1546).

$Ca_v1.3$: α1D (Q01668) is 2,161 a.a length with the DHP binding sites (1075-1165) and (1420-1486).

$Ca_v1.4$: α1F (O60840) is 1,977 a.a length with the DHP binding sites at (1060-1150) and (1397-1463).

Alignment with bacterial proteins

The 3D structure of *Escherichia coli* histidine kinase (CpxA) was obtained by blasting the 3SL2 fasta sequence against the PDB data base for protein structure, while the fasta sequences of HKs of other strains were obtained by blasting 3SL2 against the ref-seq database.

Homolgy Modeling

Homolgy model of the 3D structures of HKs of *Staphylococcus aureus, Salmonella enterica, vibrio cholerae and pseudomonas aeruginosa* were prepared using SWISS-model web server.

Superposition (docking site prediction)

The 3D structures of the six proteins were superimposed using POSA server (Partial Order Structure Alignment) to identify the conserved structurally similar sites to be prepared for docking.

Docking

Each protein structure of the six HKs was docked with Amlodipine using Autodock2.4 program and docking scores were obtained, then the types of interactions were visualized using the Accelry Discovery Studio 2.5 to reveal the H-bonding and the Pi type interaction and the Ligplot tool to reveal the hydrophobic interaction.

RESULTS

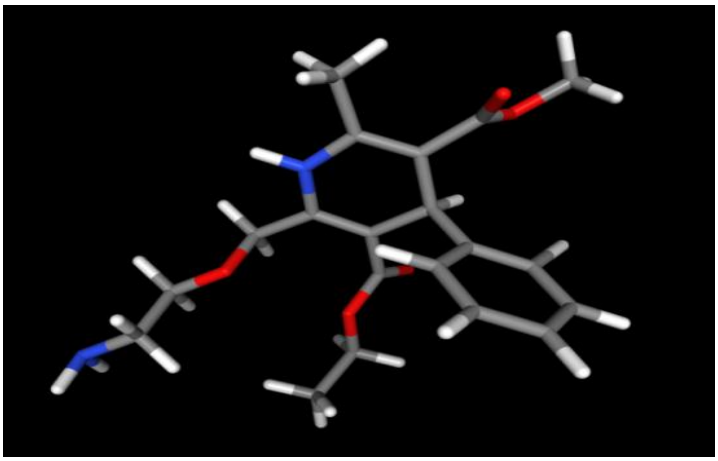
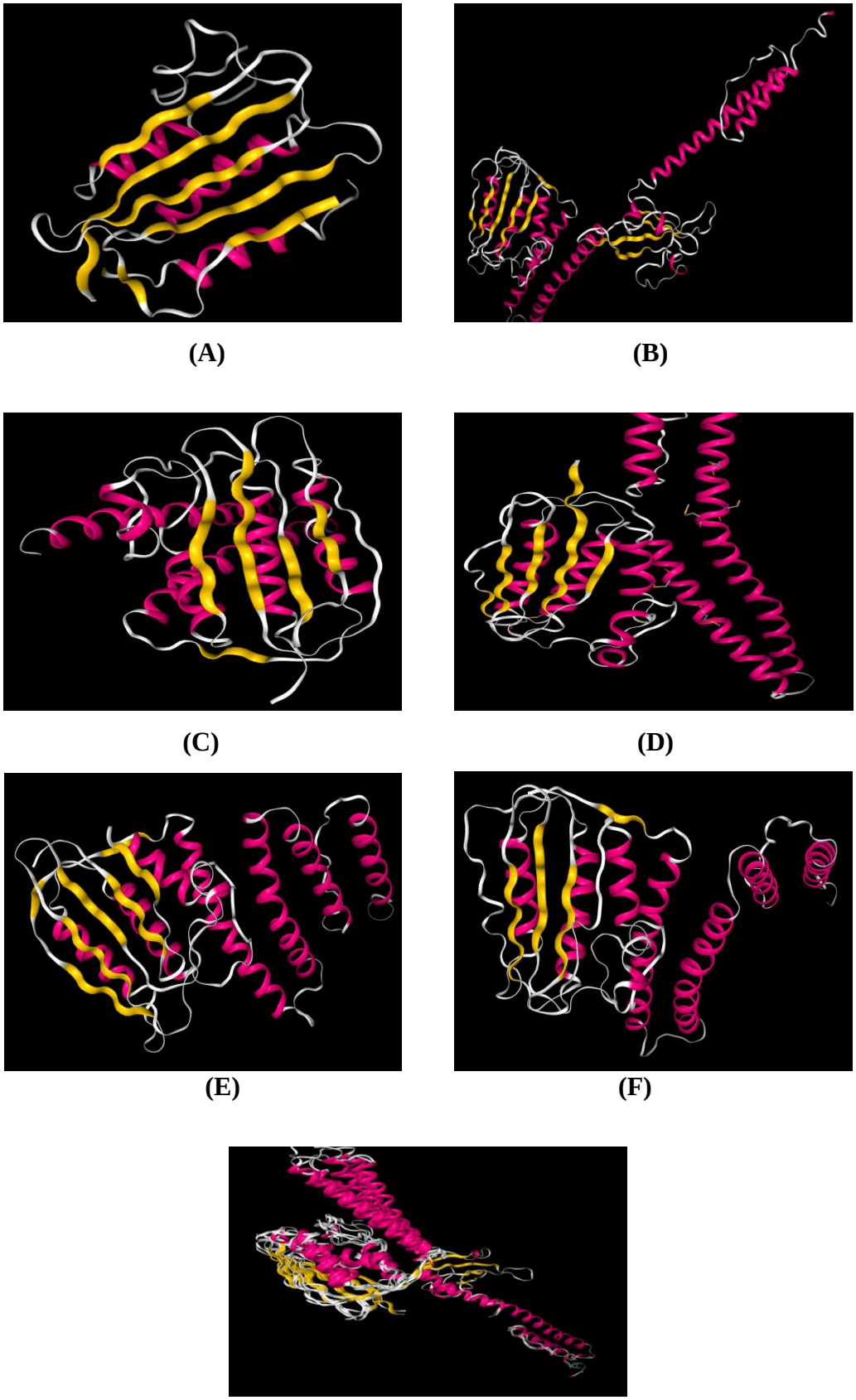


Fig.1 The 3D structure of Amlodipine as seen by NGL viewer.

Table 1: BLAST results of the catalytic domain of *B.subtilis* (3SL2) and different isoforms of the L –type Ca channel.

L-type Ca channel isoform	Uniprot ID	E- values	Identities %
α1S (988-1077) (1337-1403)	Q13698	1.6 2.6	50 24
α1C (1109-1199) (1478-1546)	Q13936	1.9 4.3	33 24
α1D (1075-1165) (1420-1486)	Q01668	1.2 2.8	33 57
α1F (1060-1150) (1397-1463)	O60840	4.5 2.7	67 24



(A) **(B)**

(C) **(D)**

(E) **(F)**

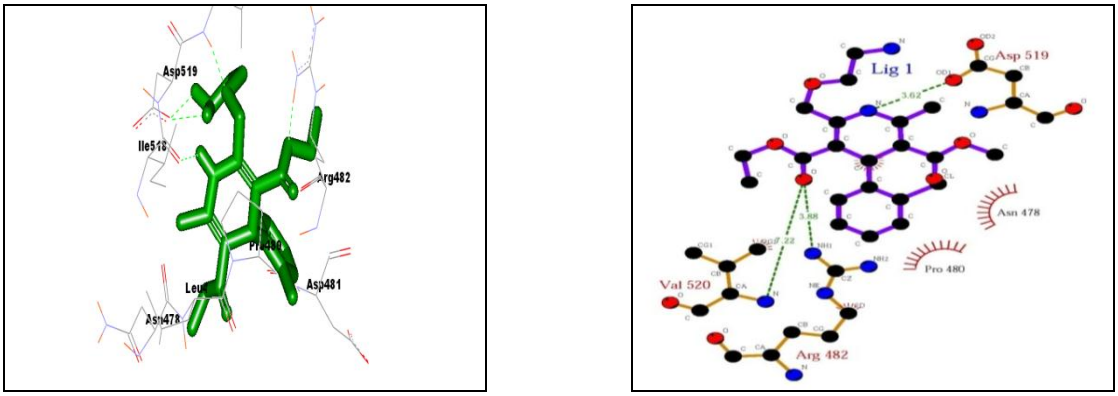
(G)

Fig. 2: Shows the 3D structures of bacterial HKs visualized by NGL viewer.

(A) PDB structure of the *Bacillius subtilis* HK (Yycg) (3SL2), (B) homology modeled structure of *Staphylococcus aureus* HK. (C) Homology modeled structure of *Vibrio cholerae*. (D) PDB structure of *Escherichia coli* HK (CpxA) (4BIV) chain A, (E) Homology modeled structure of *Salmonella enterica* HK. (F) Homology modeled structure of *Pseudomonas aeruginosa* HK (G) The superimposed structures of the six HK proteins obtained by POSA tool.

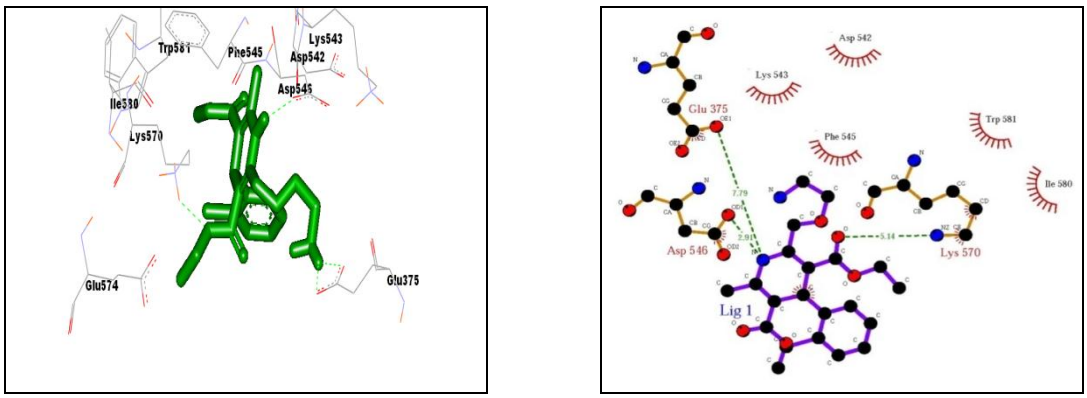
Table. 2 Shows the docking scores, the H-bonds, the PI interactions and the hydrophobic interactions of bacterial HKs with Amlodipine (LIG).

Organism	Autodock binding energy scores (Kcal/mol)	H-bond	Distance of H-bond (A◦)	Pi- interaction	Hydrophobic interaction
B.subtilis	-5.14	LIG(O):(HN1)Ile518 LIG(O):(HN1)Asp519 LIG(O):(HN2)Asp519 LIG(O):(NH)Val520 LIG(O):(HH)Arg482	2.21 2.14 2.06 1.89 2.07	NONE	Pro480,Asn478,Arg482 Val520
Staph.areus	-3.23	LIG(O):(Hz3)Lys570 LIG(HN):(OD1)Asp546 LIG(HN1):(OE1)Glu375 LIG(NH1):(OE2)Glu375	2.10 2.00 1.72 2.47	NONE	Asp542,Lys543,Phe545 Asp546,Lys570,Ile580 Trp581,Gly375
V.cholera	-3.5	LIG(O1):(NH)Glu347 LIG(H):(OE2)Glu350 LIG(H1):(OE1)Glu347	1.81 1.77 1.87	PI-sigma LIG(C):Trp347	Trp345,Val346,Glu347 Glu350,Leu376
E.coli	-4.38	LIG(O):(NH2)Ile288 LIG(N):(NH2)Ile288 LIG(NH1):(O)Leu284 LIG(NH2):(O)Arg283	2.15 2.47 2.02 1.84	NONE	Ile246,Leu250,Arg283 Leu284,Ile288,Leu291 Pro402,Phe403
Salm.enterica	-3.71	LIG(HN):(OE1)Glu256 LIG(HN1):(OE1)Glu256 LIG(HN2):(OE1)Glu256	2.25 2.14 1.99	Pi-sigma LIG(CL):Phe259 Pi-Pi LIG(C):Phe259	Lys226,Leu228,Glu256 Phe259,Ser263
P.aeurignosa	-5.54	LIG(O):(HN)Glu421 LIG(H):(OE1)Glu421	2.21 1.82	NONE	Ile420,Gln421,Leu377 Tyr379,Gly509,Arg531 HIS508,Pro530,Trp529



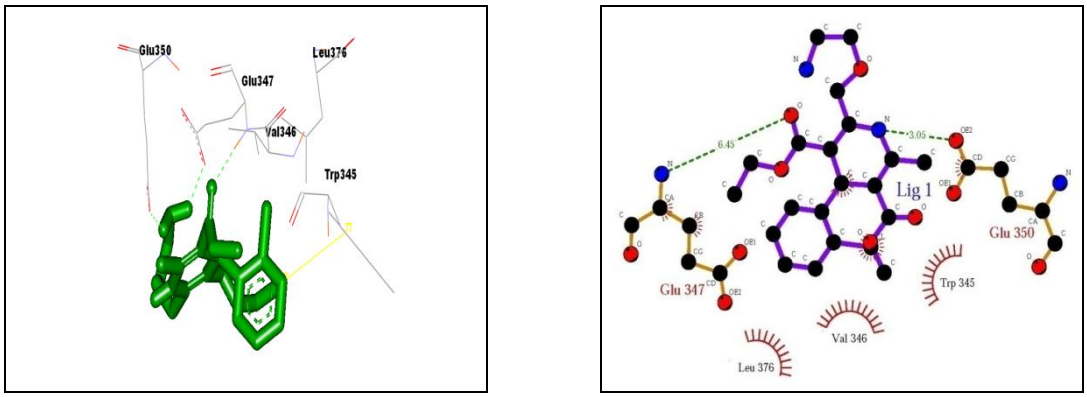
(A) **(B)**

Fig. 4: *B.subtilis* HK-AML complex (A) shows the H-bonds (B) shows the hydrophobic interactions.



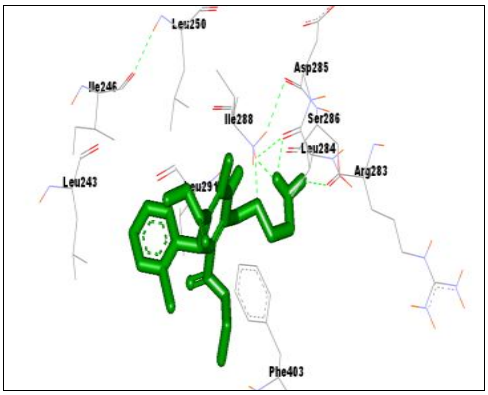
(A) **(B)**

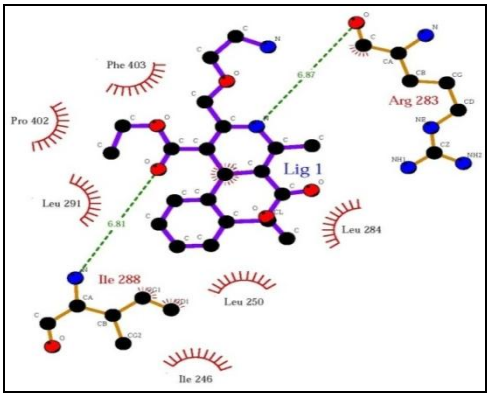
Fig. 5: *Staph.arues* HK-ligand complex (A) shows the H-bonding (B) shows the hydrophobic interactions.



(A) **(B)**

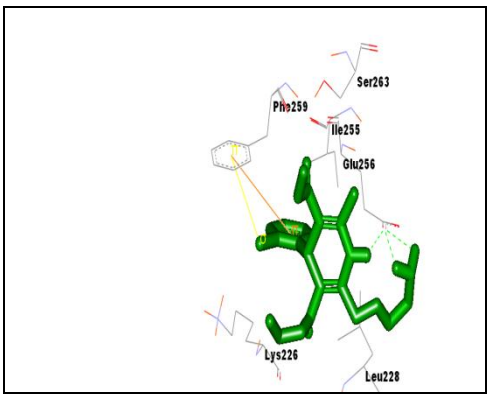
Fig. 6: *V. cholera* HK-ligand-complex (A) shows the H-bonding and Pi-sigma interaction (B) shows the hydrophobic interactions.

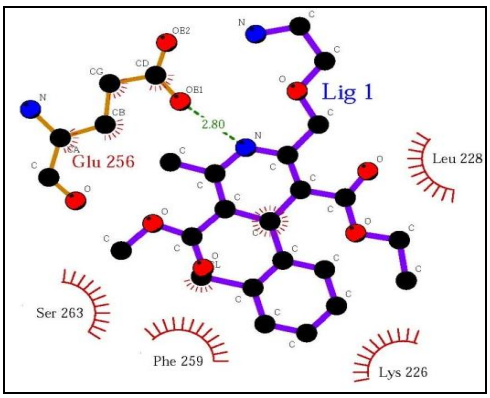




(A) **(B)**

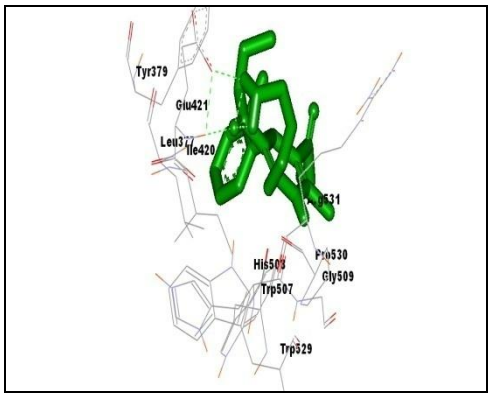
Fig.7: *E.coli* HK ligand-complex (A) shows the H-bonding (B) shows the hydrophobic interactions.

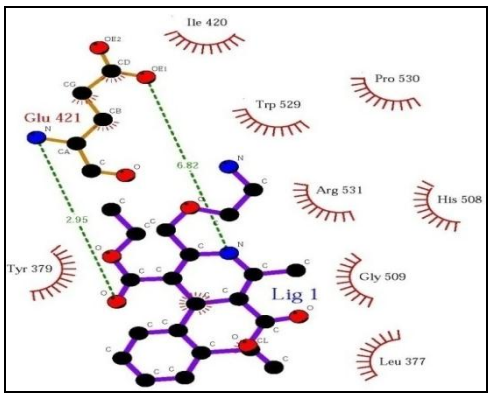




(A) **(B)**

Fig. 8: *Salmonella enterica* HK ligand-complex (A) shows the H-bonding, the Pi-Pi interaction and Pi-sigma interaction (B) shows the hydrophobic interactions.





(A) **(B)**

Fig.9: *P. aeurignosa* HK ligand-complex (A) shows the H-bonding (B) shows the hydrophobic interactions.

Fig.4,5,6,7,8 and 9 represent Amlodipine - protein interactions showing the H-bonding (green doted lines) the Pi-Pi interaction (orange) and the Pi-sigma interaction (yellow) using Accelry discovery studio 2.5,and the hydrophobic interactions (red projecting spheres)using the Ligplot.

DISCUSSION

Amlopdipine binding regions in the L-type Ca channels showed sequence similarity with the catalytic domain of bacterial histidine kinase (table.1) which is conserved between different bacterial strains. Also the 3D structures of the selected HKs were shown to superimpose at the catalytic domain provided from PDB (3SL2). These sites of superposition were used as docking sites to reveal the possibilities of ligand-protein interaction in the selected HKs. The docking parameters obtained from Autodock 2.4 and the interactions revealed by accelry discovery studio 2.5 and the ligplot (table.2) indicate suitable Amlodipine – protein interactions as the binding energies were quite low.

Binding between *Bacillus* HK and amlodipine showed 5 H-bonds with Ile518, Asp519, Val520 and Arg482, and 4 hydrophobic bonds with Pro480, Asn478, Arg482 and Val520. The interaction between AML and the modeled HK of *Staphylococcus* showed 4 H-bonds with Lys570, Asp546 and Glu375and 8 hydrophobic bonds with Asp542, Lys543, Phe545, Asp546, Lys570, Ile580, Trp581 and Gly375. Docking of AML at the modeled HK of *Vibrio cholera* showed 3 H-bonds with Glu347 and Glu350, Pi-Pi bond with Trp347 and 5 hydrophobic bonds with Trp345, Val346, Glu347, Glu350 and Leu376. The binding between AML and the HK of *E.coli* revealed 4 H-bonds with Ile288, Leu284and ARG283 and 8 hydrophobic bonds with Ile246, Leu250, Arg283, Leu284, Ile288, Leu291, Pro402 and Phe403, while the bonding between AML and modeled HK of *Salmonella enterica* showed 3 H-bonds with Glu256, Pi-Pi and Pi-sigma bonds with Phe259 and 5 hydrophobic bonds with Lys226, Leu228, Glu256, Phe259 and Ser263. Finally the interaction between AML and homology modeled protein HK of *Pseudomonas aeruginosa* showed 2 H-bonds with Glu421 and9 hydrophobic bonds with Ile420, Gln421, Leu377, Tyr379, Gly509, Arg531, His508, Pro530 and Trp529.

H-bonds and Pi bonds are indication of stable interaction between molecules unlike the hydrophobic bonds which are weaker, the more stronger interactions appeared with B.subtilis, V.cholerae and Staph.aureus while the much weaker interactions appeared with E.coli, Salmonella and pseudomonas which actually consistent with the in vitro studies.

Docking was not performed at the ATP binding sites as most of enzymes under study were homology modeled so rigid docking was achieved.

CONCLUSION

According to interactions obtained amlodipine showed promising results as HKs inhibitor that could be used alone or as adjuvant, and seems to be a good candidate for drug design as bactericidal, that may need further attention and probably modifications to enhance its antibacterial activity and to minimize its adverse effects and toxicity. Although virtual screening is a novel method for drug discovery, the repurposing of drugs already in use is still a good method as it passes the long journey for drug discovery so it can save time and cost.

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