Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 6, 2014

Original Article

IN SILICO ANALYSIS TO ACCESS THE ANTIBACTERIAL EFFECT OF THIAZIDES ON PDFS: MOLECULAR DOCKING APPROACH

TARUN AGARWAL[1], PRERAK GUPTA[1], SOMYA ASTHANA[1], ASIF KHURSHEED[2]*

[1]Department of Biotechnology and Medical Engineering, National Institute of Technology, Rourkela, Odisha, [2]Department of Bioengineering, Integral University, Lucknow, Uttar Pradesh, India.
Email: akhursheed12@gmail.com

Received: 29 Apr 2014 Revised and Accepted: 30 May 2014

ABSTRACT

Objective: Increasing prevalence of antibiotic resistant pathogenic bacteria has reached the alarming level that poses a need to identify new drugs and drug targets. Peptide Deformylase may prove to be a wise choice since it is crucial for native protein functioning in most pathogenic bacteria.

Methods: In present study, we demonstrated preexisting drug Thiazides, primarily used for hypertension and edema, as an antibacterial agent. We screened the binding affinity of Thiazides and its derivatives against seven peptide deformylases (PDF's) models retrieved from different pathogenic bacteria using Autodock 4.2 (version1.5.6). Further their interaction pattern was analyzed using Lig Plot+.

Results: S-Bendroflumethiazide (CID_6604206) and Quinethazone (DB01325) showed a considerable binding affinity against all selected protein models. Analysis of their interaction pattern revealed that DB01325 is interacting with protein models 1BSK and 1SZZ through more number of H-bonds with their active site residues. An almost same pattern was observed for CID_6604206 with 1N5N and 1SZZ protein models. The binding affinity of these ligands was significantly better than that of Actinonin (CID_443600) which is a well known natural PDF inhibitor.

Conclusion: The binding pattern of Thiazide derivatives with peptide deformylase protein models may provide a clue for designing a new potent drug to control the diseases caused by newly evolved antibiotic resistant pathogenic bacterial strains.

Keywords: Peptide deformylase (PDF), Antibacterial, Thiazides, Molecular docking, Autodock 4.2.

INTRODUCTION

An increase in frequency of antibiotic resistance has been observed in broad range of pathogenic bacterial strains since past few decades. Emergence of such resistance becomes more severe when it corresponds to several broad range antibiotics simultaneously. This situation creates a need to design more potent and effective drugs. This is quite possible if we locate a new target site for pathogen growth inhibition and a therapeutic drug molecule that can bind effectively to the target site. In this regard, peptide deformylase (PDF) may prove an intelligent choice because of the prokaryotic protein synthesis which universally initiates with an N-formylmethionine residue [**1**]. It is an essential metalloenzyme in most pathogens, which removes the formyl group from methionine at the N terminus of nascent polypeptide chains followed by protein maturation [**2**]. This protein is expressed in all pathogenic bacteria, along with Mycoplasma and Chlamydia species. Blockage of the PDF activity may result in complete inhibition of prokaryotic protein synthesis resulting in their death. In fact, the disruption of the PDF encoding gene in *E. coli* has proved critical for cell death [**1, 3**]. Moreover, cytoplasmic protein synthesis in eukaryotes has no relation with N-formylmethionine, and thus it forms a unique target for drug design to combat bacterial diseases [**4**]. In this context, pre-existing drugs for other diseases may prove efficient. Thiazides are FDA approved drugs belonging to class of diuretics, used for treatment of hypertension and edema [**5**]. Here in the present study, we explored the potential of Thiazides as an antibacterial agent through inhibition of peptide deformylase (PDF) using *in silico* docking approach.

MATERIALS AND METHODS

Retrieval of protein structure and Multiple sequence alignment

Protein sequences and corresponding structures of Protein Deformylases (PDF) from selected pathogenic micro-organisms viz. *Leptospira interrogans, Escherichia coli, Enterococcus faecium, Pseudomonas aeruginosa, Vibrio cholera, Staphylococcus aureus and Streptococcus mutans* were retrieved from RCSB Protein Data Bank (http://www.rcsb.org). Protein models with PDB I.D: 1N5N, 1BSK, 3G6N, 1SZZ, 3L87, 3QU1 and 1LQW were selected for further study (Table 1). The retrieved protein structures were further used for active site predictions and ligand docking analysis All the structures were cleaned and optimized by removing ligand and other hetero-atoms (water, ions, etc.) using Argus Lab Software. The metal ion bound to the protein was not removed while protein structure cleaning process.

In order to analyze percentage homology between retrieved PDF's multiple sequence alignment was performed using EMBL-EBI ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/)

Table 1: Protein models of Peptide Deformylase chosen for analysis

S. No.	PDB ID	Organism	Protein	Reference
1	1N5N	*Pseudomonas aeruginosa*	Peptide Deformylase	http://www.rcsb.org/pdb/explore/explore.do?structureId=1N5N
2	1BSK	*Escherichia coli*	Peptide Deformylase	http://www.rcsb.org/pdb/explore/explore.do?structureId=1BSK
3	3G6N	*Enterococcus faecium*	Peptide Deformylase	http://www.rcsb.org/pdb/explore/explore.do?structureId=3G6N
4	1SZZ	*Leptospira interrogans*	Peptide Deformylase	http://www.rcsb.org/pdb/explore/explore.do?structureId=1SZZ
5	3L87	*Streptococcus mutans*	Peptide Deformylase	http://www.rcsb.org/pdb/explore/explore.do?structureId=3L87
6	3QU1	*Vibrio cholera*	Peptide Deformylase	http://www.rcsb.org/pdb/explore/explore.do?structureId=3QU1
7	1LQW	*Staphylococcus aureus*	Peptide Deformylase	http://www.rcsb.org/pdb/explore/explore.do?structureId=1LQW

Protein Active Site Predictions

The prediction of the active site residues of selected protein models was carried out using Computed Atlas of Surface Topography of proteins (CASTp) (http://stsfw.bioengr.uic.edu/castp/calculation.php). The predicted active sites by CASTp were analyzed and the active site possessing metal binding residues was preferred for the docking of Thiazides.

The results obtained were made more precise by pocket Finder and Q site Finder. The clue regarding these metal binding amino acid residues along with their positions was obtained from Uniprot (http://www.uniprot.org/).

Ligand identification and selection

Molecules having potential to inhibit PDF protein are hydroxamic acid derivatives. Actinonin and Macrolactin N are the only naturally-occurring Peptide Deformylase inhibitors (PDI's) [**6-7**]. Here, Actinonin was taken as reference molecule for the study. The three dimensional chemical structures of Thiazides and its derivatives were screened from PubChem database (http://pubchem.ncbi.nlm.nih.gov/) and Drug Bank (http://www.drugbank.ca/) on the basis of R.E.O.S [**8**] and Lead-like Soft filters [**9-10**] present at FAF-Drug2 server. (http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/ portal.py?form=FAF-Drugs2#forms::FAF-Drugs2).

The PDB structure of thiazide derivatives were deduced using PRODRG Server (http://davapc1.bioch.dundee.ac.uk/prodrg/). The molecular structure with only polar hydrogen bond was taken for optimization using Argus Lab software.

Docking setup

Docking of thiazides along with reference molecule Actinonin (a natural PDF inhibitor), was performed on the active site of various peptide deformylases crucial for pathogenic bacteria growth, using Autodock4.2 (MGL Tools) as per the protocol and parameters followed by Agarwal *et al* [**11-12**]. Docking was carried out based on standard protocol using Lamarckian Genetic Algorithm [**13**]. Twenty five independent docking runs were performed for each ligand. Further, the interaction of ligand with all selected protein models was evaluated using Lig Plot+ [**14**].

Molecular Properties and Drug likeness

The thiazide molecules giving the best binding energies with the PDFs were examined for their drug likeness and molecular properties using Molsoft Drug-Likeness and molecular property prediction server (http://molsoft.com/mprop/).

RESULTS AND DISCUSSION

The computational methodology of molecular docking of ligands onto the protein has turned out to be an effective approach to analyze their docking patterns that gives us an idea about their binding affinity and corresponding inhibitory effect. Understanding such interactions pattern provide us an idea to target specific site of any key enzyme involved in a pathway that is vital for survival of that pathogenic bacteria. Targeting an enzyme, that is crucial for prokaryotic survival but functionally absent in humans is reasonable choice for treatment of diseases caused by these bacteria. Here in this study, we adopted molecular docking approach to explore the inhibitory effect of Thiazides and its derivatives on peptide deformylase. Moreover, deformylation is a conserved feature throughout the eubacterial kingdom [**15-16**]. Protein synthesis in bacteria, under normal conditions, is initiated by formyl-methionyl-tRNA. A formyl methionine residue is inherently incorporated at the N-terminus of all newly formed polypeptides; however, majority of the N-formyl moiety is removed from the mature proteins. The removal of this N-formyl moiety is catalyzed by peptide deformylase (PDF) [**1**], which is a crucial step for optimal protein functioning and consequent activation. Considering the necessity of PDF for protein synthesis in pathogenic bacteria, the Peptide Deformylase enzyme models from seven pathogenic microorganisms were retrieved from RCSB PDB as mentioned in Table 1. The Clustal W2.1 multiple sequence analysis showed that the Protein Deformylase sequence varied significantly between the species of micro-organisms selected for studies. The percentage homology ranged from 25.57 – 66.49% between the various pairs of the protein structure models. The percentage identity matrix generated ClustalW2.1 has been displayed in Fig. 1. The prediction of the active site in the protein structure models was carried out using CASTp calculations. Amongst ten binding sites obtained in the analysis, the site containing the metal binding resides was preferred for docking analysis of ligands. The amino acid residues present in the active site of all the seven protein models are compiled in Table 2. For virtual screening of the ligands, a total of 105 Thiazides and their derivatives from PubChem and DrugBank databases were passed through R.E.O.S and Lead-like Soft filters filter. Both filters screen out the ligands based on their molecular properties such as molecular weight, logP value, hydrogen bond acceptor, hydrogen bond donor, total polar surface area, rotatable bonds, rigid bonds, H/C Ratio and Stereocenters. Out of 105 thiazide structures, only seven were passed the filter tests. Three dimensional structures of screened ligands were retrieved from PRODRG server, minimized using Argus Lab software and used for docking and molecular property analysis.

Table 2: Active Site Residues: CASTp Calculation predictions

S. No.	Protein Model	Active Site Residues
1	1BSK	Val5, His7, Glu41, Glu42,Gly43, Ile44, Gly45, Leu46, Gln50, Ile86, Glu 87, Glu88, Gly89, Cys90, Leu91, Ile93, Pro94, Glu95, Gln96, Arg97, Leu125, Ile128, Cys129, His132, Glu133, His136, Leu161, Asp162, Lys165
2	1SZZ	Glu45, Gly46, Val47, Gly48, Gln53, Tyr71, Thr74, Phe97, Trp98, Glu99, Gly100, Cys101, Leu102, Val104, Pro105, Gly106, Met107, Arg108, Tyr136, Ile139, Val140, His143, Glu144, Asn166
3	1N5N	Met39, Tyr40, Pro43, Gly44, Ile45, Gly46, Gln51, Asp62, Ser64, Glu65, Lys67, Tyr88, Gln89, Glu90, Gly91, Cys92, Leu93, Val95, Pro96, Gly97, Phe98, Tyr99, Leu127, Val130, Cys131, His134, Glu135, His138
4	1LQW	Ile77, Pro78, Asp80, Ser82, Gly83, Lys84, Tyr86, Tyr88, Cys111, Arg127, Ile128, Gln141, Leu142, Arg143, Leu144, Lys145, Gly146, Tyr147, Pro148
5	3L87	Arg68, Gly70, Val71, Gly72, Leu73, Gln77, Leu125, Glu129, Gly130, Leu132, Ser133, Val134, Arg136, Arg144, Tyr167, Ile170, Val171, His174, Glu175, Asp177, His178, Ile182, Met183, Phe184,
6	3QU1	Met1, Ala2, Val3, Leu4, Glu5, Ile6, Leu7, Thr8, Thr37, Leu38, Tyr39, Ala40, Thr41, Asp42, Asn43, Gly44, Ile45, Asp62, Leu63, Ser64, Aso65, Asn66, Arg67, Gly90, Cys91, Leu92, Val94, Pro95, Asp96, Tyr97, Tyr98, Phe126, His162, Val163, Arg166, Arg168
7	3G6N	Arg56, Gly57, Gly58, Val59, Gly60, Gln65, Leu108, Glu112, Gly113, Cys114, Leu115, Tyr150, Ile153, Val154, His157, Glu158, His161

For docking analysis, the 3D structures of the screened ligands were docked onto the predicted active sites of each protein model using Autodock4.2 software. For each ligand, 25 independent docking runs were carried out. The minimum binding energies of all 7 ligands with protein models under study are enlisted in Table 3. Also, the docking of the reference molecule Actinonin (a natural PDF

inhibitor) onto the same active sites demonstrated the minimum binding energies as displayed in Table 4. The efficacy of final protein ligand complex was evaluated on the basis of two essential criteria's: (1) the minimum binding energy and (2) the interaction of the ligand with the active site residues. A ligand undergoes either hydrogen bonding or hydrophobic interactions or both while docking into the active site. Docking analysis of the Thiazides and their derivative revealed that S-Bendroflumethiazide (CID_6604206) and Quinethazone (DB01325) showing an effective and efficient binding with all selected PDF models under study (Fig. 2). Although it was found that S-Bendroflumethiazide (CID_6604206) is showing almost constant binding affinity with all protein models with a binding energy of above -8.0kcal/mol whereas Quinethazone (DB01325) was found to be more effective against protein models 1BSK and 1LQW with a minimum binding energies of -9.35kcal/mol and -9.55kcal/mol respectively. Interaction pattern analysis revealed that Quinethazone interacted with protein model 1BSK with 7 H-bonds (Leu91, Gln50, His132, His136, Glu133, Gly45, Cys90 residues) with active site residues while on the other side, same ligand interacts with 1LQW and 1SZZ through 7 hydrogen bonds that includes some of the active site residues (Table 5). Interaction profile of S-Bendroflumethiazide (CID_6604206) and 1N5N (Binding energy: -8.92kcal/mol) revealed the presence of 7 H-bonds with active site residue (Cys131, Cys92, His134, Ile45, His138, Leu93, Gly46) (Fig. 3). Diazoxide (DB01119) was found to be minimally effective against all selected protein models. Among other ligands tested, Chlorothiazide (DB00880**),** Hydrochlorothiazide (DB00999) and Methyclothiazide (DB00232) were found to be effective against 1BSK **(***Escherichia coli* PDF**)** with minimum binding energies of -9.22kcal/mol, -8.75kcal/mol and -8.60kcal/mol respectively. Hydrochlorothiazide (DB00999) showed a significant efficacy against 1LQW (*Staphylococcus aureus* PDF) with -8.85kcal/mol binding energy. It is important to mention that the standard inhibitor, actinonin showed a lower affinity with all the protein models used in the analysis. The interaction pattern of actinonin was also found to weaker in comparision to the thiazide derivatives.

```
Percent Identity  Matrix - created by Clustal2.1


  1: 1N5N_A|P.            100.00   57.23   48.21   40.96   26.06   25.57   28.57
  2: 1BSK_A|ECOLI          57.23  100.00   52.73   34.34   29.63   27.16   29.75
  3: 3QU1_A|V.CHOLERAE     48.21   52.73  100.00   34.55   28.66   27.11   28.75
  4: 1SZZ_A|LEPTOSPIRA     40.96   34.34   34.55  100.00   30.59   32.16   31.33
  5: 3G6N_A|E.             26.06   29.63   28.66   30.59  100.00   66.49   53.01
  6: 3L87_A|S.             25.57   27.16   27.11   32.16   66.49  100.00   48.63
  7: 1LQW_A|S.AUREUS       28.57   29.75   28.75   31.33   53.01   48.63  100.00
```

Fig. 1: Multiple Sequence Alignment: ClustalW2.1 analysis

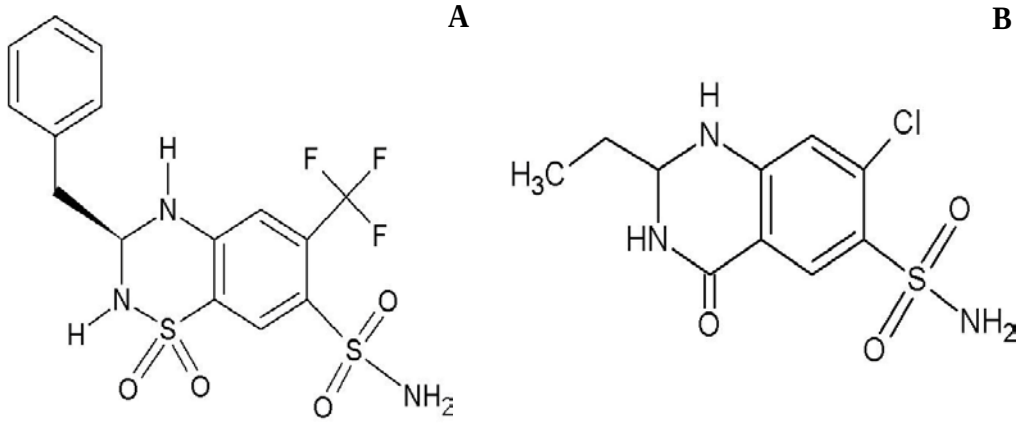

Fig. 2: Chemical structures of Thiazide derivatives demonstrating higher binding affinity with the protein models, A) S-Bendroflumethiazide (CID_6604206) and B) Quinethazone (DB_01325).

Table 3: Binding Affinity of screened Thiazides with protein models (kcal/mol)

Protein	DB00232	DB00774	DB00880	DB00999	DB01119	DB01325	CID_6604206
1BSK	-8.6	-7.98	-9.22	-8.75	-6.03	-9.35	-8.83
1SZZ	-7.96	-7.42	-7.34	-7.51	-6.39	-8.44	-8.67
1N5N	-7.48	-6.66	-7.03	-7.14	-6.74	-7.78	-8.92
1LQW	-7.32	-7.59	-6.99	-8.85	-6.09	-9.55	-8.09
3QU1	-8.13	-8.28	-8.64	-8.52	-6.56	-8.78	-8.91
3G6N	-7.84	-7.32	-7.7	-7.7	-6.47	-7.73	-8.96
3L87	-7	-6.21	-7	-6.96	-6.09	-7.3	-8.22

Table 4: Binding Affinity of reference ligand Actinonin (CID_443600) (kcal/mol)

S. No.	Organism	PDB ID	Binding Energy
1	*Pseudomonas aeruginosa*	1N5N	-7.66
2	*Escherichia coli*	1BSK	-7.23
3	*Enterococcus faecium*	3G6N	-6.61
4	*Leptospira interrogans*	1SZZ	-7.38
5	*Streptococcus mutans*	3L87	-6.01
6	*Vibrio cholerae*	3QUI	-7.55
7	*Staphylococcus aureus*	1LQW	-6.96

Table 5: Hydrogen bond analysis of Thiazides with selected PDF models

Protein Model	Quinethazone	(S)-Bendroflumethiazide
1BSK	Leu91, Gln50, His132, His136, Glu133, Gly45, Cys90	Glu42, Cys129, Glu133
1LQW	Gln65, Cys111, Leu112, Glu155, Gly60, His154, His158	Leu112, His154, His158, Cys111, Glu155, Gly60
1N5N	Cys131, Glu135, Ile45	Cys131, Cys92, His134, Ile45, His138, Leu93, Gly46
1SZZ	Gln53, Cys101, Leu102, Glu144, His143, Asp146, His147	Gly48, Val47, Leu102, Glu45, Tyr71, Ala44
3G6N	Gly60, Glu158, Gly111, Val59	Val59, Gly60, Glu158, Arg56
3L87	Val71, Gly72, Gly128, Glu175	Val71, Gly72, Glu175
3QUI	Leu92, Gly46, Ile45, Gln88, Glu134	Leu92, Cys91, His137, His133, Gly46

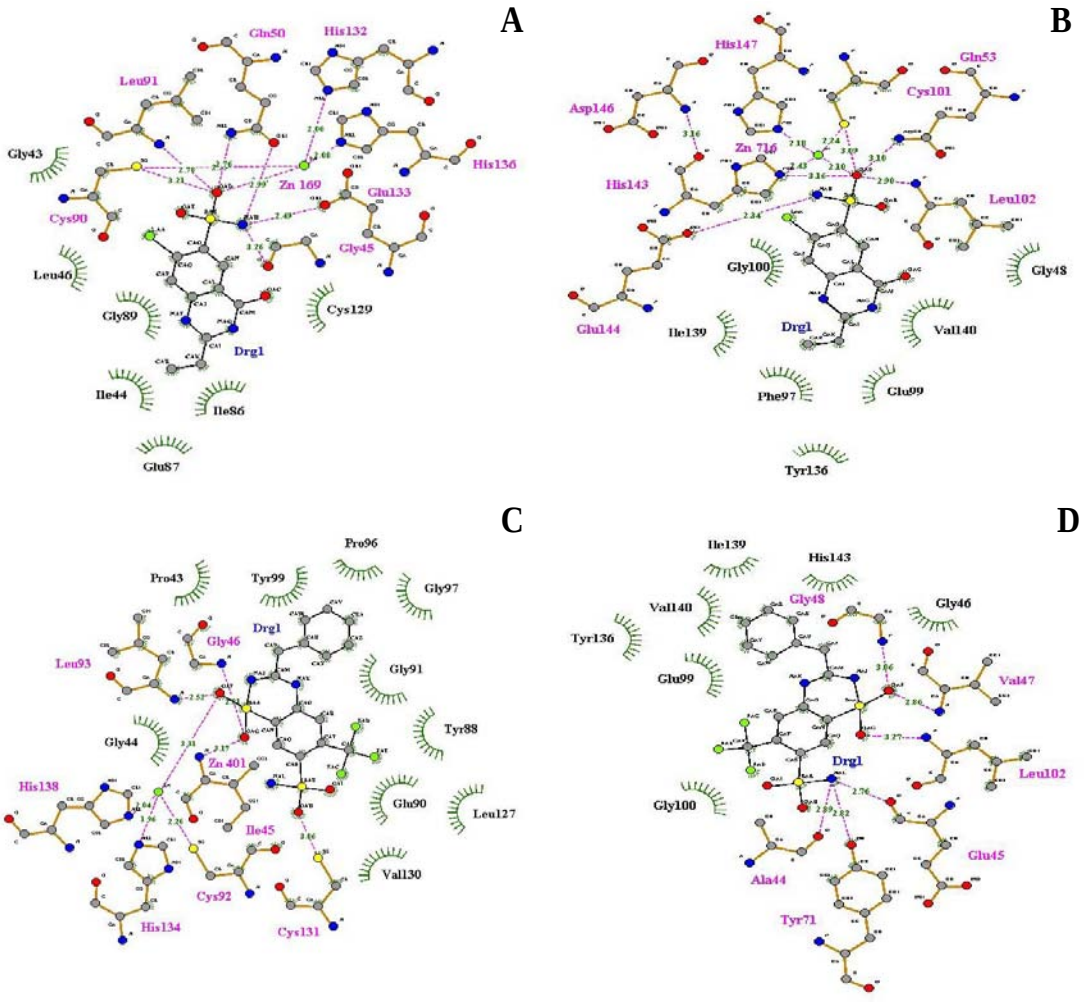

Fig. 3: Molecular interaction pattern of Thiazides with Peptide Deformylase protein models. A) DB01325 with 1BSK, B) DB01325 with 1SZZ, C) CID_6604206 with 1N5N and D) CID_6604206 with 1SZZ

The analysis of the molecular properties of S-Bendroflumethiazide and Quinethazone demonstrated that both the ligands possess a good drug likeness, without any mutagenic, carcinogenic or irritant effects (Table 6). This clearly suggests that both the drugs could be used as antibacterial agents without any toxic effect to humans.

Table 6: Molecular properties of S-Bendroflumethiazide and Quinethazone

Molecular Properties	S-Bendroflumethiazide	Quinethazone
Molecular Formula	C15H14F3N3O4S2	C10H12ClN3O3S
Molecular Weight	421.04	289.03
Number of HBA	6	4
Number of HBD	4	4
MolLogP	2.15	1.2
MolLogS (mg/L)	9.17	31.83
MolPSA A^2	102.92	87.46
MolVol A^3	315.61	235.41
Number of stereo centers	1	1
Drug likeness	0.72	1.15

CONCLUSION

Since past few years, the emergence of antibiotic resistant pathogenic bacterial strains has posed a great threat to the human community. So, in order to cope up with such scenario more research groups are being involved to identify new potential drug targets against these organisms. Still there remains a necessity to deal with same situation. In this regard, Peptide Deformylase has proved to be a potential target since its activity is vital for most pathogenic bacterial strains. The use of preexisting drugs may become a wise choice for the same. Thiazides are FDA approved drugs mainly used for hypertension treatment. Here, we showed the potential of Thiazide and its derivatives as an antibacterial agent using *in silico* approach. Our results demonstrated that two Thiazide derivatives S-Bendroflumethiazide and Quinethazone were showing better binding affinity with PDF as compared to its natural inhibitor Actinonin. Further, *in vivo* and *in vitro* validations of the results are required for its application in treatment of the bacterial diseases.

REFERENCES

1. Meinnel T, Blanquet S. Evidence that peptide deformylase and methionyl-tRNA (fMet) formyltransferase are encoded within the same operon in Escherichia coli. J Bcateriol. 1993;175(23):7737-40.
2. Takeda M, Webster RE. Protein chain initiation and deformylation in B. subtilis homogenates. PNAS. 1968;60(4):1487-94.
3. Mazel D, Pochet S, Marliere P. Genetic characterization of polypeptide deformylase, a distinctive enzyme of eubacterial translation. EMBO J. 1994;13(4):914-23.
4. Meinnel T. Peptide deformylase of eukaryotic protists: a target for new antiparasitic agents? Parasitol Today. 2000;16(4):165-68.
5. Whitworth JA. World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension 2003. J Hypertens. 2003;21(11):1983-92.
6. Chen DZ, Patel DV, Hackbarth CJ, Wang W, Dreyer G, Young DC, *et al.* Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. Biochem. 2000;39(6):1256-62.
7. Yoo J-S, Zheng C-J, Lee S, Kwak J-H, Kim W-G. Macrolactin N, a new peptide deformylase inhibitor produced by Bacillus subtilis. Bioorg Med Chem Lett. 2006;16(18):4889-92.
8. Walters WP, Namchuk M. Designing screens: how to make your hits a hit. Nat Rev Drug Discov. 2003;2(4):259-66.
9. Oprea TI, Davis AM, Teague SJ, Leeson PD. Is there a difference between leads and drugs? A historical perspective. J Chem Inf Comput Sci. 2001;41(5):1308-15.
10. Baell JB. Broad coverage of commercially available lead-like screening space with fewer than 350,000 compounds. J Chem Inf Model. 2012;53(1):39-55.
11. Agarwal T, Singh A, Asthana S. Study of binding mode of Betulin (lup-20(29)-ene-3β,28-diol) on Plasmepsin II from *Plasmodium Falciparum* : An Antimalarial Studies. Int J Curr Research. 2012;4(12):505-08.
12. Agarwal T, Asthana S, Gupta P, Khursheed A. In silico study to elucidate inhibitory effect of Thiazides on Plasmepsins: Implications of new Antimalarial drug design. Int J Pharm Pharm Sci. 2014;6(2):379-82.
13. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, *et al.* Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998;19(14):1639-62.
14. Laskowski RA, Swindells MB. LigPlot+: multiple ligand–protein interaction diagrams for drug discovery. J Chem Inf Model. 2011;51(10):2778-86.
15. Verma SK, Jat R, Nagar N. A novel antibacterial target: peptide deformylase. Pharmacophore J. 2011;2(2):114-23.
16. Meinnel T, Blanquet S, Dardel F. A new subclass of the zinc metalloproteases superfamily revealed by the solution structure of peptide deformylase. J Mol Biol. 1996;262(3):375-86.