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Full Length Research Paper

In silico designing selective inhibitor of drugs, medicinal plants compounds and experimental ligands for pteridine reductase targeting visceral leishmaniasis

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This research aimed to generate and validate the three-dimensional structure of mostly targeted proteins of *leishmania* using homology modeling tools and then virtual screening and docking the available chemical and medicinal plants compounds to the generated proteins as the receptor. Overall, 12 drugs and 10 medicinal plants compounds were selected for internal and comparative study. Then 18 experimental ligands were docked using AutoDock 4.3 program into the active sites of *leishmania donovani* pteridine reductase 1, which was modeled using homology modeling programs. Out of 28 templates, the best one (PDB ID: 1W0cA) showed 97.17% sequence identity, E-value of 9.13483e-147 and final total model energy of -12574.484 kJ/mol. Validation of different models of pteridine reductase 1 (PTR1) was carried out and ramachandran plot was computed, which showed 96.75% residues in favored and allowed regions. Furthermore, ERRAT program observed the overall quality factor of the prepared model as 92.937. Vinblastine and diospyrin in the medicinal plants group showed the highest biding affinity. Compounds 11 and 3 are more active on *leishmania* PTR1 with IC_{50} of 25 and 35 µM, respectively. The hope of identifying novel drug targets and vaccine candidates against parasitic protozoan is laying on their peptides, currently. The homology or comparative modeling due to its simplicity to predict the structure of the target proteins or peptides with the help of available protein structure as temples has come as a rescue. Thus, it could be concluded that our generated experimental drug compounds could have potential as pharmacological tools against the visceral leishmaniasis.

Key words: Pteridine reductase 1, anti-leishmanial drugs, vinblastine, diospyrin, experimental ligands, homology modeling, docking.

INTRODUCTION

Leishmania is a parasite from a genus of trypanosome protozoa and it cause a number of diseases in humans called leishmaniasis (Myler and Fasel, 2008; Handman, 2001). The disease is prevalent in large number of countries, which approximately infect 400000 of people annually (Armijos et al., 2003; Ashford et al., 1992). Chemotherapy is considered as the main therapeutic approach used against *Leishmania* due to the absence of effective vaccines and vector control. Therefore, the development of drug resistance is a serious problem (Pérez-Victoria et al., 2001). Drug treatments especially

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vaccines against leishmaniasis are under development due to sever toxic side effects and high cost (Armijos et al., 2004; Coler and Reed, 2005). *In Silico* tools offer an attractive alternative strategy to the cumbersome experimental approaches, which have metamorphosed over the years into complex algorithms that attempt to predict the binding of peptides to receptors, efficiently (Rahim, 2008; 2010). Currently hope lies in researches, that will help *In Silico* and bioinformatics tool towards identification and characterization of novel drug candidates that affect the parasite growth and survival in mammalian to decrease the side effects and costs of development (Kaur et al., 2011).

One of the putative targets is (PTR1, EC 1.5.1.33), a NADPH dependent short-chain reductase responsible for the salvage of pterins in the leishmania, that acts as a metabolic bypass for drugs targeting dihydrofolate reductase (Kumar et al., 2008). Folate analogue inhibitors and non-folate compounds are potential antiparasitic drug candidates for combined therapy of *leishmania* major PTR1 (Cavazzuti et al., 2008; Ferrari et al., 2010). The plant kingdom is undoubtedly valuable as a source of new medicinal agents, natural products and medicinal plants are attractive drug candidates due to their structural diversity for many diseases such as leishmaniasis (García et al., 2010; Rocha et al., 2005; Fournet and Muñoz, 2002). The X-ray crystallographic structure of the important proteins of *leishmania* which plays the roles as the drug candidates or targets unfortunately is not available. The main purpose of this research was to generate and validate the three-dimensional structure of mostly targeted proteins of *leishmania* using homology modeling tools in order to find out the key amino acids involved in ligand- protein interaction and then virtual screening and docking the available chemical and medicinal plants compounds to the generated proteins as receptor.

MATERIALS AND METHODS

Template selection and validation, and homology modeling

The protein sequences of *l. donovani*, *leishmania* major, *leishmania* tropica, *l. infantum* and *leishmania* braziliensis were retrieved in Gen Bank Flat File (GBFF) from the National Center for Biotechnology Information (NCBI) using protein Basic Local Alignment Search Tool (BLAST) and Los Alamos National Laboratory websites (Pruitt et al., 2009; 2007). Multiple sequence alignment was conducted using ClustalW (Version 1.8) to locate the interested regions. The three-dimensional structure models of PTR1 were generated using Swiss-Model, Geno3D and EasyModeller software (Schwede et al., 2003; Combet et al., 2002; Kuntal et al., 2010). The models were validated using a set of structural validation programs which include PROCHECK, WHATIF, ERRAT (Colovos and Yeates, 1993) and HARMONY.

Phylogenetic analysis

Based on the retrieved and multiple sequences alignment outputs an un-rooted phylogenic tree was constructed using MPI-PHYLIP program (Ropelewski et al., 2010). The final tree diagram was generated using PHYLODENDRON program.

Virtual screening

The three-dimensional of all the anti-*leishmania* drugs and medicinal plants from PubChem and Drug Bank databases were screened with 12 drugs and 10 medicinal plants compounds and were selected for internal and comparative study. Then the 18 experimental ligands also were prepared for docking study. The virtual screening was performed using PyRx software.

Docking

The grid maps of all PTR1 molecules were calculated individually using AutoGrid part of AutoDock tools, focusing on sufficient large to include active site and significant part of surface as well. All the selected drug compounds and experimental ligands were docked into the active sites of PTR1 using AutoDock 4.3 program. Automated docking was performed using AutoDock 4.2 with Lamarckian genetic algorithm (LGA) to model ligand- PTR1 interaction and binding, in which 100 multiple, independent docking runs were carried out to increase the performance of docking programs. Finally, cluster analysis was carried out on the observed docking values base on the root mean square (RMS, 0.5 Á). Consequently, binding affinity and free energy charge of binding were calculated using AutoDock Vina and AutoDock 4.2 (Trott and Olson, 2010). The final dock energy and estimated free energy charge of binding were calculated using the following formulas:

Final dock energy = sum of final intermolecular energy and final internal energy of ligand

Estimated free energy charge of binding = sum of final intermolecular energy and torsional free energy of ligand.

Data analysis

All calculations were carried out on Intel core i7 Pentium 4 1.73 GHz based machine running Micro Soft Windows 7 as operating system.

RESULTS

The PTR1 of different *leishmania* strains and 3 different protozoans were compared to each other using BLAST and ClustalW analysis (Figure 1A). Phylogenic relationship between the members of PTR1 in *leishmania* and other protozoan families show the close similarity between *leishmania* major**,** *Leishmania tropica*, *Leishmania infantum*, *Leishmania* braziliensis and *L. donovani* compared to other protozoan families and make a separate cluster distinct (Figure 1B). The homologous to target sequence was selected for modeling using SWISS-MODEL and Geno3D. Out of 28 templates, the best one was 1W0cA (PDB ID) with 97.17% sequences identity and E-value of 9.13483e-147 and final total model energy of -12574.484 kJ/mol (Figure 2A and D). Validation of different models of PTR1 was carried out and ramachandran plot was computed, which showed

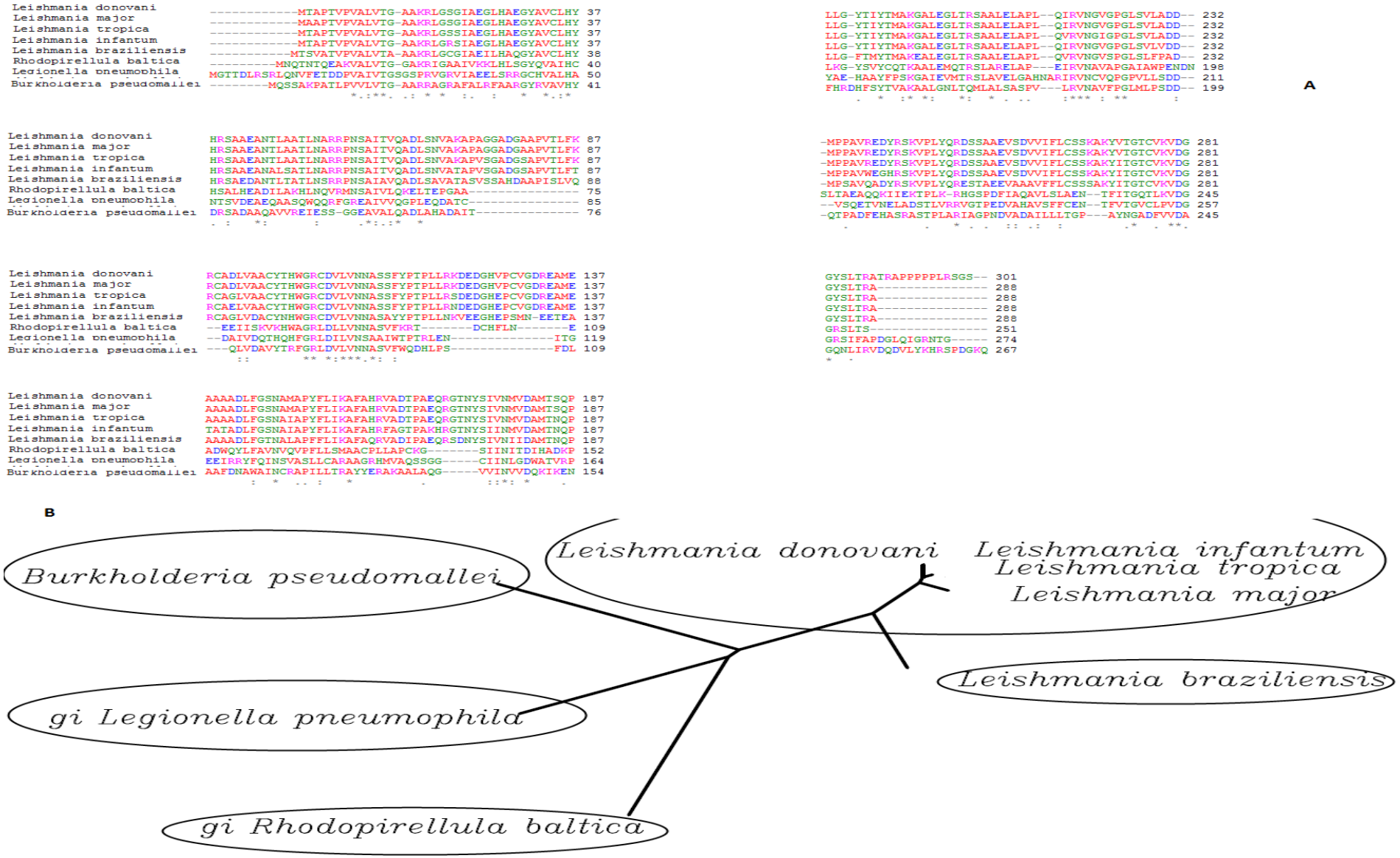


Figure 1. A, PTR1 of different *leishmania* strains and 3 different protozoans were compared to each other using BLAST and ClustalW analysis and **B**, Phylogenic relationship between the members of PTR1 in *leishmania* and other protozoan families.

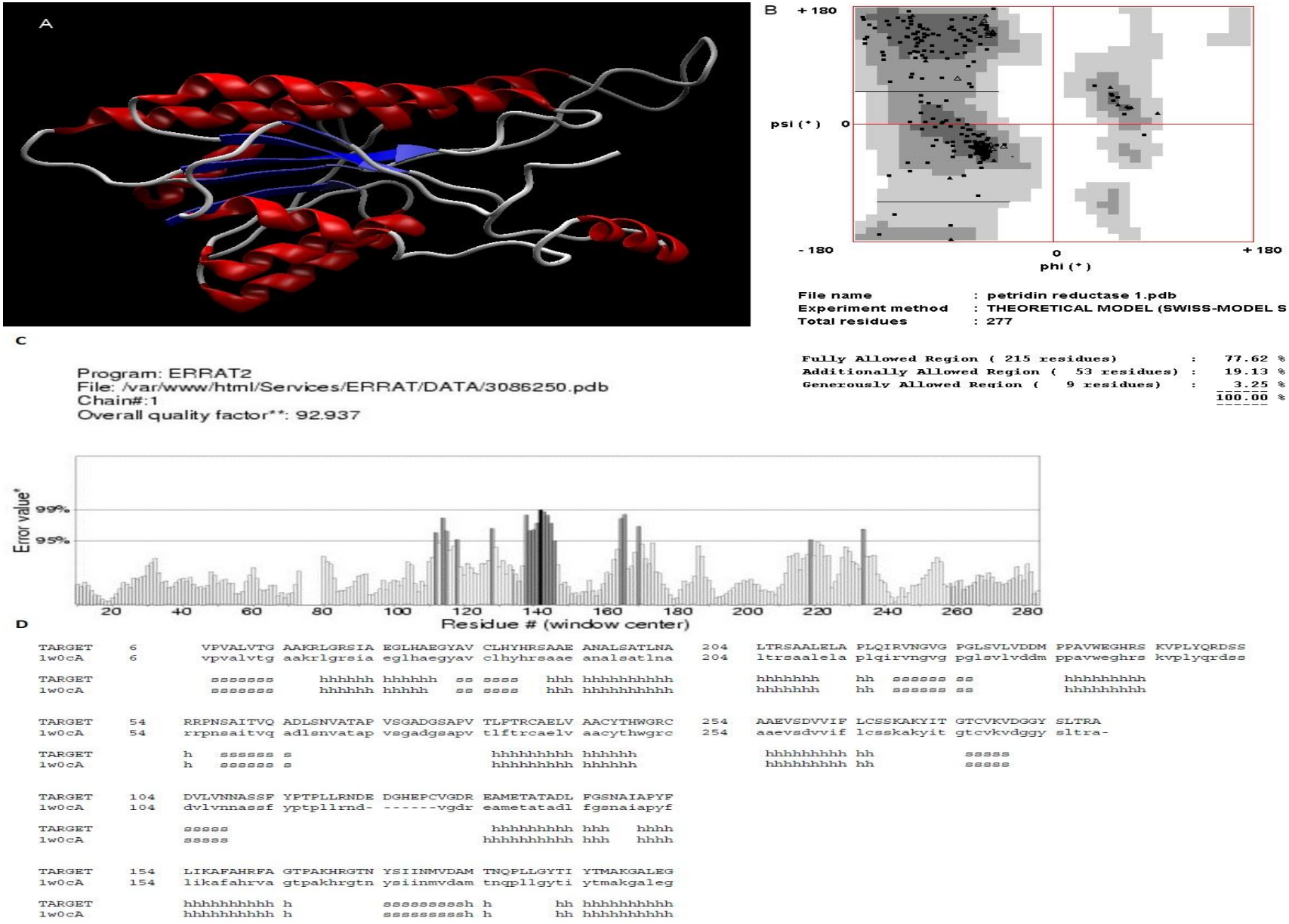


Figure 2. A, a model of PTR1 that was carried out using homology modeling software; **B**, Ramachandran plot of validate model; **C**, The validation of created model PTR1 using ERRAT;**D**, The pair-wise alignment of target and template sequences.

96.75% residues in favored and allowed regions (Figure 2B). ERRAT program were computed the overall quality factor of the prepared model as 92.937, which indicated the error value of individual residue are negligible (Figure 2C).

The backbone conformation of the refined model of PTR1 also was almost as good as X-ray crystallographic structure of template with the weighted root-mean-square deviation (RMSD) of Cα trace of 2.60 A°. Side chain and loop modeling refinements did not reveal any further change in model properties. More than 150 dockings have been performed for each ligand taken for this study. Different ligand selected for this study in the drug compound group include sitamaquine, parmomycine, miltefosine, vinblastine, allopurinol, ketoconanazole and captopril and medicinal plant compound include berberine, dephanandrine, akuammicine, harmaline, obaberine, akuammicin, jacaranone, labdane, lapachol, plumbagin, diospyrin and 18 experimental compounds. The structures, IC_{50} and percent of cell survival of 18 experimental ligands had been described in detail (Table 1). The binding affinities of all drugs, experimental ligands and medicinal plants compound to PTR1 were calculated, which vinblastine showed the highest value (Figure 3A).

The best biding affinities of experimental ligands belong to four compounds which are 18, 17, 11 and 3 (Figure 3B). The best binding affinity in medicinal plant compounds belong to diospyrin (Figure 3C), which forms H-bond with Lys 268^{th}, Glu 29^{th} and Ala28 (Figure 4C). Vinblastine is likely to form H-bonds with $Glu29^{th}$, Lys 268^{th} and pro 7^{th}, $Thr172^{th}$, Val 6^{th} amino acids. Two different biding sites in PTR1 were observed (Figure 4D). Out of 18 experimental ligands only four showed most binding affinity to PTR1, which is in comparison of the final dock and binding energies, also these four compounds were in concern (Table 2). Among these five compounds, CP3 and CP11 had less IC_{50} (Table 2). This finding indicated that compounds 11 and 3 are more active on *leishmania* PTR1 with IC_{50} of 25 and 35 µM, respectively (Figure 4A and B).

DISCUSSION

The hope of identifying novel drug targets and vaccine candidates against parasitic protozoan is laying on their peptides, currently. In case of *leishmania*, limited numbers of peptides of parasitic protozoan have received considerable attentions that include serine type oligopeptidase B (Mottram et al., 2004), membrane bound zinc metalloprotease (Yao et al., 2003), lysosomal cysteine peptidases (de Andrade et al.,1998) and leucylaminopeptidase (Morty and Morehead, 2002). Recently, ACE- related dipeptidyl carboxylpeptidase (Baign et al., 2010) and PTR1 (Kaur et al., 2011) in *l. donovani* are demonstrated using *in Silico* methods. PTR1 is a NADPH dependent short-chain reductase responsible for the salvage of pterins in the *Leishmania*, which acts as a metabolic bypass for drugs targeting dihydrofolate reductase. In this study, we have attempted to utilize the available drugs and medicinal plants compounds in order to find a selective inhibitor and rational design new drug to identify novel compounds with optimal selectivity, efficacy and safety that bind to target peptide in *leishmania* parasites as well.

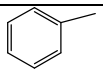
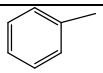
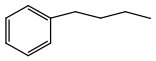
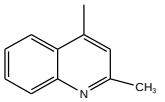
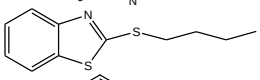
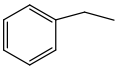
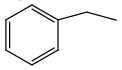
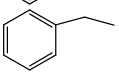
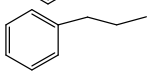
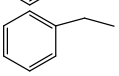
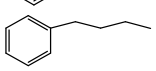
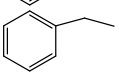
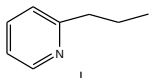
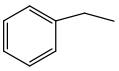
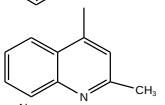
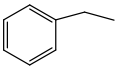
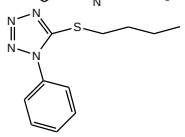
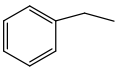
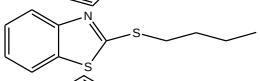
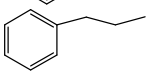
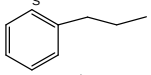
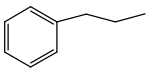
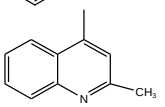
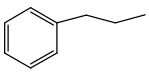
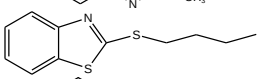
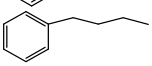
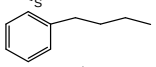
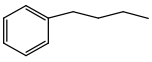
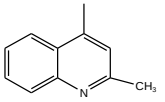
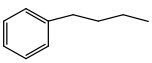
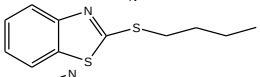
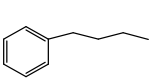
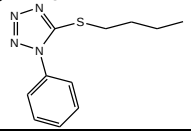
In a study conducted recently, the kinetoplastide protein of different *leishmania* strains, showed that vinblastine has no interaction with protein as no binding site has yet been found (Sahoo et al., 2009). Hence, it is known from this *In Silico* study not only vinblastine has an effect on PTR1 protein, but its effect and biding affinity is the highest. The microtubule drugs such as vinblastine were identified as the antiparasitic drug targets, especially in *leishmania* (Chatterji et al., 2011; Hiam et al., 2006). Vinblastine, an anti-cancer drug, binds to tubulin and inhibits microtubule formation, resulting in disruption of mitotic spindle assembly and arrest of tumor cells in the M phase of the cell cycle (Werbovetz et al., 1999). However, the multidrug resistance 1 protein, has been shown to confer resistance to vinblastine and the new antileishmanial lipophilic drugs, miltefosine and edelfosine (Pérez-Victoria et al., 2001; Gueiros-Filho et al., 1995). We have recently studied the anticancer effects of the selected compounds on breast cancer cell line (Fouladdel et al., 2010).

So, our selected compounds have shown the same effects on PTR1 as a target from *leishmania* with an acceptable IC_{50} in comparison to vinblastine. Diospyrin, a plant derivative, had significant activity against promastigotesandisa specific inhibitor of the type I deoxyribonucleic acid (DNA) topoisomerase enzyme of *L. donovani*as well (Hazra et al., 1987; Ganapaty et al., 2006). We have observed a significant affinity of diospyrin on PTR1 in comparison to other available medicinal plant compounds. The relative binding modes and site interactions of amino derivatives of diospyrin with the crystal structure of human and *L. donavani* were studied extensively and reported previously (Chhabra et al., 2007). Based on the docking results, binding modes of diospyrin with the leishmanial PTR1 were predicted in the present study. The present study provides an understanding of the structural basis of ligand binding to the PTR1 receptor, which may be used for the structure-based design of potent and novel ligands for anticancer and antileishmanial therapy. To our knowledge, this is the first report of a binding mode exploration study for diospyrin and its derivatives as inhibitors of the leishmanial PTR1.

Conclusion

The lack of crystal structure and structural data for validated targets are the major problem that faced

Table 1. Structures, IC_{50} and percent of cell survival of 18 experimental ligands.

Compound name	Compound Structure		[a]IC_{50} (µM)	[b]Survival (%)
	R	R'		
Cp1			60	82.35
CP2			50	75.23
CP3			35	67.25
CP4			35	64.1
CP5			75	91.3
CP6			55	75.97
CP7			70	88.5
CP8			75	79.22
CP9			25	49.8
CP10			50	73.62
CP11			25	54.32
CP12			50	78.76
CP13			10	28.15
CP14			45	79.82
CP15			70	84.53
CP16			20	45.72
CP17			60	74.92
CP18			55	67.86

[a] IC_{50} of compounds was determined at 2 days exposure using MTT assay; [b] Percent survival of T47D cells following exposure to 25µM concentration of compounds was determined after 2 days exposure using MTT assay.

application based drug designing. The homology or comparative modeling due to its simplicity to predict the structure of the target proteins or peptides with the help of available protein structure as temples, here comes to

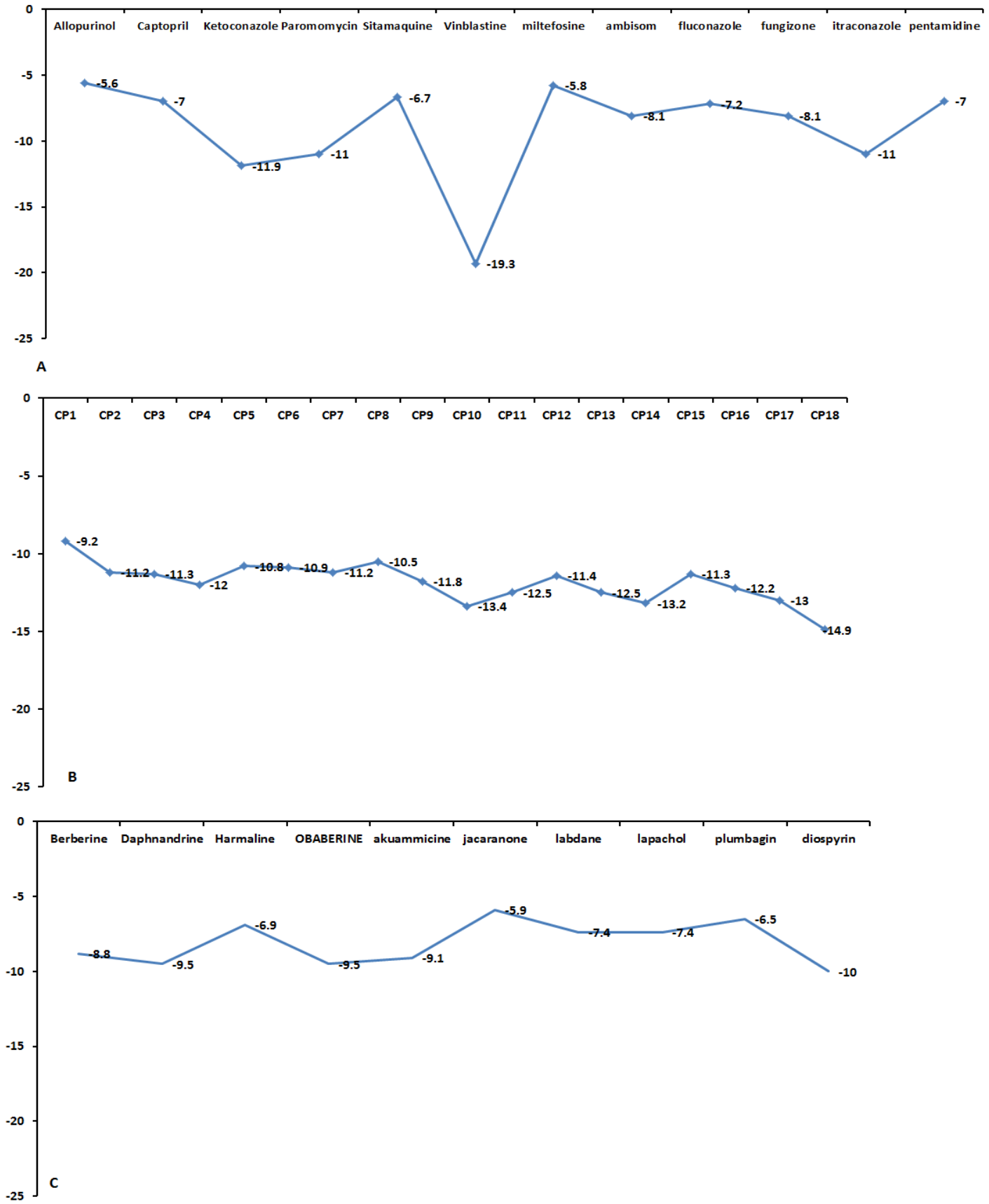


Figure 3. Comparison of the binding affinity of drug **A**, Experimental ligands; **B**, and medicinal plant; **C**, compounds.

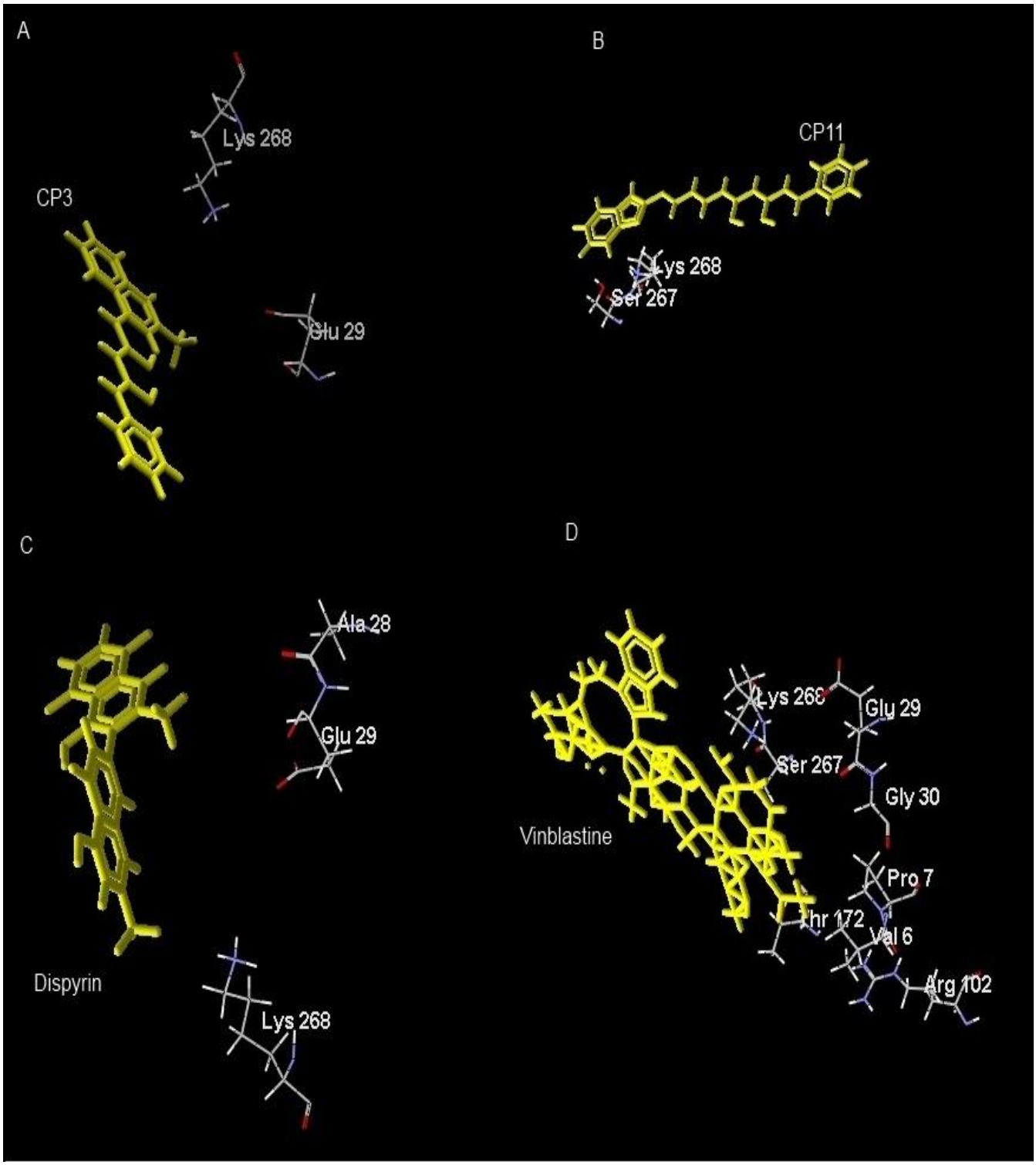


Figure 4. A, Experimental ligand No. 3 (CP3) that showed H-binding to Lys 268th and Glu29th; **B**, experimental ligand No. 11 (CP11) that showed H-binding to Lys 268th and Ser267th; C, diospyrin which forms H-bond with Lys 268th, Glu 29th and Ala 28th; D, Vinblastine forms H-bonds with Glu 29th, Lys 268th and pro 7th, Thr 172th, Val 6th amino acids as two different biding sites in PTR1.

rescue. Present study demonstrated that PTR1 of *leishmania* significantly differs from the other protozoan similar proteins in several structural properties at the active site of these functionally similar enzymes. However, this developed and modeled structure indeed prospects the possibility of using in selective and novel

Table 2. Simulation data from binding of drugs, medicinal plants and experimental compounds to PTR1.

	Pubchem /drug bank ID	Binding energy (Kcal/mol)	Final dock energy
akuammicine	CID 10314057	-6.99	-8.01
Allopurinol	CID 2094	-2.79	-3.18
Berberine	CID 2353	-7.38	-8.15
Captopril	CID 44093	-3.02	-5.94
Daphnandrine	CID 442214	-6.55	-7.91
Diospyrin	CID 308140	-8.37	-10.46
Harmaline	CID 5280951	-5.83	-6.19
Jacaranone	CID 73307	-4.72	-5.94
Ketoconazole	CID 456201	-3.47	-5.68
Labdane	CID 9548711	-7.0	-8.45
Lapachol	CID 3884	-6.84	-8.67
Miltefosine	CID 3599	-3.26	-11.23
Obaberine	CID 100231	-4.83	-6.64
Paromomycin	CID 165580	-4.18	-7.33
plumbagin	CID 10205	-6.0	-6.65
Sitamaquine	CID 42548	-6.26	-10.17
Vinblastine	CID 241903	-8.45	-11.17
Pentamidine	CID 4735	-3.27	-6.64
Fungizone	CID 5386092	-3.77	-7.95
Ambisome	CID 5280965	-3.37	-7.63
Fluconazole	CID 3365	-2.99	-5.07
Itraconazole	CID 55283	-2.72	-7.67
Cp1	----------	-8.33	-11.80
CP2	----------	-8.66	-9.26
CP3	----------	-9.41	-10.30
CP4	----------	-9.20	-10.10
CP5	----------	-8.20	-8.79
CP6	----------	-8.25	-8.85
CP7	----------	-8.54	-9.14
CP8	----------	-7.85	-8.45
CP9	----------	-8.25	-9.42
CP10	----------	-9.21	-10.11
CP11	----------	-9.51	-10.41
CP12	----------	-8.27	-8.86
CP13	----------	-8.93	-9.83
CP14	----------	-9.93	-10.82
CP15	----------	-9.22	-9.82
CP16	----------	-9.16	-10.05
CP17	----------	-10.31	-11.19
CP18	----------	-10.85	-11.74

CP, Compound; Final dock energy, sum of final intermolecular energy and final internal energy of ligand; Binding energy, sum of final intermolecular energy, Internal energy and torsional free energy of ligand minus unbound energy.

PTR1 inhibitor that may represent the important step towards the assessment of anti-leishmanials chemotherapy. Thus, it could be concluded that our generated experimental drug compounds could have potential as pharmacological tools against the visceral leishmaniasis.

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