In silico Based Designing of Short Peptide and Ligand for Calcineurin Receptor

*V. V. Bhandare¹, T. G. Jagtap², M. M. Pillai³ and P. S. Patil³

1. Center for Bioinformatics, Pondicherry University, Pondicherry

2. National Institute of Oceanography, Dona Paula, Goa

3. KIT's College of Engineering, Gokul Shirgaon, Kolhapur

(*corresponding author Email: vishwayogi@bicpu.edu.in)

ABSTRACT

Calcineurin is a target protein for Cyclosporin A (CsA) an immunosuppressive drug which prevents transplant rejection. CsA has complex structure of 11 amino acid, cyclic structure with uncommon amino acids, it can't be chemically synthesized. CsA can be synthesized commercially by using fermentation. One of the report show chemical synthesis of CsA with partial success (Jason and Diver, 2002). In view of this it is proposed to model a peptide and ligand which is alternate for CsA with same function and can be chemically synthesized. Target for Cyclosporin is Cyclophilin which forms ternary complex with calcineurin (CN). Insilico study concludes that all the four peptides (1 wild + 3 mutant) were found to bind in the catalytic site of calcineurin. Out of this, mutant3 shows better binding than the rest of the peptides with CN. Two experimental ligand molecules were selected using virtual screening technique. Further, molecular properties and drug likeliness followed by ADMETox prediction were studied. The designed biomolecule will have great commercial value in pharmaceutical and clinical fields, and could serve as an alternative for natural Cyclosporin A obtained from biological resources. Chemosynthesis of the designed molecule will be economically feasible, and could be produced in adequate amount.

INTRODUCTION

Biotic recourses from different habitats, including various marine habitats, form a source of potential bioactive molecules as well as chemical compounds of commercial importance (Cuomo et al, 1995). CsA compound of clinical applications usually extracted from biological sources, particularly from fungi (Nielsen et al, 1999). Recently the same compound was extracted from a marine fungi *Microdochium nivale* (Bhosale, 2003). Though CsA is clinical importance, its production remains uneconomical as it could only be obtained from biological source. It is therefore, very essential to search for an alternative molecule or compound for CsA that could be chemically synthesized. An effort has been made to investigate an alternative compound for CsA; however desired compound is yet to be found. Some of the pharmaceutical companies have achieved success in obtaining an immunosuppressive compound, however its mechanism differs that from CsA. (Kallen et al, 2005). Naturally occurring CsA has some side effects such as renal dysfunction, tremor, hirsutism, hypertension, and gum hyperplasia toxicity (<http://www.rxlist.com/sandimmune-drug.htm>). Inspite of these side effects it is being commonly used in various clinical applications, mainly because of its effective immunosuppressive action.

METHODOLOGY

PEPTIDE DESIGNING

1. Reported peptide inhibiting Calcineurin is G-P-H-P-V-I-V-I-T-G-P-H-E-E
2. 40 different mutants were designed by considering properties of amino acid with reference peptide
3. Blast analysis
4. Homology modeling of peptide showing maximum similarities
5. Probable binding site was determined by using PPIpred server which shows affinity measure
6. These peptides were used for docking study using Z-dock software through cluspro server
7. Docking Result Analysis
8. Mutation of peptides at particular point using specific residue to increase docking score
9. Homology modeling of mutated sequence
10. Docking of these peptides and analysis

LIGAND DESIGNING

1. Molecules that has been reported as a commercial drug was identified i.e. CsA and FK506 identified
2. Similar experimental molecules were screened by using Zinc (<http://zinc.docking.org/choose.shtml>) and Drug bank (<http://www.drugbank.ca/>) database
3. Virtual screening technique was applied to screened experimental molecules using iGEMDock software
4. Result analysis
5. Two molecules were chosen based on fitness
6. These two molecules were separately docked (Fig. b) using docking server (<http://www.dockingserver.com/web>)
7. Molecular properties of these two molecules were calculated by using Molinspiration server.
8. ADMETox prediction

RESULTS AND DISCUSSION

Insilico based designing of short peptide was done with better result. First we have designed a wild peptide which was showing poor affinity towards CN. Thus, three mutants of wild peptide were designed just to increase binding affinity by studying interactions of wild peptide. Out of three mutants, third mutant shows better binding. Insilico study concludes that all the four peptides (1 wild + 3 mutant) were found to bind in the catalytic site (Fig. a). Out of this, mutant3 shows better binding than the rest of the peptides with CN (Fig. c). Further wet lab study or cell line study is recommended to confirming its effectiveness in immunosuppressive activity.

Two experimental ligand molecules were selected using virtual screening technique. Interactions of these two ligands with respective target have been studied using docking server. Further, molecular properties and drug likeliness (Fig. d) followed by ADMETox prediction (Table 2) were studied. Out of these two molecules ligand 1 obeys most of the rules (ADMETox) required for an effective drug. Results of which were found to be very similar to that of commercial drugs i.e. CsA and Tacrolimus (FK506). Thus we can partially predict that ligand 1 can serve as an immunosuppressive drug. This prediction can be confirmed using wet lab studies.

Fig a: Phosphate binding site in calcineurin

Fig b: Docked pose of two molecules screened

Fig c: Docked poses and interactions of wild + mutant

Fig d: Molecular Properties and Drug likeliness

Peptide type	Sequence	Binding energy
Wild	PGTITCLGIGPAPE	-10.40 kcal/mol
Mutant1	PGTILCLGIGPAPE	-11.83 kcal/mol
Mutant2	PGTITCLGIGPAQE	-10.62 kcal/mol
Mutant3	PGTILCLGIGPAQE	-11.97 kcal/mol

Table 1: Binding energy of peptide + protein interactions

ADMETox Prediction

Properties	Standard Values	Ligand 1	Ligand 2
Molecular wt	Min 200.0 Max 600.0	459.3	921.4
Hydrogen donor	Min 0 Max 6	4	13
Hydrogen acceptor	Min 0 Max 12	10	27
Flexible bonds	Min 0 Max 15	10	24
Rigid Bonds	Min 0 Max 50	21	30
Ring number	Min 0 Max 7	3	3
Ring Length	<10	6	6
Ring size	Min 0.0 Max 12.0	23	
Atom Number	Min carbon 5.0 Non carbons 2.0	23 10	37 27
Ratio of Carbon and hetero atom	Min 0.1 Max 1.0	0.434783	0.729730
Charge number	Min 0.0 Max 3.0	0	0
Total Charge	Min -2.0 Max 2.0	0	0
LogP	Min -2.0 Max 6.0	1.0700	-6.6600
Polar surface area	Min 0.0 Max 150.0	159.060	419.3600

Table 2: ADMETox Prediction

CONCLUSION

A number of drugs of medicinal importance could not be synthesized chemically nor is it feasible to have from natural biological resources. Drug discovery is continuous efforts to identify a new compound that could be effective in various medical applications. In view of this the present investigations signifies importance of bioinformatics in finding alternate drugs of at most values in clinical application. The efforts made had lead to the discovery of an alternate molecule for cyclosporine A (which could only be extracted from biological resources), using various bioinformatics tools.

ACKNOWLEDGEMENT

*Author is thankful to Director, National Institute of Oceanography for giving an opportunity to work in CSIR laboratory and Head, Dr. D. Y. Patil University, Navi Mumbai for providing a special training on request related to drug designing. Finally I, specially thanks to Dr. Ashokan K.V. for helping continuously throughout the work.