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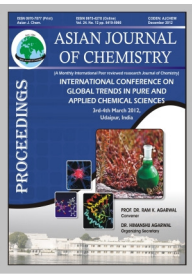

In silico Identification of Drug Analogues for Antifertility†

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The identification of new, clinically relevant, molecular targets is of utmost importance for the discovery of innovative drugs. In this paper natural products (plant species) are selected for comparative study in order to analyze the compounds showing antifertility property with respect to the metabolic pathways involved. The two enzymes are identified as targets namely, L-aspartate dehydrogenase (EC 1.4.1.21) and *trans*-hexaprenyl-*trans*-transferase (EC No. 2.5.1.30) from nicotinate and nicotinamide metabolism and biosynthesis of steroids respectively. It has been found that there are no inhibitors existing for these enzymes so it becomes necessary to identify and design the new inhibitors. Further in order to design and model the analogues on screening various libraries we have identified most potent ligands using auto-dock. The most appropriate inhibitors for the targets in terms of binding affinity and enzyme inhibition constant (Ki) have been identified.

Key Words: *In silico*, Identification, Drug, Antifertility.

INTRODUCTION

Proteomics and Genomics are rapidly changing the very foundation of several aspects of drug discovery research, one of them being systems biology and bioinformatics approach for rational identification of drug targets. The targets are evaluated using two criteria *i.e*. essentiality and selectivity. In order to understand the phenotype of any living system, it is essential not only to investigate genes, but also the specific metabolic pathway variant of the organism of interest, ideally in comparison with other organisms. Nowadays thousands of drugs are in market but still there are many which need improved efficacy (minimization of biological toxicity effects and side effects)[1,2]. In present study clomifene drug used for fertility regulation treatment is studied. Two enzymes, namely, L-aspartate dehydrogenase (EC no. 1.4.1.21) and *trans*-hexaprenyl-*trans*-transferase (EC no. 2.5.1.30), were identified as novel drug targets from the metabolic pathway analysis. Validation of the essential proteins identified through metabolic pathway comparison was done based on the literature information. Comparisons of differential reaction content of various metabolic pathways of the 13 plant species led to the identification of 2 unique enzymes, which may be responsible for the antifertility property namely, *trans*-hexaprenyl-*trans*-transferase or heptaprenyl diphosphate synthase (EC no. 2.5.1.30), which is involved in the biosynthesis of steroids and L-aspartate dehydrogenase (EC no. 1.4.1.21), which is involved in the biosynthesis of nicotinate and nicotinamide. The Kyoto encyclopedia of genes and genome pathway database was the source of metabolic pathway information. Protein sequences and classification numbers of unique enzymes exclusively present in certain plant species were identified using the expert protein analysis system[3].

The molecular modeling of the PDB structures with very low sequence similarity with know PDB templates is a bit tougher task as for these identified targets no PDB structures exists. An attempt has been made to model the tertiary structure of drug targets through multiple templates with incorporation of fold assignment and secondary structure information[4]. After model assessment, active site characterization, analogs generation, analog selection has been done through various offline and online softwares and tools. Furthermore to explore the efficacy of clomifene, a comparative analysis of generated analogs has been performed with clomifene.

EXPERIMENTAL

The information about the drug clomifene along with its PDB structure was obtained from drug bank. The two unique

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enzymes were taken as drug targets which were obtained by differential reaction content analysis of different metabolic pathways[3].

Modeling of drug targets: The PDB structures of both drug targets are not available. So, modeling of both the target proteins were performed using MODELLER. A template search has been performed through BLAST and PSIBLAST programs[5]. Global alignment method was used for comparison between the target template sequences. Gaps with variable gap penalty function are included for structural loops and core regions, in order to get maximum correspondence between the sequences. Alignment file for MODELLER was prepared by CLUSTALW[6]. Parameters like covalent bond distances and angles, stereo-chemical validation, atom nomenclature were validated using PROCHECK and overall quality factor of non bonded interactions between different atoms types were measured by ERRAT program[7]. Functionally important residues (active site) were identified through POCKETFINDER.

Analog generation characterization and docking: Drug analogs were created through mono-substitution in the hydrophilic region on the target molecule with other functional groups (I group to + I group) through CHEMSKETCH 10.0. Generated analogs were further analyzed by comparative study of molecular descriptors and various energies. Drug binding specificity of target to analogs was also performed with docking through AUTODOCK 4.0[8]. In the effective analog development the mono-substitution studies have more relevant results. The most important step is the prediction and identification of potential drugs and non-drugs from generated analogs[9].

RESULTS AND DISCUSSION

The modeling of target protein, model verification, active site characterization, analogs generation and docking studies, resulted through various softwares and tools available online and offline are as follows.

Models of target proteins (enzymes): The three dimensional structure of drug targets are shown in Fig. 1a and b.

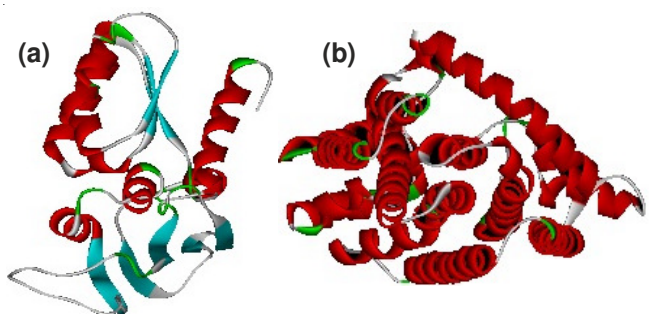

Fig. 1. (a) and (b) shows three dimensional visualization of the modeled structure of drug targets (Model a and Model b) for L-aspartate dehydrogenase (EC 1.4.1.21) and Trans hexaprenyltranstransferase (EC No. 2.5.1.30)

The generated 3D model (Fig. 1 a and b) of target proteins was checked by Ramachandran plot [Fig. 2 (a) and (b)] through PROCHECK program.

In Ramachandran plot for model-a and model-b, 91.1 % and 93.8 % residues were found in most favoured region respectively. Besides very low sequence similarity and sequence coverage to PDB templates, the overall quality factor for drug target model-a (75.127 %) and model-b (85.262) were reported through structure validation server ERRAT [Fig. 3(a) and (b)].

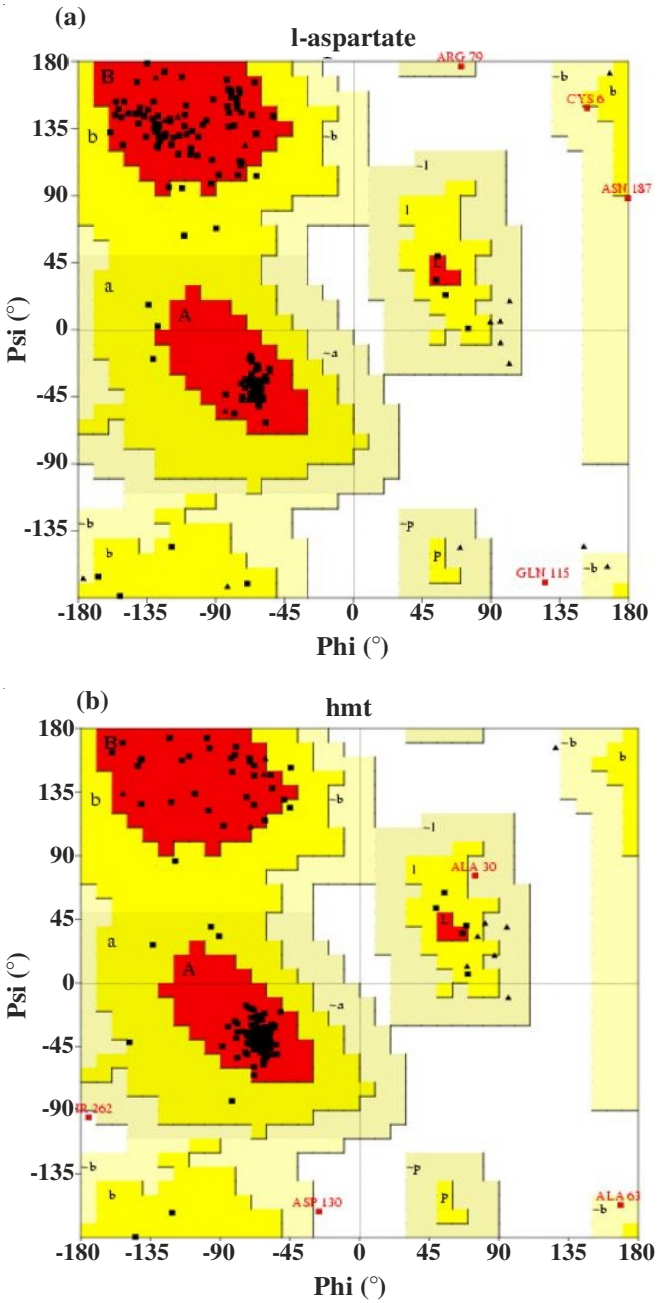

Fig. 2. (a) and (b) shows torsion angles of f and y in the generated models through Ramachandran plot of generated model a and b

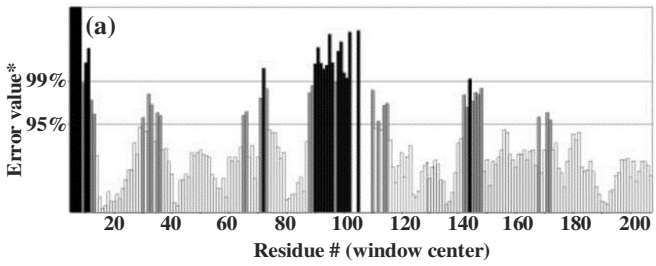

Fig. 3. (a) shows the overall quality factor of generated model-I of drug target of Clomifene through structure validation server ERRAT

TABLE-1
SHOWS LIST OF SELECTED ANALOGS THEIR BEST BINDING CONFORMATIONS, BINDING ENERGY AND INHIBITION CONSTANT

Analogues	Best Binding conformations out of 10 (appx.)	Binding energy (Kcal/mol)		Inhibition constant	
		Target-I	Target-II	Target-I	Target-II
Ref. com.	5	2.67	1.69	$+ 7.15^{-4}$	$+ 5.35^{-4}$
$-CCl_3$	8	1.48	1.09	+ 0.18	+ 0.15
$-CCl_2OH$	4	1.56	1.98	+ 0.23	+ 0.35
$-CH_3$	9	1.19	2.07	$+ 3.18^{-4}$	$+ 2.15^{-4}$
$-CH_2OH$	5	1.26	0.79	+ 0.42	+ 0.56
$-CH_2 CH_3$	4	1.13	3.63	+ 0.13	+ 0.48

Dark black regions were showing high error due to high structural variability in generated models.

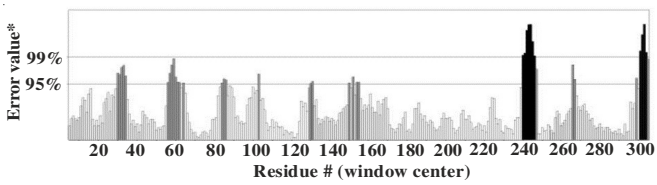

Fig. 3. (b) shows the overall quality factor of generated model-II of drug target of Clomifene through structure validation server ERRAT

Analogues generation and optimization: Drug analogs were created through mono-substitution in the hydrophilic region on the drug molecule with other functional groups (I group to + I group) through CHEMSKETCH 10.0 and compatibility of analogs was checked through AUTODOCK 4.0 against modeled structures of target proteins (target-I + target-II) with binding energies.

Conclusion

It is clearly indicated that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities, toxicity and Lipinski evaluation to speed up drug discovery process by eliminating less potent compounds from synthesis. In present work efforts have been made to identify new candidate compounds for existing drug through modeled structure of drug targets. Both drug target of clomifene were generated using MODELLER (Fig. 1a and b) and validated by PROCHECK and ERRAT programs. For model-I, 91.1 % residues were lying in most favoured region whereas 93.8 % residues were lying in most favoured region in model-II in Ramachandran plot (Fig. 2a and b) which were good indicator of fitness of stereo-chemical quality of protein structures. Besides very low sequence similarity and sequence coverage to PDB templates, the overall quality factor for model-I (75.127 %) and model-II (85.262) were reported through structure validation server ERRAT (Fig. 3a and b). Binding affinities compatibility of analogs was checked through AUTODOCK 4.0 with model-I and II (Table-1). Total 151 analogs were generated and five are selected for comparative study. On the basis of docking studies it may be concluded that the enzyme targets binds to the drug analogs efficiently and their activity is inhibited.

Future aspects: Toxicity of analogs with reference compound is to be checked. On the basis of binding energy, inhibition constant and Lipinski evaluation, out of the selected clomifene drug analogs the one showing theoretically more superior results can be selected for toxicity analysis. The present model can be further extended with some modifications, if necessary for analysis of other drug compounds which have more side effects. All these analysis and predictions are made on the basis of bioinformatics tools and techniques by statistical analysis. Theoretical calculation may vary from software to software but the protocol which is developed is up to mark for the new drug discovery. This knowledge will contribute positively to develop new drug analogs with more efficacies as well as less side effects.

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