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DESIGNING A POTENT INHIBITOR FOR HUMAN EIF2AK3 (PERK) - A THERAPEUTIC TARGET FOR PROGRESSIVE SUPRANUCLEAR PALSY

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ABSTRACT

For a typical drug action, proteins are considered as the major targets as they are the functional molecules in living systems. Conventional laboratory techniques takes minimum ten years in designing a single drug. This time-span is drastically reduced via the use of computational/in-silico tools. Rational designing of a drug starts with finding out the proteins having the potential to be considered as drug targets in disease pathogenesis. Proteomics plays a big role in identification of the same and thus aid in multi-step drug-development process. The process basically comprises of identification and validation of target, selection of lead, molecular screening and optimization, and toxicity testing. This paper aims at designing a computer-assisted, structure based drug of an effective inhibitor of human EIF2AK3 (PERK) gene, a target protein for Progressive Supranuclear Palsy (PSP), a degenerative neurological disorder of uncertain aetiology, caused mainly by mutation of tau gene. Discovery of potent and selective inhibitors which has the potential to inhibit PERK activity in cells and display growth inhibition of human tumour has helped in identifying selective lead compounds as drug candidates. The drug selected via virtual screening and ADME analysis though having a low drug score of 0.27 had no toxicity risks and was good enough for inhibition of the protein expression, so can be used for drug development for further application. The study also provides hints for the future design of new derivatives with higher potency and specificity.

KEYWORDS: Proteomics, Virtual screening, Docking, Drug designing, Drug target.

INTRODUCTION

Computational tools present the advantage of new drug candidates getting delivered more quickly and at a lower cost (Naveekaran *et al.*, 2011). Structure-based computational methods show ever-increasing utility for the discovery and refinement of lead compounds, along with re-engineering of drugs to overcome certain types of resistance. The application of harmonizingexperimental and informatics techniques has increased the success rate in various stages of the drug discovery process, from target identification and clarification of their functions to the discovery and development of lead compounds with desired properties. Structure based drug design (SBDD) facilitates in the evolution of new drug compounds by using the recognized 3D geometrical shape or structure of proteins. Nuclear magnetic resonance (NMR) or X-ray crystallography are the techniques by which these 3D structures are mostly derived. These have the ability to resolve the protein structure to a resolution of a few angstroms, i.e. about 500,000 times smaller than the diameter of a human hair (Casey, 2006). This resolution level can effectively help researchers examine the interactions between targeted protein atomsand potential drug compounds that bind to it. This ability to study interaction at high resolution makes SBDD one of the most robust methods in drug designing. In other case, molecular modeling offers the best substitute when a three dimensional structure of the target protein is not obtainablefrom experimental techniques. That being said theuse of insilico methods in drug design has been embraced by many pharma companies to complement high throughput screening (HTS) methods (Heal, 2003).

PROGRESSIVE SUPRANUCLEAR PALSY (PSP)

PSP is a rare, progressive neurological disease (Timothy, 2013) which is characterized by motor and visual symptoms which includes problems in balance and gait, slowing or lack of ability to give rise to voluntary saccadic eye movements, axial rigidity and frontal lobe deficits (Maria *et al.*, 2010). It belongs to the family of tauopathy and involves both cortical and subcortical structures. Loss of neurons accompanied by presence of numerous neurofibrillary tangles composed of hyper phosphorylated tau are common. Tau is a microtubule-binding protein that is normally present in abundance in

neurons. In typical PSP, pathological tau is composed of aggregated 4- repeat (E1 0+) forms that accumulate in cells and glia in the brain.So the gene encoding tau has been considered a candidate gene for PSP.

POTENTIAL DRUG TARGET

Literature survey revealed that there are 4 loci that contribute to risk of developing this disease. These genes are *MAPT*, *EIK2AK3*, *MOBP*, and *STX6* (Gerard, 2011). Here the study has been focusedon EIK2AK3, a gene that encodes the protein eukaryotic translation initiation factor 2-alpha kinase, also known as PERK. Genotypes at this gene confer a modest risk for developing PSP (odds ratio = 1.33, confidence interval = 1.23 - 1.45, P = 3.2 x 10-13). PERK is a component of the endoplasmic reticulum (ER) unfolded protein response (UPR). When unfolded proteins accumulate in the ER, they are detected by the chaperone BIP, or by PERK directly. When activated, PERK dimerizes, is then autophosphoryated, and subsequently phosphorylates EIF2A (eukaryotic translation initiation factor 2A) thereby inhibiting protein synthesis (Atkins *et al.*, 2013). The UPR can activate autophagy, a method that potentially rides the cytoplasm of abnormal proteins such as tau. This may be how this system functions in PSP pathogenesis.

METHODOLOGY

The work commenced with the identification of the protein targeted, in this case, EIF2AK3 (PERK) for which protein sequence was recovered from NCBI and its homology was established using NCBI blast. Bestsequence matching the query sequence was extracted and then homology modelling was done using MODELLER. The generated models were then analysed by using SAVS (PROCHECK) followed by validation via loop-building and energy-minimization. Best model was chosen, which acted as the receptor protein. For accurate and efficient binding, the best pocket in the receptor, where the inhibitor could attach was then searched via LIGSITE. Now, for insilico drug designing, ligands were selected based on existing PERK inhibitorspresent.GSK2060414 (7- Methyl-5-(1-{[3-trifluoromethyl) phenyl] acetyl}-2, 3-dihydro- 1 H-indol-5-yl) - 7 H-pyrrolo [2, 3- d] pyrimidin-4-amine) is a Potent and Selective First-in-Class Inhibitor of PERK (Axten *et al.*, 2012). Similar compounds to this inhibitor were searched on PUBCHEM which showed a listing of 92 compounds. Applying the Lipinski's Rule of 5 (Drug selection criteria) brought the list down to 72 structures.Then virtual screening was performed on these 72 compounds via docking software GLIDE, with the optimized PERK structure acting as the substrate for these ligands. The compound with the minimum binding energy is selected as the ideal lead compound.Thenparameters as absorption, distribution, metabolism, and excretion (ADME) properties were checked on OSIRIS, MOLSOFT and MOLINSPIRATION. These are very critical for drug design (Butina *et al.*, 2002). Finally, selection of an inhibitor with no toxicity risks was confirmed and study concluded.

RESULTS AND DISCUSSIONS

The structure of the PERK was downloaded in FASTA format from NCBI which acted as the query sequence. This sequence was then compared with the database of sequences using BLAST (Altschul et al, 1990). Sequences having 90% similarity with the query sequences were selected as templates which acted as input for MODELLER (Sali *et al.*, 1995, Sanchez *et al.*, 1997 and Eswar *et al.*, 2006). It is a software which automatically calculates a model containing all non-hydrogen atoms.Analysis of the best model obtained was done using Swiss PDB viewer. Ramachandran plot in Swiss PDB viewer (Guex et al, 1997) enabled the refinement of the model by loop-building and side chain packing for energy minimization (Fig. 1). Therefined model obtained acted as our receptor protein.

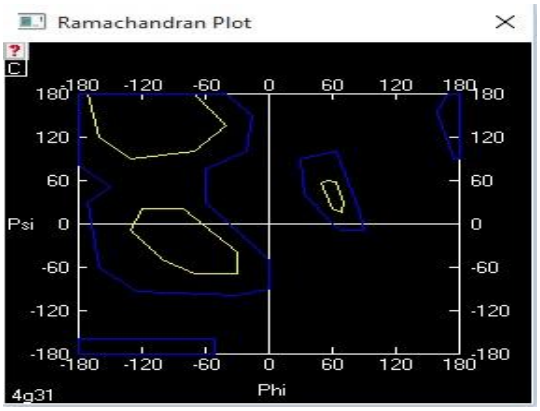
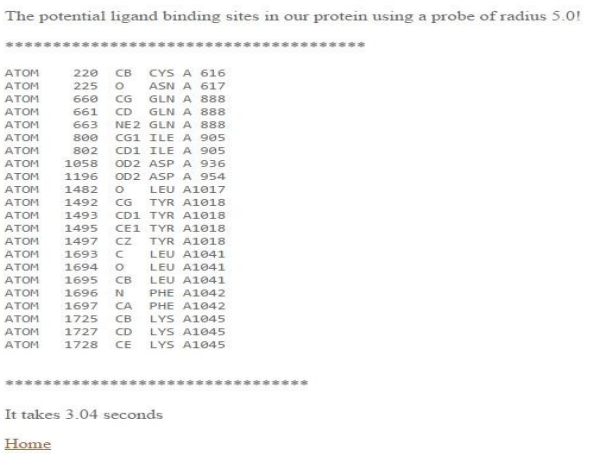


Fig. 1. Visualization of Ramachandran plot of the model using SPDB viewer.

Molecular cavities helped in the prediction of active amino acid sitesof the receptor which would act as best binding site where targeting of the ligand protein molecule would be done. LIGSITE (Hendlich et al, 1997) online software was used to identify the pockets (Fig. 2).


The potential ligand binding sites in our protein using a probe of radius 5.0!

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ATOM    220  CB  CYS A 616
ATOM    225  O   ASN A 617
ATOM    660  CG  GLN A 888
ATOM    661  CD  GLN A 888
ATOM    663  NE2 GLN A 888
ATOM    800  CG1 ILE A 905
ATOM    802  CD1 ILE A 905
ATOM   1058  OD2 ASP A 936
ATOM   1196  OD2 ASP A 954
ATOM   1482  O   LEU A1017
ATOM   1492  CG  TYR A1018
ATOM   1493  CD1 TYR A1018
ATOM   1495  CE1 TYR A1018
ATOM   1497  CZ  TYR A1018
ATOM   1693  C   LEU A1041
ATOM   1694  O   LEU A1041
ATOM   1695  CB  LEU A1041
ATOM   1696  N   PHE A1042
ATOM   1697  CA  PHE A1042
ATOM   1725  CB  LYS A1045
ATOM   1727  CD  LYS A1045
ATOM   1728  CE  LYS A1045

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It takes 3.04 seconds

Home

Fig. 2. Identification of pockets using LIGSITE.

Molecular docking is a procedure often hired to help determining the way of interaction of a particular drug lead with a binding pocket. The ligand and receptor PDB files were opened in GLIDE followed by pre-adjustment of different docking parameters. Now the selection of an effective lead compound which would likely act as drug candidate was done via virtual screening (Fig. 3). It yielded the best compound having the minimum binding energywith the receptor as compared to others.

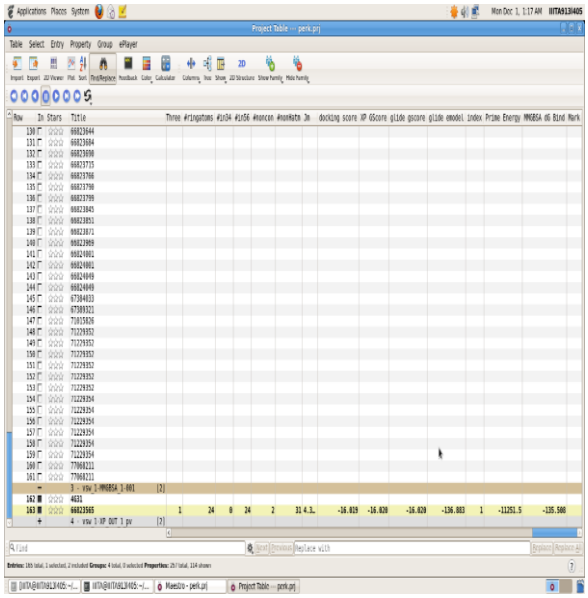

Fig. 3. Virtual screening done via GLIDE.

We finally got a best selected molecule as a result of the above procedure.

Ligand PubChem id: 66823565.

Obtained binding energy: -135.508 kJ/mol.

Its molecular structure in conjunction with potential amino acid binding sites has been observed. (Fig. 4).

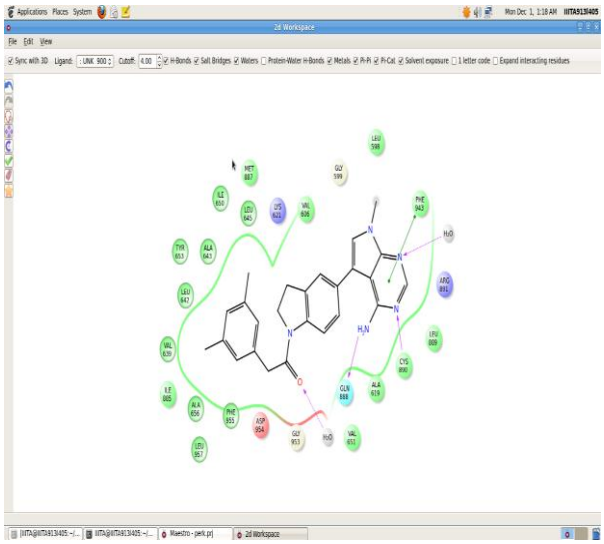

Fig. 4. Molecular structure of the selected ligand.

Now we proceeded to calculate and analyse the drug effectiveness via online softwares as OSIRIS (Sander, 2014), MOLSOFT (Ruben, 1994,) and MOLINSPIRATION (Nova, 1986).

OSISRIS Observation

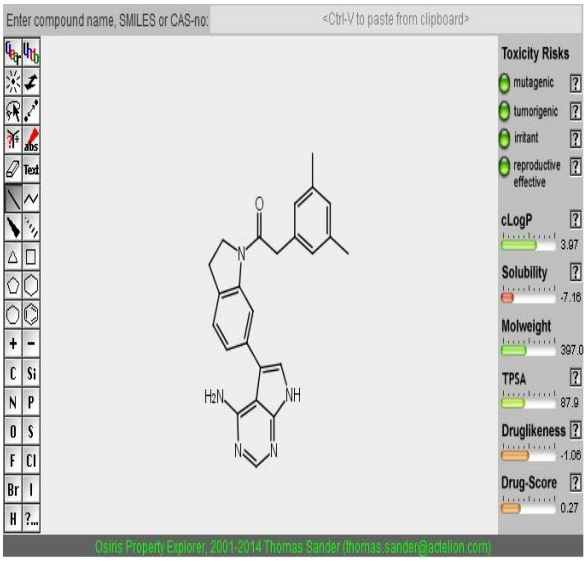

Fig. 5. Observation via OSIRIS.

Analysis of drug properties using OSIRIS was done. (Fig. 5). Scores were highlighted in two colours: green and red. Those properties with high chances of unwanted effects like mutagenicity or a poor intestinalabsorption were indicated in red, and a green colour indicated drug-complyingbehaviour. Measurement of parameters signifying toxicity risks as shown in figure was done. Noirritating, mutagenicity ortumorigenicity was observed in the candidate molecule. The value of clog P is a measure of compound's hydrophilicity which was less than 5. This indicated that the drug has a reasonable probability of being well absorbed. Its absorption is directly related to the solubility of a compound in aqueous solutions. Lower the solubility, lesser the absorption. Molecular weight was also noted to be low, which is a good thing since compounds with less molecular weight are more likely to be absorbed faster. The scores from all the parameters are summed up in drug-score (Fig. 6).

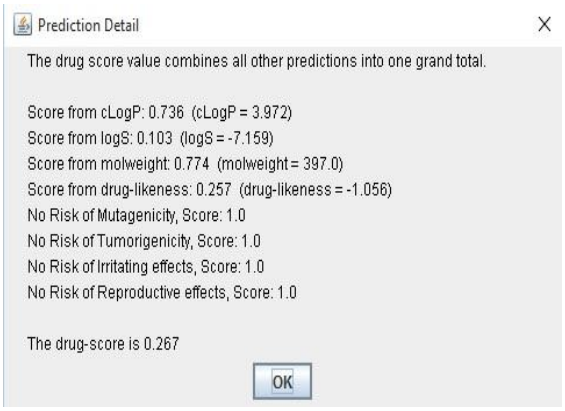

Fig. 6. Calculation of the drug-score.

MOLSOFT Observation

MOLSOFT (Fig. 7) provides various tools and services catering to proteomics, bioinformatics, rational drug design, etc. Measurement of chemical Parameters like Molecular Formula, Molecular Weight, Number of Hydrogen Bond Acceptors (HBA), Number of Hydrogen Bond Donators (HBD), mol Log P (octanol/water partition coefficient), mol Log S (water solubility), Polar Surface Area (mol PSA), Volume, Number of Stereo Centers, and Drug Likeness Model Score of the lead candidate were done. Its drug-likeness graph showed that the compound is ideal for future drug development.

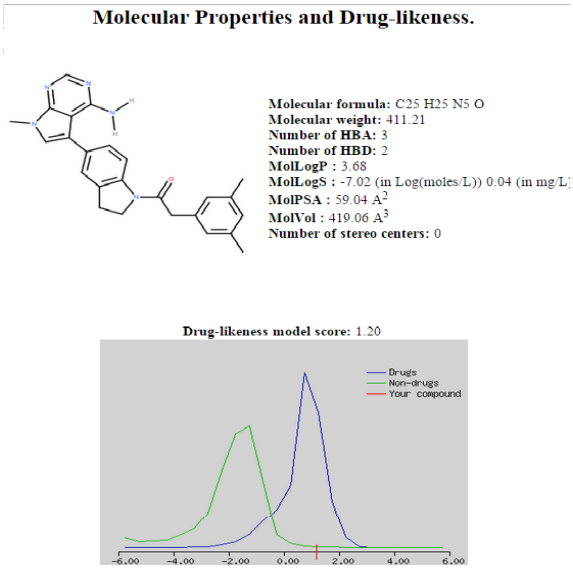

Fig. 7. Observation of chemical properties via MOLSOFT.

MOLINSPIRATION Observation

Activity score and drug likeness were calculated using MOLINSPIRATION by choosing the "Predict Bioactivity" option (Fig. 8).

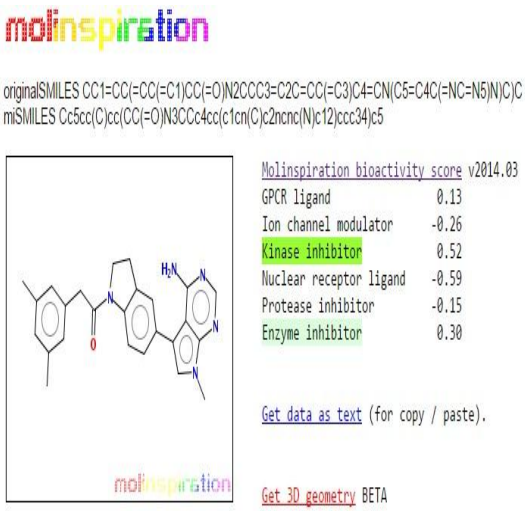

Fig. 8. MOLINSPIRAION Bioactivity score.

The tool alsocalculated the molecular physicochemical properties relevant to drug design and QSAR, including log P, molecular polar surface area (PSA), and the rule of five descriptors, by choosing the "Calculate properties" option. (Fig. 9).

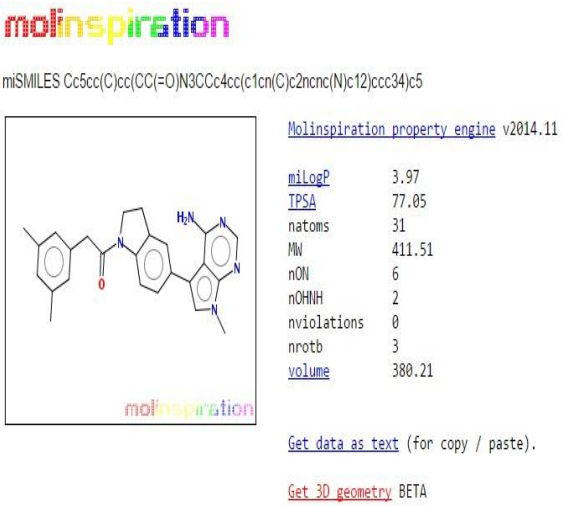

Fig. 9. MOLINSPIRAION Properties calculation.

The candidate molecule was, as discussed, pre-selected by applying **Lipinski's Rule of Five (**Leeson, 2012). Thisis a rule of thumb to assess drug likeness, or decide whether a biologically- or pharmacologically active chemical is in possession of properties that would probably make it an orally active drug in humans. Lipinski's rule for classification of an orally active drug:

- Less than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms).
- Less than 10 hydrogen bond acceptors (nitrogen or oxygen atoms).
- A molecular weight under 500 daltons.
- An octanol-water partition coefficient log *P* of less than 5.

CONCLUSION

In conclusion, our study gives an idea about the interaction between the active site residues and the substrate which is explained on the basis of size & hydrophobicity of the binding pocket. The molecules that showed less binding energy and showed better interactions with protein are not yet tested in the laboratory and the autoflourescence data for these molecules is not available. The extent of the work stretches to the in-silico approach for determining the binding mode. Further there is need to generate in vitro and in-vivo activity of thegenerated data to synthesize and test so to design drugs with better specificity and metabolism.

REFERENCES

1. Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ Basic local alignment search tool. J. Mol. Biol., 1990; 215(3): 403–410.
2. Butina D, Segall MD, and Frankcombe K. Predicting ADME properties in silico: methods and models. Drug Discovery Today., 2002; 7(11): S83-8.

3. Charity Atkins et al. Characterization of a Novel PERK Kinase Inhibitor with Antitumor and Anti-Angiogenic Activity, 10.1158/0008-5472.CAN-12-3109.
4. Eswar N, Marti-Renom M A, Webb B, Madhusudhan M S, Eramian D, Shen M, Pieper U and Sali A (2006) Comparative Protein Structure Modeling With MODELLER. Current Protocols in Bioinformatics, John Wiley & Sons, Inc., 2006; Supplement 15: 5.6.1 -5.6.30.
5. Gerard D. Schellenberg (2011) The Genetics of Alzheimer's Disease, Progressive Supranuclear Palsy, and other Proteinopathies Cure PSP 2011 International Research Symposium.
6. Guex N and Peitsch M C SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. Electrophoresis, 1997; 18(15): 2714–2723.
7. Hendlich M, Rippmann F and Barnickel G LIGSITE: automatic and efficient detection of potential small molecule – binding sites in proteins. J Mol Graph Model., 1997; 15(6): 359-63, 389.
8. Jeffrey M. Axten, Jesus R. Medina, Yanhong Feng, Arthur Shu, Stuart P. Romeril, Seth W. Grant, William Hoi Hong Li, Dirk A. Heerding, Elisabeth Minthorn, Thomas Mencken, Charity Atkins, Qi Liu, Sridhar Rabindran, Rakesh Kumar, Xuan Hong, Aaron Goetz, Thomas Stanley, J. David Taylor, Scott D. Sigethy, Ginger H. Tomberlin, Annie M. Hassell, Kirsten M. Kahler, Lisa M. Shewchuk, and Robert T. Gampe (2012) Discovery of 7- Methyl-5-(1-{[3-(trifluoromethyl)phenyl]acetyl}-2,3- dihydro-1 H- indol-5-yl)- 7 H- pyrrolo[2,3- d]pyrimidin-4-amine (GSK2606414), a Potent and Selective First-in-Class Inhibitor of Protein Kinase R (PKR)-like Endoplasmic Reticulum Kinase (PERK), dx.doi.org/10.1021/jm300713s | J. Med. Chem., 2012; 55: 7193−7207.
9. Kopp J and Schwede T Automated protein structure homology modeling: A progress report Pharmacogenomics J., 2004; 5(4): 405-416.
10. Kramer B, Metz G, Rarey M and Lengauer T Ligand docking and screening with Flex X, Med. Chem. Res., 1999; 9(7/8): 463–478.
11. Maria Stamelou et al. Rational therapeutic approaches to progressive Supranuclear palsy, Brain, 2010; 133: 1578–1590.
12. Navkiran Dosanjh, Anju Sharma, Bhardwaj, S.V., Vinay Randhawa, Harpreet Singh (2011) Insilico structure based drug designing of a potent inhibitor for human notch1 - a therapeutic target for lymphoblastic leukemia, I.J.S.N., 2011; 2(4): 705-712.
13. Prudence M. Stanford et al. (1999) Progressive supranuclear palsy pathology causedby a novel silent mutation in exon 10 of the *tau*gene Brain, 2000; 123(5): 880-893.
14. Rarey M, Kramer B, Lengauer T and Klebe G A fast flexible docking method using an incremental construction algorithm, J. Mol. Biol., 1996; 261(3): 470–489.
15. Tuccinardi T Docking-Based Virtual Screening: Recent Developments. Combinatorial Chemistry & High Throughput Screening, 2009; 12: 303-314.