Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(6):353-363
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Target identification and validation for diabetic nephropathy using molecular docking studies

Ramanjaneyulu M.*, K. Ananth Kumar, M. Shubash Kumar, Shivakanth Reddy, Divyadhatri Kara, Pravallika Mukthala, Rohith Raj and Chandaka Madhu

Department of Pharmacology, SRM College of Pharmacy, SRM University, Kattankulathur, Chennai, Tamilnadu, India

---

ABSTRACT

Diabetic nephropathy is one of the chronic complications of diabetes mellitus. Inflammatory mediators are believed to play a vital role as predictors of low grade systemic inflammation in diabetic nephropathy. Drug designing, one of the hottest topics have found its new pathway to create a history in the field of medical science. The lead compound analysis starts with CADD, assisting to identify and to optimize the right compound. The technique helps in generating a suitable compound specific to the disease; thereby an effective treatment is achieved. Molecular modeling method has been used for modeling a new molecule for Diabetic nephropathy using Lisinopril , a drug that's already designed. This drug is drawn using hyperchem, and its R group is modified by replacing different functional groups like OH, Br, CH_2CH_3, CH_3, Cl, F, H, and NH2 , etc in its place and docked by using gold software.. The molecules designed as such are optimized using different algorithms and their affinity is checked with protein. The binding free energy of the protein is calculated by performing docking process. The molecule with minimum binding energy will have the maximum binding affinity. The binding free energy is calculated by the formula Z = Sum of the energy of optimized ligand devoid of solvation parameters and the energy of the protein - ligand optimization. The binding free energy of the designed molecules is obtained by eliminating the energy of the main molecule i.e. Lisinopril .From the results obtained it's clear that ligand 1 & 5 (-3.65 & -2.73) for Diabetic nephropathy have the maximum binding affinity. So these molecules are determined as the best lead molecules targeting computationally.

Keywords: Diabetic Nephropathy, Inflammatory mediator, CADD, Lisinopril, Hyperchem

---

INTRODUCTION

Diabetic nephropathy (*nephropatia diabetic*), also known as Kimmelstiel - Wilson syndrome , or nodular diabetic glomerulosclerosis and intercapillary glomerulonephritis, is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. It is characterized by nephrotic syndrome and diffuse glomerulosclerosis. It is due to longstanding diabetes mellitus, and is a prime indication for dialysis in many Western countries[1].

Lisinopril, an angiotensin-converting enzyme (ACE) inhibitor, is used to treat hypertension, congestive heart failure (CHF), postmyocardial infarction, and diabetic nephropathy or retinopathy. Although it is the lysine ester of enalaprilat, the active form of the prodrug enalapril, lisinopril is active unchanged [2].

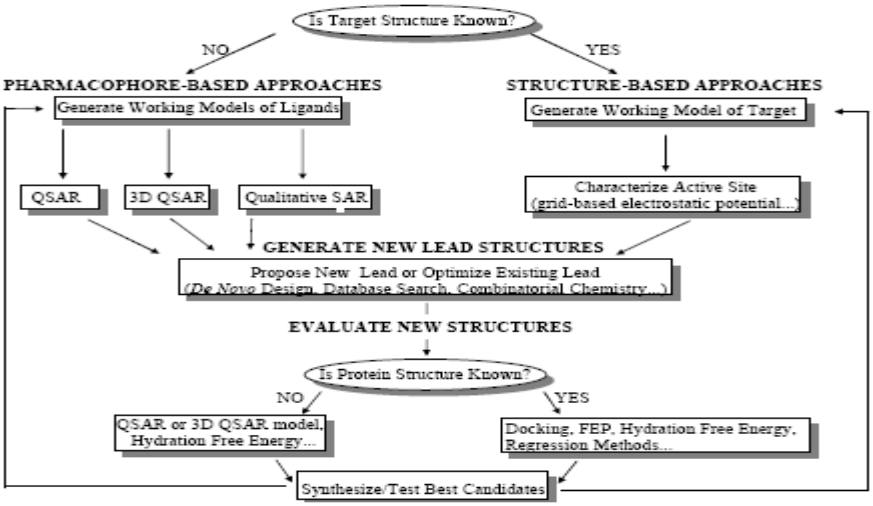
Bioinformatics

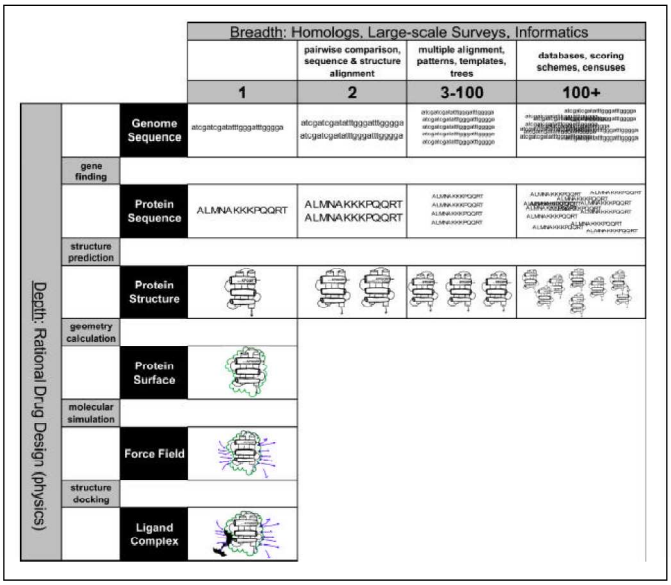
Bioinformatics is conceptualizing biology in terms of molecules (in the sense of physical chemistry) and applying " *informatics techniques* " (derived from disciplines such as applied math's, computer science

and statistics) to *understand* and *organize* the *information* associated with these molecules, on a *large scale*. In short, Bioinformatics is a management information system for molecular biology and has many *practical applications.*

Applications of Bioinformatics

- Database query tools
- Sequence analysis and molecular Evolution
- Genome mapping and comparison
- Gene identification
- Structure prediction
- Drug design and drug target identification [3]





Computer aided drug design (CADD)

Drug design is the approach of finding drugs by design, based on their biological targets. Typically a drug target is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology, or to the infectivity or survival of a microbial pathogen. Computer – assisted drug design (CADD), also called computer - assisted molecular design (CAMD), represents more recent applications of computers as tools in the drug design process. In most current applications of CADD, attempts are made to find a ligand (the putative drug) that will interact favorably with a receptor that represents the target site. Binding of ligand to the receptor may include hydrophobic, electrostatic, and hydrogen - bonding interactions. In addition, solvation energies of the ligand and receptor site also are

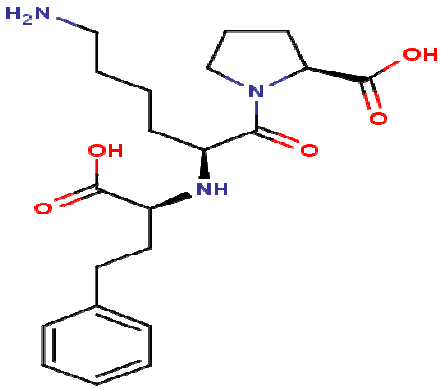
important because partial to complete desolvation must occur prior to binding. This approach to CADD optimizes the fit of a ligand in a receptor site. However, optimum fit in a target site does not guarantee that the desired activity of the drug will be enhanced or that undesired side effects will be diminished. Moreover, this approach does not consider the pharmacokinetics of the drug.

Benefits of CADD

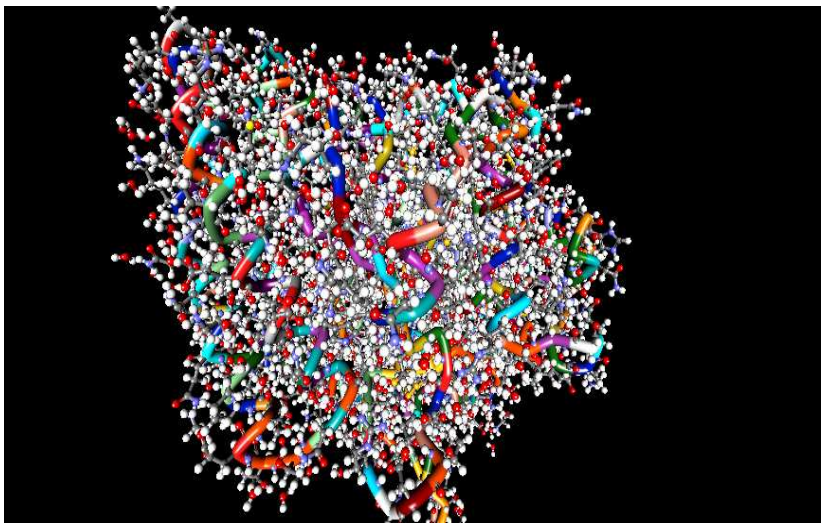
CADD methods and Bioinformatics tools offer significant benefits for drug designing programs. Cost Savings. Many biopharmaceutical companies now use computational methods and Bioinformatics tools to reduce cost burden. Only the most promising experimental lines of inquiry can be followed and experimental dead – ends can be avoided early based on the results of CADD simulations. Time – to - Market. The predictive power of CADD can help drug research programs choose only the most promising drug candidates. By focusing drug research on specific lead candidates, biopharmaceutical companies can get drugs to market more quickly. One of the non -quantifiable benefits of CADD and the use of Bioinformatics tools is the deep insight that researchers acquire about drug – receptor interactions. When we show researchers new molecular models of their putative drug compounds, their protein targets and how the two bind together, they often come up with new ideas on how to modify the drug compounds for improved fit [4].

The present study of " Target identification and validation for diabetic nephropathy using molecular docking studies " requires Bioinformatics and CADD techniques. Here we used the different computer aided softwares to achieve the appropriate design of the new drug by modifying the selected drug for a particular disease. We used the Hyperchem software for energy calculations of the ligands , GOLD software for docking and somemore softwares used based on their priority. Later, we analysed the protein using different databases. Drugs like lisinopril ($C_{21}H_{31}N_3O_5$) selected for Diabetic Nephropathy . Our aim is trying to increase the binding affinity of the designing drugs using free energy calculations, because binding affinity is directly proportional to effect of the drug.

Protein View



STRUCTURE OF LISINOPRIL



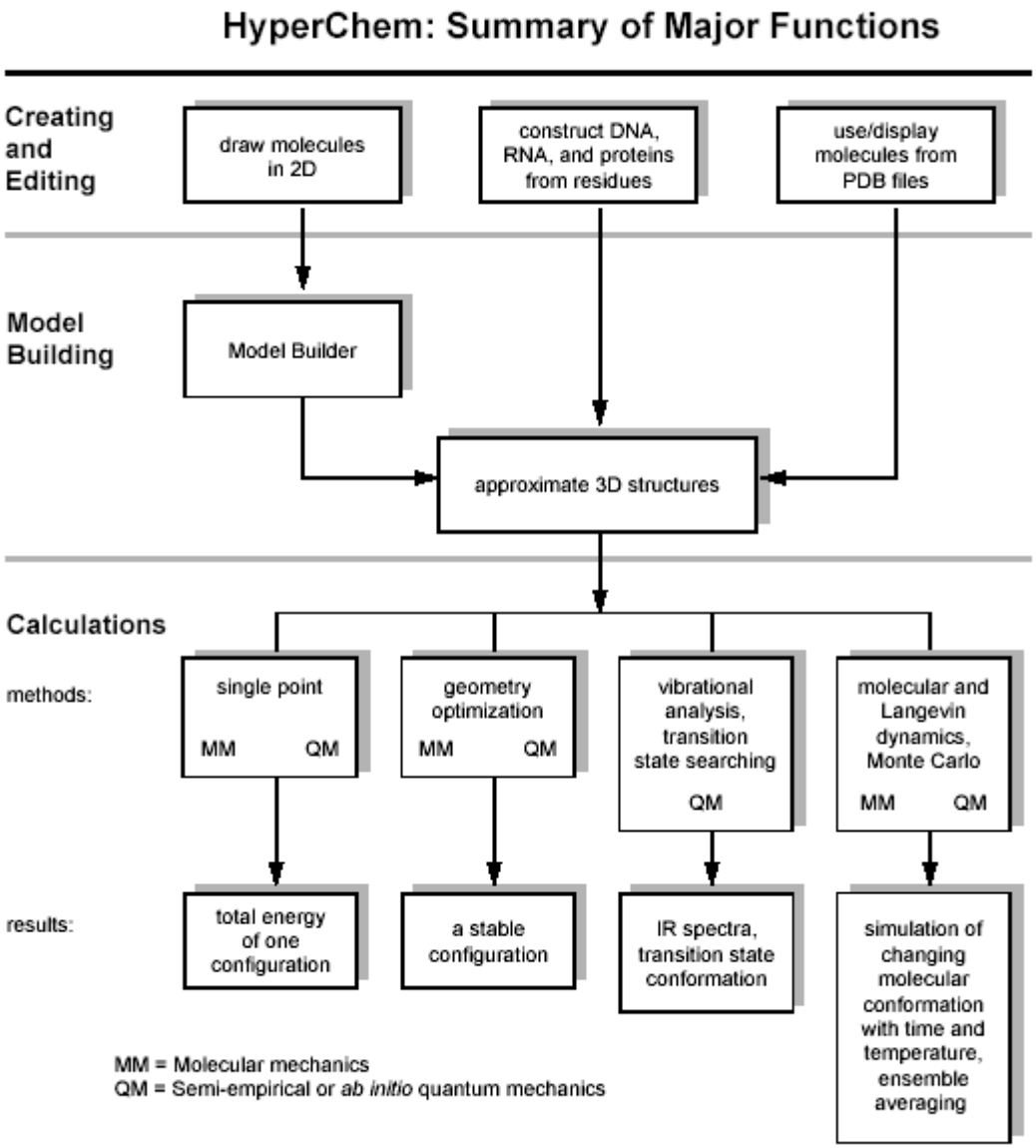
Plan of Work

- Energy Calculations of Ligand in Air by Single Point, Geometry Optimisation, Molecular Dynamics, Monte Carlo
- Energy Calculations of Ligand with different replaced groups
- Energy Calculations of Ligands (Solvent Intra)
- Energy Calculations of Ligands (Protein Intra)
- Docking
- Free Energy Calculations for more effective drug
- Protein Analysis by different Databases

MATERIALS AND METHODS

Softwares used

- HYPERCHEM
- GOLD
- SPDBV
- CHEM OFFICE



HyperChem

HyperChem is a *versatile molecular modeler and editor* and a powerful *computational package*. It offers many types of molecular and quantum mechanics calculations. For optimization of small molecules in solution and protein complex the intramolecular energies of ligand. Solvent and ligand protein will be calculated using molecular mechanics calculations of HyperChem software.

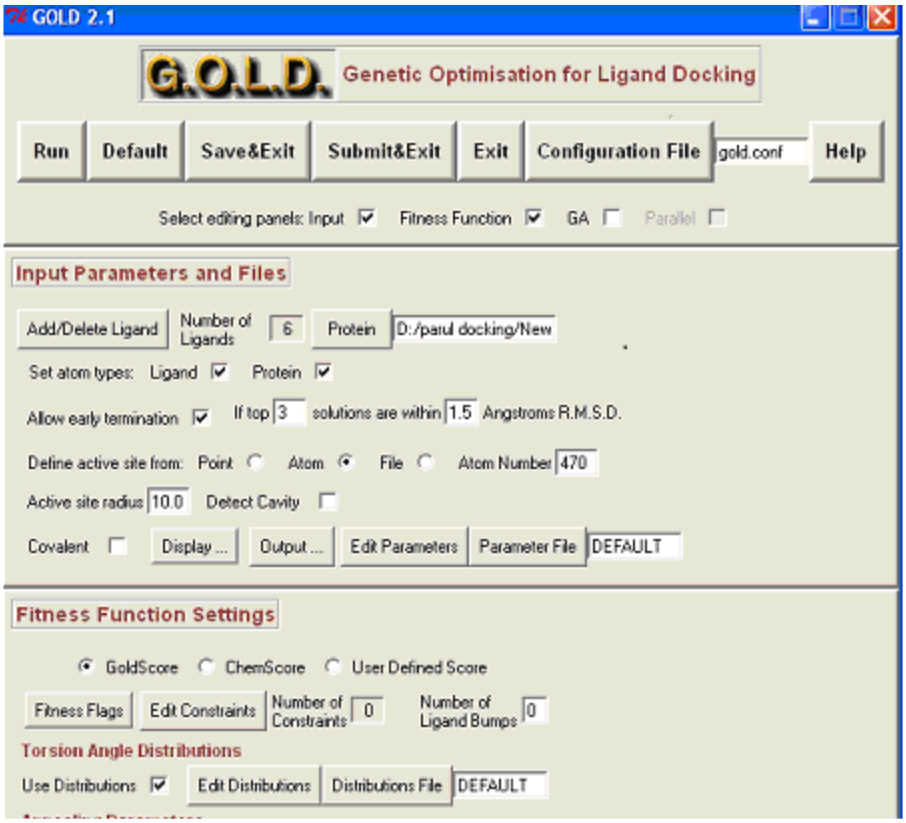
HyperChem includes these functions

- Drawing molecules from atoms and converting them to three dimensional (3D) Models
- Constructing proteins and nucleic acids from standard residues
- Using molecules from other sources; for example, Brookhaven Protein Data Bank (PDB) files
- Rearranging molecules by, for example, rotating and translating them
- Changing display conditions, including stereo viewing, rendering models, and structural labels
- Setting up and directing chemical calculations, including molecular dynamics, by various molecular mechanical or *ab initio* or DFT or semi empirical quantum mechanics methods
- Determination of isotope effects in vibrational analysis calculations for semi -empirical and *ab initio* SCF methods
- Graphing the results of chemical calculations
- Solvating molecules in a periodic box [5]

GOLD (Genetic Optimization for Ligand Docking)

Gold uses *genetic algorithm* to provide *docking of flexible ligand and a protein with flexible hydroxyl groups*. Otherwise the protein is considered to be *rigid*. This makes it a good choice when the binding pocket contains amino acids that form hydrogen bonds with the ligand.

GOLD offers a choice of scoring functions: Gold Score, Chem Score and User Defined Score. The solutions are known to have *70-80% accuracy* when tested on complexes extracted from PDB. GOLD will only produce reliable results, if it is used properly and correct atom typing for both protein and ligand is particularly important. We work with *GOLD version 2.1* [6]



Gold is a program for calculating the docking modes of small molecules into protein binding sites. The product of collaboration between the University of Sheffield, Glaxosmith klineplc and CCDC ,

GOLD is very highly regarded with in the molecular modeling communities for its accuracy and reliability.

Ligand – protein interactions (Inter – Protein) (Docking)
For docking of small molecules into the protein active site, the VDW, hydrogen bonds and hydrophobic energies of ligand – protein interaction will be calculated using GA of Gold software [7]

SPDBV (SWISS PROTEIN DATA BANK VIEWER)
To see and identify the protein report and active sites of protein for docking.

METHODOLOGY
COMPUTER AIDED DRUG DESIGN APPROACHES
Computational assessment of the binding affinity of enzyme or receptor (protein) inhibitors prior to synthesis is an important component of computer - aided drug design (CADD) paradigms. In this study , the molecular mechanics (MM) method is used for the estimation of relative binding affinities of inhibitors to an enzyme or receptor. calculating the following energy variables:

$$E_{bind}\ (intra) = E_{com}\ (intra) - E_{sol}\ (intra) \quad \ldots\ldots\ldots\ldots\ (1)$$
$$E_{bind}\ (inter) = E_{com}\ (inter) - E_{sol}\ (inter) \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, E_{bind} (intra) and E_{bind} (inter) are relative intra and intermolecular binding interaction energies of a ligand, respectively, and where E_{com} (intra), E_{com} (inter), E_{sol} (intra), and E_{sol} (inter) are intra and intermolecular interaction energies of a ligand in the complexed and solvated states, respectively. Relative differences in intra, intermolecular and total binding interaction energies for a pair of ligands L1 and L2 are given by,

$$E_{bind}\ (intra: L1, L2) = E_{bind}\ (intra: L2) - E_{bind}\ (intra: L1) \ldots\ldots\ldots\ldots\ (3)$$
$$E_{bind}\ (inter: L1, L2) = E_{bind}\ (inter: L2) - E_{bind}\ (inter: L1) \quad \ldots\ldots\ldots\ldots\ (4)$$
$$E_{bind}\ (tot: L1, L2) = E_{bind}\ (intra: L1 - L2) + E_{bind}\ (inter: L1 - L2) \ldots\ldots\ldots (5)$$

Where, E_{bind} (tot: L1 L2) is the total relative difference in the binding energies of L1 and L2. Hence, an agreement in the overall trends between the experimental measurements and the energy minimization results were expected. In the Table 2, the relative differences in the binding affinities measured experimentally (E_{bind} (expt)) are compared with the relative binding affinities calculated using minimization methods and for all the cases the minimizations results provided qualitative agreement with experimental results. Energy components calculated by performing molecular mechanics calculations both in explicit solvent and complex states are sufficient to estimate the binding free energy differences between two inhibitors qualitatively.

These qualitative methods will continue to improve and become more accurate as;
1) force field parameters become more refined,
2) Other variables important for binding such as entropy are included,
3) Methods for estimating relative binding entropy changes improve,
4) Docking and scoring procedures improve, and
5) Average molecular dynamics simulations are used to obtain energy variables.

These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities of enzyme inhibitors for more quantitative analysis of the most promising candidates.

Lead generation
The following three methods are often used for discovery of lead compound.

1.DE - NOVO drug design methods
De novo drug design requires the 3-dimensional structure of the target protein. A few successes are reported but overall de novo design represents a goal and not a reality. De novo molecular design methods have been used to design new structures by sequentially adding molecular fragments to a growing structure, by adding functionality to an appropriately – sized molecular scaffold, or by adding fragments building toward the center of a molecule starting from distant sites thought to interact with the target (Van Drie *et al*, 1997, Hahn *et al*, 1997). These approaches can be used for generating diverse molecular structures [8]

2. Database searches

In some cases, new lead compounds have been identified by screening structures found in databases of known (Bohm et al, 1995, Westhead *et al*, 1995) commercial as well as proprietary chemical databases for particular structural features using three dimensional structure of a target protein with known active site. In addition, database search methods have been developed that search databases for compounds that have particular molecular functionality separated by physicochemical properties, including solvent interactions and a specified number of bonds or distance ranges. More chemically intuitive database search methods search for chemicals with particular steric and electrostatic fields (Thorner et *al*, 1997).

3. Combinatorial methods

This method doesn't require target protein structure, which is the main requirement for other two methods. Combinatorial chemistry helps to create a large library of structures with a great deal of diversity. A growing number of drug leads are being generated by combinatorial methods in combination with high - throughput screening (Agrafiotis, et al, 1997, Varr, et al, 1997) [9]

Optimization of lead compounds

Optimization of lead compounds is often a step - wise process using computational methods in combination with SAR information to determine areas on the molecule to expand, contract, or modify. Accordingly, the challenge is, to prioritize a large diverse set of molecules to a small set of compounds that have the highest likelihood to bind. Methods that rapidly and accurately predict absolute binding affinities represent the long - term goals. Currently, the methods range from being able to provide qualitative rank ordering of a large number of molecules in a relatively short period of time (Holloway, *et al*., 1999) that generate quantitatively accurate predictions of relative binding affinities for structurally related molecules (Merz, Erion, Reddy, 1989, 2000, 2001).

A large percentage of the proposed analogs can usually be eliminated by evaluating their expected binding affinities based on docking (Kurtz, 1994, Bohacek, 1992) graphical analysis, desolvation costs and conformational analysis. The remaining analogs are prioritized using one or all of the following methods, depending on the availability of computational power, time and resources: i) Free Energy Perturbation (FEP) calculations, which provide accurate predictions, but are computationally very expensive (Erion, 1997, Reddy, 2000, Van Drie, Hahn, 2001), ii) molecular mechanics calculations, which provide rapid qualitative predictions (Holloway, 1995, Viswanadhan, 1996), and iii) regression methods (Holloway, 1995) that incorporate interaction variables and ligand properties, which provide semi – quantitative predictions and are much faster than FEP calculations. The top scoring compounds are synthesized and tested for activity. The process is repeated in an interactive fashion until potential drug candidates are identified with the desired biological activity.

Computational details

All molecular mechanics calculations were carried out with the HyperChem program using an all atom force field (Weiner *et al.,* 1984 & Singh *et al.,* 1986) and the SPC/E model potential (Berendsen *et al.,* 1987, Reddy *et al.,* 1989) to describe water interactions. Electrostatic charges and parameters for the standard residues were taken from the Hyperchem database. For non – standard solute atoms, partial charges were obtained by fitting wave functions calculated with Gaussian 94 (*Frisch et al., 1994*) *ab initio* 6-31 G* basis set level with CHELP (Chirlian *et al.,* 1987). All equilibrium bond lengths, bond angles, and dihedral angles for non - standard residues were taken from *ab initio* optimized geometries. Missing force field parameters were estimated from similar chemical species within the Hyperchem database. Molecular mechanics calculations (energy minimizations) on all the structures were also performed using the Hyperchem program [10].

RESULTS

LISINOPRIL

DRUG NAME	**:-**	LISINOPRIL
CHEMICAL FORMULA	**:-**	$C_{21}H_{31}N_3O_5$
MOLECULAR WEIGHT	:-	405.48
IUPAC NAME	**:-**	(2S)-1-[(2S)- 6 amino-2[((2s)-1-hydroxy-1-oxo-4-phenyl butan-2-yl]amino]hexanoyl]pyrrolidine-2-carboxylic acid
UNITS	**:-**	ENERGY –Kcal/mol
GRADIENT	**:-**	Kcal/(mol-A^0)
EXPERIMENTAL PDB ID	**:-**	1UZF

STEP 6 OF ALL MOLECULES

TABLE 1:- SOLVENT (INTRA)

MOLECULE	INTRA ENERGY(x_2)
R=Cl	-23.16
R=CH3	-90.90
R=F	-23.86
R=NH2	-23.74
R=H	-23.97
R=CH2CH3	-91.78

TABLE 2:- ENERGY OF LIGAND IN AIR (X_1)

MOLECULE	Energy in Air (x_1)
R=Cl	22.63
R=CH3	90.31
R=F	23.97
R=NH2	23.74
R=H	24.09
R=CH2CH3	23.34

TABLE 3:- PROTEIN (INTRA)

MOLECULE	INTRA ENERGY(y_1)
R=Cl	16.09
R=CH3	83.97
R=F	16.50
R=NH2	16.63
R=H	16.67
R=CH2CH3	16.46

TABLE 4:- DOCKING (INTER)

MOLECULE	DOCKING(y_2)
R=Cl	-44.92
R=CH3	-41.10
R=F	-40.37
R=NH2	-41.10
R=H	-43.93
R=CH2CH3	-42.74

TABLE 5:-

MOLECULE	SOLVENT($X=x_1+x_2$)	PROTEIN($Y=y_1+y_2$)
R=Cl	-0.53	-28.83
R=CH3	-0.59	42.87
R=F	0.104	-23.87
R=NH2	0.00	-24.47
R=H	0.12	-27.26
R=CH2CH3	-68.44	-26.28

TABLE 6:- BINDING FREE ENERGY CHANGES

S.NO	*MOLECULES*	*Z-VALUES(Y-X)*	*E BIND(Z_2-Z_1)*
1	R=Cl	*-28.30 (Z_2)*	*-3.65*
2	R=CH3	*43.46 (Z_3)*	*68.11*
3	R=F	*-23.97 (Z_4)*	*0.67*
4	R=NH2	*-24.47 (Z_5)*	*0.18*
5	R=H	*-27.38 (Z_6)*	*-2.73*
6	R=CH2CH3	*42.16 (Z_7)*	*66.81*
7	$C_{21}H_{31}N_3O_5$(Standard)	*-24.65(Z_1)*	*0.00*

Docking(y_2) :

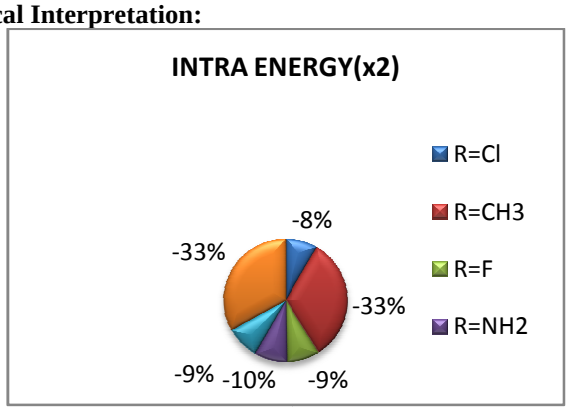
Fitness (y_2) = S (hb – ext) + 1.3750 × S (vdw - ext) + S (hb - int) + 1.0000 × S (vdw - int)

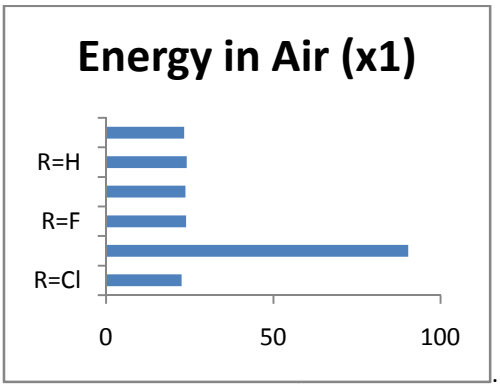
(Solvent) $X = x_1 + x_2$ (Protein) $Y = y_1 + y_2$

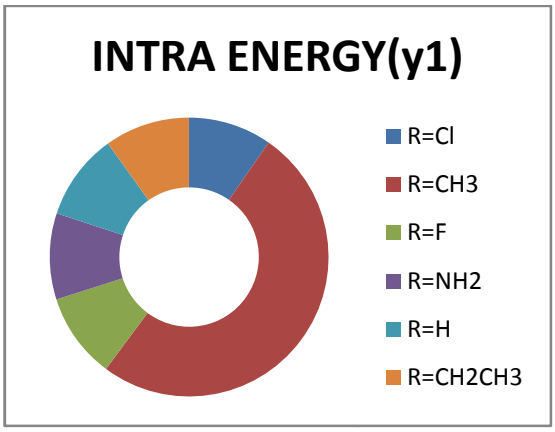
Binding free enrgy $Z = Y - X$

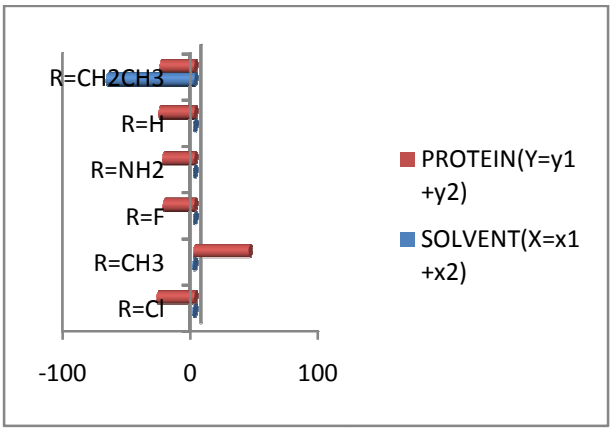
E bind $= Z_2 - Z_1$

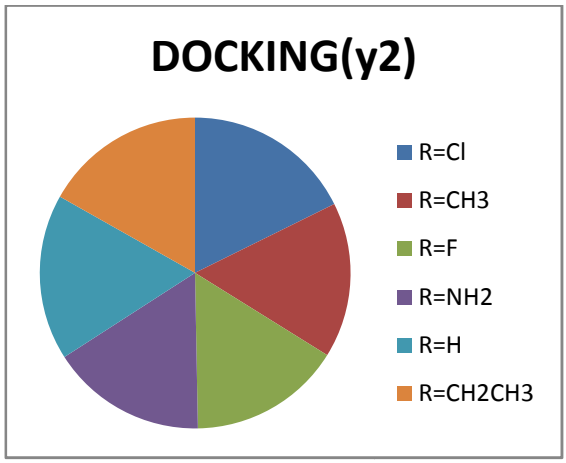
Statistical Interpretation:

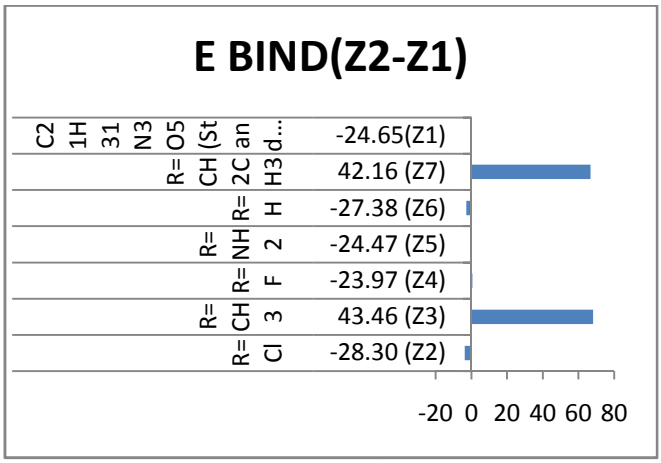






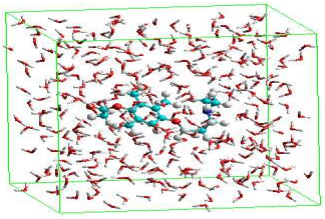




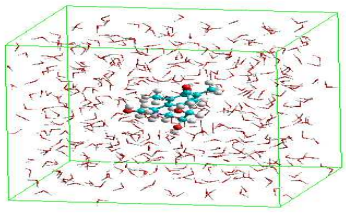


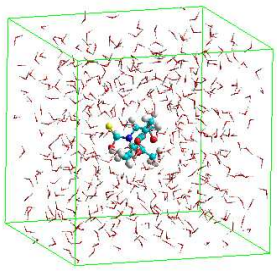
Solvent Interactions:

Molecule 1

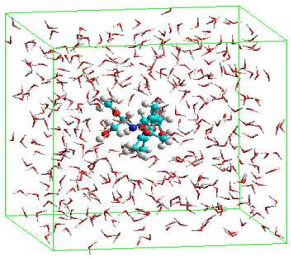


Molecule 2

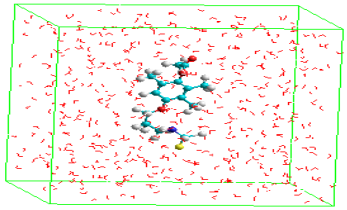




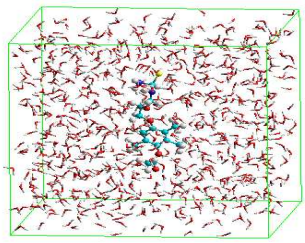
Molecule 3



Molecule 4

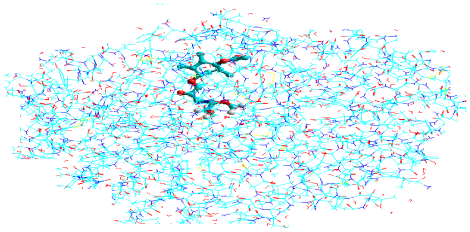


Molecule 5

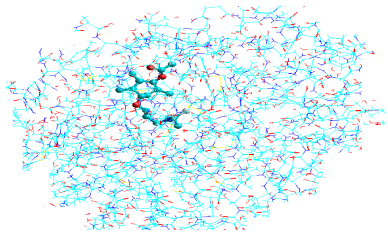


Molecule 6

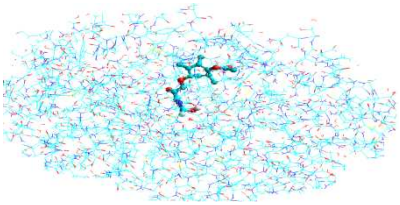
Protein- Ligand Interactions:



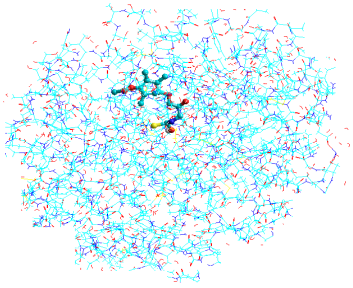
Molecule 1



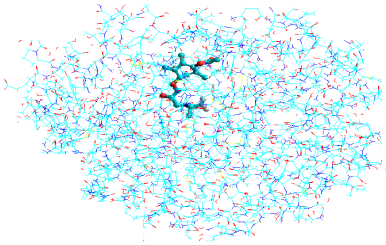
Molecule 2



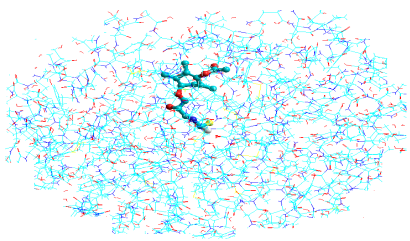
Molecule 3



Molecule 4



Molecule 5



Molecule 6

DISCUSSION

Diabetes is one of the world's fastest growing metabolic disorders. While the knowledge of the heterogeneity of this disease increase , so also is the need for more appropriate therapies increases. Moreover diabetic nephropathy is a

major cause of morbidity and mortality , affecting 35% of insulin dependent and 3 - 17% of non insulin dependent diabetes mellitus patients . For the treatment of diabetic nephropathy there is need of more than one drugs,hypoglycemic agent for maintaining blood glucose level normal and ACEI for prevention of renal damage and control of blood pressure.Drug designing, one of the hottest topics have found its new pathway to create a history in the field of medical science. The lead compound analysis starts with CADD, assisting to identify and to optimize the right compound. The technique helps in generating a suitable compound specific to the disease ; thereby an effective treatment is achieved. Molecular modeling method has been used for modeling a new molecule for DIABETIC NEPHROPATHY using Lisinopril , a drug that's already designed. This drug is drawn using hyperchem, and its R group is modified by replacing different functional groups like Cl,CH_3,F,NH_2,H,CH_2CH_3 in its place and docked by using gold software. The molecules designed as such are optimized using different algorithms and their affinity is checked with protein. The binding free energy of the protein is calculated by performing docking process. The molecule with minimum binding energy will have the maximum binding affinity. The binding free energy is calculated by the formula Z = Sum of the energy of optimized ligand devoid of solvation parameters and the energy of the protein - ligand optimization. The binding free energy of the designed molecules is obtained by eliminating the energy of the main molecule i.e.Lisinopril .From the results obtained it's clear that ligand 1 & 5 for DIABETIC NEPHROPATHY have the maximum binding affinity. So these molecules are determined as the best lead molecules targeting computationally. We can findout the drug binding affinity by using fitness of the drug , which can bind to target protein during the docking process and second way is using Gibbs free energy calculations.According to this more negative value, we can consider as more effective drug. Here the following replacement groups for Diabetic nephropathy such as Cl & H found to be -3.65 & -2.73. So we can predict the above mentioned replaced groups found to be more effective than standard drug.

CONCLUSION

Calculations of binding affinities, binding free energies changes for structurally similar Inhibitors to LISINOPRIL indicates that the molecular mechanics methods gave suitable analogues. These results clearly indicate that before synthesis and biochemical testing of new analogs, one can use molecular mechanics based methods for qualitative assessment of relative binding affinities for speeding up drug discovery process by eliminating less potent compounds from synthesis. The Lisinopril inhibitors 1 & 5 with the substituent's R=Cl & R=H are identified as the most suitable analogues in the present study need to be further evaluated in laboratory.

REFERENCES

[1] Http://en.wikipedia.org/wiki/Diabetic_nephropathy.
[2] Http://www.drugbank.ca/drugs/DB00722.
[3] Http://en.wikipedia.org/wiki/Bioinformatics.
[4] Http://en.wikipedia.org/wiki/Computer-aided_design.
[5] Hyperchem software 7.5 version.
[6] GOLD software 2.1 version.
[7] Fischer. E., (**1894**),"Einfluss der Configuration auf die Wirkung derEnzyme.Ber.Dtsch.Chem.Ges" vol.27, pp.2985.
[8] Http://en.wikipedia.org/wiki/Drug_design.
[9] Http : // citeseerx. ist. psu. edu / viewdoc / summary ? doi = 10.1.1.90.2169.
[10] Http : // towhee. sourceforge. net / forcefields / weiner **1984**. html.