



Research Article

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Molecular level targeting of hepatic glucokinase by computational docking approach in the treatment of type 2 diabetes

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ABSTRACT

Diabetes is a metabolic disorder characterized by high blood sugar due to insufficient insulin or the cells do not respond to the insulin produced. There are several causes, out of which Glucokinase has a major impact in the development of the disease and the gene responsible for the synthesis of this protein is GCK. In normal conditions Glucokinase helps in the conversion of glucose to glucose-6-phosphate. In β -cells, insulin secretion results from the co-ordinated activity of K^+ -ATP channels and voltage-gated Ca^{2+} -channels. They are "fine-tuned" by the glycolytic flux, which is dependent on the plasma glucose concentration and glucokinase activity. In this view, glucokinase plays the preeminent part, serving as a glucose sensor, a veritable "glucose receptor" in β -cells, because the rate of conversion of glucose to G6P is the rate-limiting step in glucose-stimulated insulin secretion, regulated in a concentration-dependent manner and triggered at a threshold of 5 mM. The present work deals with the designing a suitable drug by molecular docking which acts on the glucokinase receptor to regulate the secretion of insulin. The glucokinase receptor selected was 1V4S which was minimized and evaluated with the help of various softwares and databases and a suitable drug was designed which acts on the receptor, taking metformin as a template for the designing of the library for the compound. Out of the various compounds designed the best molecule which was obtained was found to be *N*-(*N,N* dimethylcarbamiimidoyl) carbamiimidic acid.

Keywords: Diabetes, Glucokinase, GCK, Docking, 1V4S.

INTRODUCTION

The major cause for type-2 diabetes is either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced [1,2]. After eating, the pancreas automatically releases an adequate quantity of insulin to move the glucose in our blood into the cells, and lowers the blood glucose levels. Glucokinase (EC 2.7.1.2) is an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate. This enzyme is encoded by a gene GCK which is located at 7p13 allele of chromosome 20 [4-6]. Mutations of the gene for this enzyme can cause unusual forms of diabetes or hypoglycemia. Glucokinase (GK) is a hexokinase isozyme, related homologously and by evolution to at least three other hexokinases [7-8]. All of the hexokinases can mediate phosphorylation of glucose to glucose-6-phosphate (G6P), which is the first step of both glycogen synthesis and glycolysis. However, glucokinase is coded by a separate gene and its distinctive kinetic properties allow it to serve a different set of functions. Glucokinase has a lower affinity for glucose than the other hexokinases do, and its activity is

localized to a few cell types, leaving the other three hexokinases as more important preparers of glucose for glycolysis and glycogen synthesis for most tissues and organs. Because of this reduced affinity, the activity of glucokinase, under usual physiological conditions, varies substantially according to the concentration of glucose [9-13]. In β -cells, insulin secretion results from the coordinated activity of K-ATP channels and voltage-gated Ca^{2+} -channels. They are "fine-tuned" by the glycolytic flux, which is dependent on the plasma glucose concentration and glucokinase activity [3,14-16]. Any alterations in the GCK gene results in the abnormal production of insulin which results in the development of diabetes. So by targeting the GCK gene we can develop a potent drug for the treatment of diabetes.

EXPERIMENTAL SECTION

TARGET IDENTIFICATION

There may be several targets for a particular disease but the selection of an individual target is of utmost important. Target identification extract useful knowledge from the raw data and help to focus on the relevant items of data. Knowledge on the three-dimensional structure (fold) of a protein provides clues on its function and aids in the search for inhibitors and other drugs. The target selected was GCK gene whose protein sequence and relevant data are validated by using several computational tools like NCBI, UniProtKB, GeneCards, KEGG etc.

CHEMICAL LIBRARY

A chemical library or compound library is a collection of stored chemicals usually used ultimately in high throughput screening. The chemical library can consist in simple terms of a series of stored chemicals. Each chemical has associated information and its physiochemical properties with information such as the chemical structure, molecular formula, weight, logP, hydrogen bond donor, hydrogen bond acceptor, characteristics of the compound etc. For this library of screening Accelrys Discovery Studio, ChemSpider, PubChem, ChemBank, etc. databases were used. There are millions of compounds available in these databases. Through the help of these tools we can find a new compound against a GCK gene and tested for their ability to modify/inhibit the target protein. In compound screening the major part to test that compound is having druglikeness or must passed ADME properties. We have used Accelrys Discovery Studio for the present work.

LEAD OPTIMIZATION

There are many tools available for designing of lead/drug such as Discovery Studio, HyperChem, ChemDraw, ChemSketch, etc. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. Activity is generally dosage-dependent and it is not uncommon to have effects ranging from beneficial to adverse for one substance when going from low to high doses. Activity depends critically on fulfillment of the ADME criteria. To be an effective drug, a compound not only must be active against a target, but also possess the appropriate ADME (Absorption, Distribution, Metabolism, and Excretion) properties necessary to make it suitable for use as a drug. The drug must possess the TOPKAT parameter for its novel properties. TOPKAT is nothing but the properties prediction of that drug. The properties such as molecule's bioavailability, it is carcinogenic or not, lethal dose (LD50), value of developmental toxicity prediction etc. The all values are calculated by protocols of Discovery studio. The lead taken here was **METFORMIN** whose skeleton was taken as the basic nucleus.

Molecular simulation and docking

High-throughput screening (HTS) of compound libraries is used to discover novel leads for drug development. When a structure is available for the target, computer-based screening using molecular docking may also be considered. Molecular docking is to compute simulation procedure to predict the conformation of a receptor-ligand complex, where the receptor is usually a target protein and the ligand is either a small designed molecule. It can also be defined as a simulation process where a ligand position is estimated in a predicted or pre-defined binding site. Molecular docking simulations may be used for reproducing experimental data through docking validation algorithms, where protein-ligand conformations are obtained in-silico and compared to structures obtained from X-ray crystallography or nuclear magnetic resonance. Furthermore, docking is one of main tools for virtual screening procedures, where a library of several compounds is "docked" against one drug target and returns the best hit. Before docking study, we

need to minimize the energy of both molecule (ligand) and receptor (target molecule). All these studies were carried out through Discovery studio. With the help of this tool we can see the proper intermolecular bonds between ligand-receptor complexes. There were three intermolecular hydrogen bonds seen in the complex of receptor and screened molecule.

RESULTS

From the designed library of molecule very few candidates screened out from the ADME and TOPKAT parameter. The best candidate molecule has been selected for further analysis. By using molecular simulation and docking technique the best drug candidate were identified which is satisfied the all rules and possess the inhibitor property. The inhibitor shows the highest binding affinity towards the receptor cavity is chosen for the best drug candidate molecule among synthesized library. The drug **N'(N,N dimethylcarbamimidoyl) carbamimidic acid** was the best of all the compounds from the library of the compounds whose chemical structure, TOPKAT and ADME Parameters are shown below.

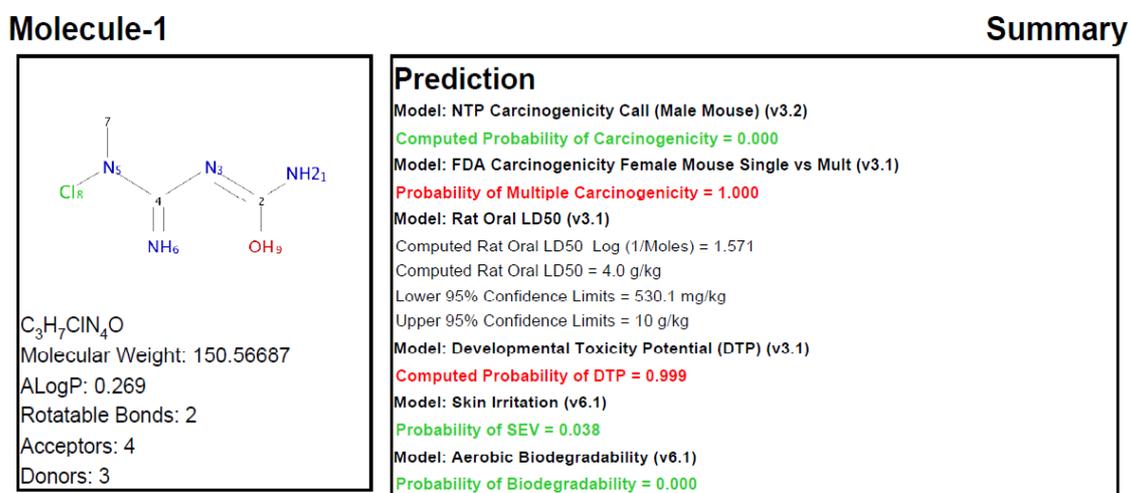


Fig 1: Chemical structure, Molecular properties with TOPKAT results showing carcinogenicity, biodegradability, Rat oral LD₅₀ and skin irritation properties of designed drug molecule of N'(N,N dimethylcarbamimidoyl) carbamimidic acid.

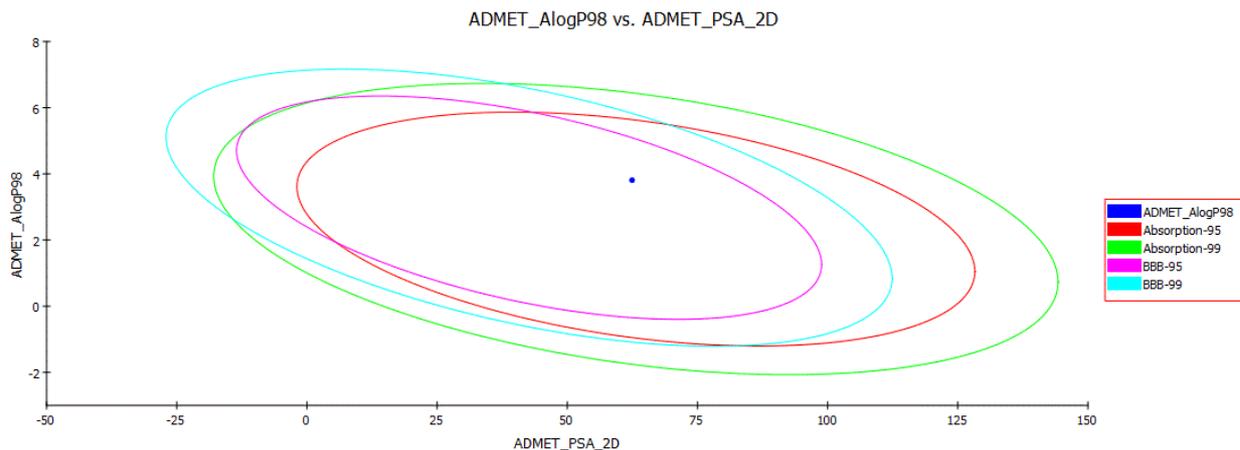


Fig 2: Graphical representation of ADMET absorption.

The following table shows a list of designed compounds showing their absorption and hepatotoxicity results and the compound 4 is N'(N,N dimethylcarbamimidoyl) carbamimidic acid which has good absorption and very low hepatotoxicity as shown in the results, the compounds with zero values are good.

Compound Name	BBB Level	Absorption Level	Solubility	Solubility Level	Hepatotoxicity	Hepatotoxicity Level
Cmp1	3	0	-0.323	4	1	0.9
Cmp2	3	0	-1.176	4	1	0.973
Cmp3	3	0	-3.807	3	1	0.94
Cmp4	3	0	-0.383	4	0	0.420
Cmp5	3	0	-1.41	4	1	0.88
Cmp6	3	0	-1.372	4	1	0.615

Table 1: List of designed compounds showing their absorption and hepatotoxicity.

Docking Results:

This above drug is minimized and is docked with the active minimized site of the receptor molecule whose Pdb id is 1V4S and the dock score was found to be and the docking was found between the **hydrogen 16 of the molecule with ALALINE 114 Oxygen molecule and hydrogen 16 of the molecule with THERONINE 118 Oxygen molecule.**

Compound Name	C Docker Energy	C Docker Interaction Energy	Pose No
Molecule-4	-469.099	-148.836	1
Molecule-4	-469.794	-148.558	2
Molecule-4	-478.414	-150.445	3
Molecule-4	-489.199	-149.499	4
Molecule-4	-494.313	-149.815	5
Molecule-4	-535.869	-150.302	6
Molecule-4	-541.109	-149.837	7
Molecule-4	-544.947	-135.686	8
Molecule-4	-545.33	-135.565	9
Molecule-4	-547.535	-113.561	10

Table 2: The docking results with their C-Docker interaction energy to active site of target receptor

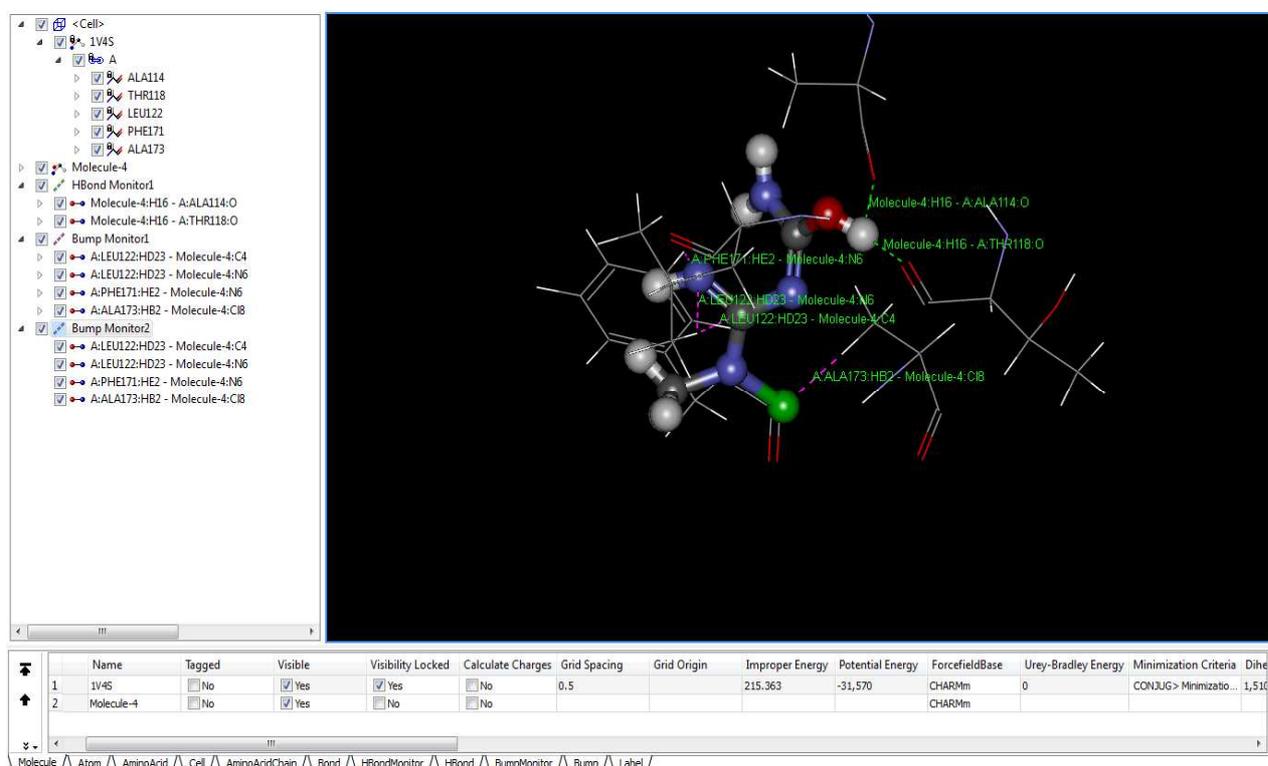


Fig 3: Binding orientation of designed compound N'(N,N dimethylcarbamimidoyl) carbamimidic acid with the target associated protein 1V4S.

PHARMACOPHORE:

The docked compound with binding pocket of receptor can be easily visualized on three feature of pharmacophore model. The hydrophobic region feature (blue), hydrogen bond acceptor feature (red) and green shows the bond.

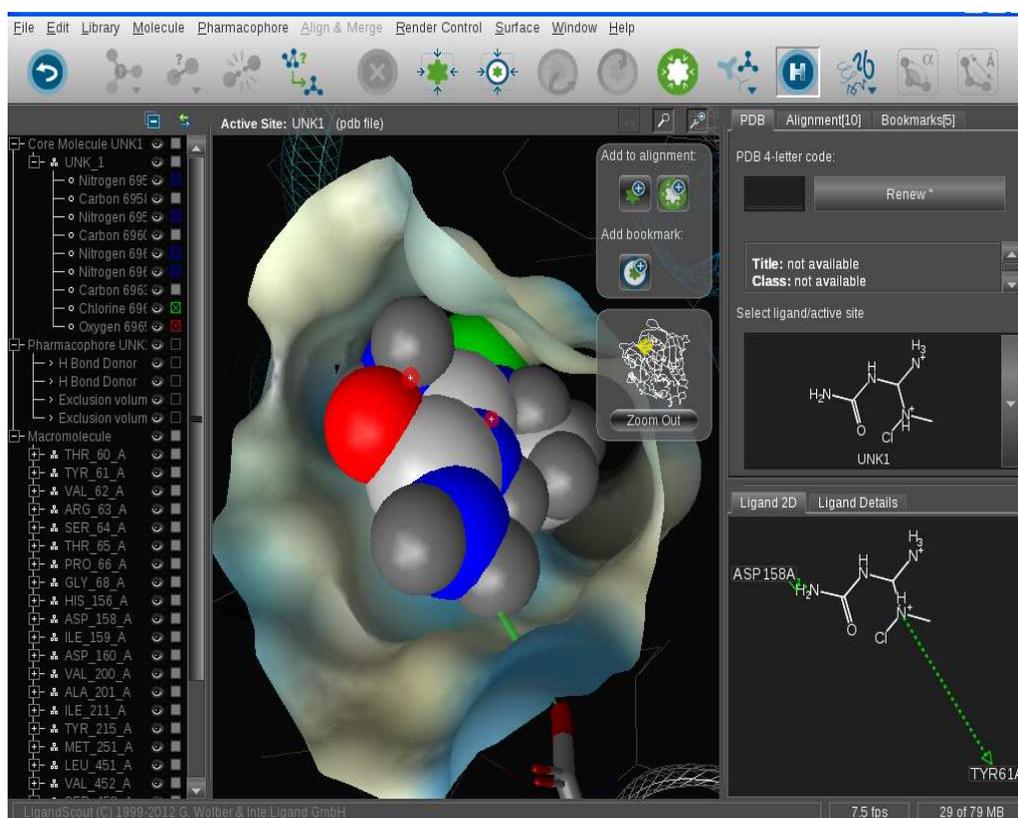


Fig 4: The designed compound with binding pocket of target receptor.

DISCUSSION

The interactions between designed potent inhibitor and receptor were studied by using various computational methods. Based on binding energy, and hydrogen bond formed, docking results were analyzed to find out the best ligand which was **N'(N,N dimethylcarbamidoyl) carbamimidic acid** has high values to potentiate the target among all ligands. Thus the *in-silico* method adopted in the present study helped in identifying the ligands using the commercial software and online tools for the treatment of diabetes. This method reduces the time and cost in designing a drug as well as in analyzing the drug likeliness before it enters the clinical trials. The further studies were carried out by preclinical trials.

CONCLUSION

The developed drug **N'(N,N dimethylcarbamidoyl) carbamimidic acid** was found to have all characteristics and which can act on GCK gene and potentiate the secretion of insulin and thus be an effective drug for the treatment of diabetes.

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