MOLECULAR LEVEL DOCKING OF HEPATOCYTE NUCLEAR FACTOR (HNF)-1 ALPHA IS ASSOCIATED WITH MATURITY-ONSET DIABETES OF THE YOUNG (MODY) 3

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ABSTRACT
Heterozygous germline mutation of hepatocyte nuclear factor (HNF)1 alpha are associated with mature onset of diabetes of the young (MODY) 3 in recently gene also reported liver adenoma it characterized by the presence of numerous adenoma within normal hepatic parenchyma. Transcription factor is important for cell differentiation in pancreatic beta cells that impair the cell mass and function which lead to rises of blood glucose level in young’s. By treating these condition the cell mass and function which lead to rises of blood glucose level in young’s. The present work deals designing a suitable drug with molecular target to perform docking which is act on HNF1alpha receptor to control the differentiation of pancreatic beta cells regulate insulin secretion for controlling blood glucose level. By using different tools, data bases minimizing the targeted molecule and design a compound library. Out of the various compounds designed the best molecule which was obtained was found to be 2-amino nanic acid.

KEYWORDS: MODY, molecular docking, HNF-1A, ADMET study.

INTRODUCTION
MODY is a rare form of diabetes which is different from both Type 1 and Type 2 diabetes, it is caused by a mutation (or change) in a single gene. If a parent has this gene mutation, child they has a 50% chance of inheriting it from them. If a child does inherit the mutation, they will generally go on to develop MODY before age 25, whatever their weight, lifestyle, ethnic group etc. There are six different types of MODY caused by changes in six different genes. Treatment varies, depending on the type of MODY. For example, MODY 2 can usually be managed by eating right and exercising regularly. MODY 1, 3, and 4 can usually be managed with a type of medicine called sulfonylurea therapy, MODY 5, 6 often need a variety of treatments because it may cause other medical problems unrelated to the blood sugar level.

MODY 1 is due to a loss-of-function mutation in the HNF4α gene on chromosome 20 that codes for transcription factor 14 (TCF14). HNF4α controls function of HNF1α (see MODY 3) and perhaps HNF1β (MODY 5) as well. This transcription network plays a role in the early development of the pancreas, liver, and intestines. In the pancreas these genes influence the release of insulin, the principal glucose transporter (GLUT2), and several proteins involved in glucose and mitochondrial metabolism. The capacity for insulin production declines Diabetes (persistent hyperglycemia) typically develops by early adult years. Many patients with MODY 1 are treated with sulfonylureas for years before insulin is required.

MODY 2 is due to any of several mutations in the GCK gene on chromosome 7 for glucokinase. They serve as the glucose sensor for the beta cell. Normal glucokinase triggers insulin secretion as the glucose exceeds about 90 mg/dl (5 mM). These loss-of-function mutations result in a glucokinase molecule that is less sensitive or less responsive to rising levels of glucose. The beta cells in MODY 2 unable to secrete insulin, causes high threshold glucose level (e.g., 126-144 mg/dl, or 7-8 mM). This produces chronic, mild hyperglycemia which is usually asymptomatic.

MODY 3 is caused by mutations of the HNF1α gene, a homeobox gene on chromosome 12. This is the most common type of MODY in populations with European ancestry, accounting for about 70% of all cases in Europe. HNF1α is a transcription factor (also known as transcription factor 1, TCF1) that is thought to control a regulatory network (including, among other genes, HNF1α) important for differentiation of beta cells. Mutations of this gene lead to reduced beta cell mass or
impaired function. MODY 1 and MODY 3 diabetes are clinically similar. About 70% of people develop this type of diabetes by age 25 years.

MODY 4 arise from mutations of the IPF1 homeobox gene on chromosome 13. IPF1 is a transcription factor vital to the development of the embryonic pancreas. Even in adults it continues to play a role in the regulation and expression of genes for insulin, GLUT2, glucokinase, and somatostatin. So rare that only a single family has been well-studied. A child born with pancreatic agenesis (absence of the pancreas) was found to be homozygous for IPF1 mutations.

HNF1β-related MODY5 is one of the less common forms of MODY, with some distinctive clinical features, including atrophy of the pancreas and several forms of renal disease. HNF1β, also known as transcription factor 2 (TCF2), is involved in early stages of embryonic development of several organs, including the pancreas, where it contributes to differentiation of pancreatic endocrine Ngn3+ cell progenitors from non-endocrine embryonic duct cells. The gene is on chromosome 17. Diabetes develops due to deficiency of insulin production on beta cells of the pancreas.

MODY 6 arise from mutations of the gene for the transcription factor referred to as neurogenin 1 gene on chromosome 2. NeuroD1 promotes transcription of the insulin gene as well as some genes involved in formation of beta cells and parts of the nervous system. Mutation on gene blocks the differentiation to develop diabetes few required insulin for beta cells of the pancreas.

Experimental section
Target identification
There may be several targets for a particular disease but the selection of an individual target is out most 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 physicochemical 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 Accelyrs 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 drug likeness or must passed ADME properties. We have used Accelyrs 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 (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 chlorpropamide (sulphonyl urea derivative) 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 2-aminonanoic 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 2-aminonanoic acid.

Physicochemical Parameters of Designed Compounds
Table 1: List of designed compounds showing their molecular properties.

<table>
<thead>
<tr>
<th>compound</th>
<th>Alogp</th>
<th>Molecular weight</th>
<th>Num_H_Acceptors</th>
<th>Num_H_Donors</th>
<th>Num_Rotatable Bonds</th>
<th>Num_Rings</th>
<th>Num_Aromatic Rings</th>
<th>Molecular_Fractional Polar Surface Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>-3029</td>
<td>147.172</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>Compound 2</td>
<td>2.058</td>
<td>163.238</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.558</td>
</tr>
<tr>
<td>Compound 3</td>
<td>1.825</td>
<td>165.618</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0.361</td>
</tr>
<tr>
<td>Compound 4</td>
<td>0.561</td>
<td>173.253</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0.311</td>
</tr>
<tr>
<td>Compound 5</td>
<td>-0.356</td>
<td>173253</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0.313</td>
</tr>
</tbody>
</table>

ADMET result
Table 2: List of designed compound showing absorption and hepatotoxicity.

<table>
<thead>
<tr>
<th>Compound name</th>
<th>BBR level</th>
<th>Absorption level</th>
<th>Solubility level</th>
<th>Hepatotoxicity level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound 1</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Compound 2</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
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<tr>
<td>Compound 3</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
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<tr>
<td>Compound 4</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Compound 5</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
Docking result
Energy minimization of receptor molecule

Figure 2: Result of energy minimizing receptor molecule.

INFERENCES
1. Accelry Discovery Studio is used for energy minimization of receptor.
2. Binding site of the receptor is edited i.e., is enclosed by a sphere and charm force field is applied.
3. Conjugate gradient algorithm is used for minimization.

Energy minimization of drug molecule

Figure 3: Result of energy minimized drug molecule.

INFEERENCE
1. The drug qualifies both ADMET and TOPKAT test were minimized using Accelrys Discovery Studio.
2. The drug or ligand molecule was minimized in the same way as the receptor only with the exception there was no sphere enclosing the drug molecule.

Pharmacophore

INFEERENCE
1. 1 hydrophobic residues found – PHE389X
2. 1 hydrogen bond acceptor found – HOH 14A
3. 1 H bond donor found – PRO 389X

DISCUSSION
Mody 3 mainly causes due to HNF1 alpha gene mutation. Identify the gene target based on their structural analysis identify the binding site. Design the compound library by using computational tools, to carry out physicochemical properties and carcinogenic parameters of designed molecules by using different data bases. From that result identify a best molecule among the designed molecule. Activate receptor target site and drug molecule and to perform a docking study drug with targeted molecule. Based on binding energy, hydrogen bonding the best ligand molecule is 2-amino-nanoic acid high potential molecule among the designed molecules. These studies help to identify the ligand by using commercial soft ware and online tools for treating mature on set of diabetes in young ones. This method reduces the time and cost in designing a drug as well as
in analyzing the drug likeness before it enters the clinical trials.

CONCLUSION
The developed drug 2-amino-nanoic acid was found to have all characteristics and which can act on HNF1-alpha gene and potentiate the secretion of insulin by activation of pancreatic beta cells and thus be an effective drug for the treatment of mature onset of diabetes on young’s. Finally conclude that synthesis the compound in wet lab carry out characterization, invivo biological activity on synthesized pure compound.

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