Repurposing and in-vitro evaluation of existing drugs for the management of diabetes

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***Abstract*- Diabetes is one of the largest global public health concerns, imposing a heavy global burden on public health as well as socio-economic development. Diabetes is one of the top 10 causes of death globally. Individuals with diabetes have 2-3 fold risks of all-cause mortality. The global burden of diabetes has increased significantly in recent decades and will continue to soar in the next few decades. Patients suffering from diabetes has to take medications in the form of oral tablets or insulin injection. In case, if a patient suffers from more than one disease such as hypertension, cardiovascular disease, etc. along with diabetes, then the patient has to take multiple drugs for living a normal life. The consumption of multiple drugs on a daily basis causes non-compliance in the patients. To overcome this problems repurposing of drugs is helpful for the management of diabetes mellitus. Many drugs which are used for the treatment of some other disease or disorder, can show its usefulness in the management of diabetes mellitus. In this research work, 24 drugs have been docked with alpha-amylase to form a ligand-protein complex. The docking has been performed to obtain mol dock score which is compared with standard acarbose drug. 2 drugs namely Ketoconazole and Domperidone have been found to be compatible for the purpose of management of diabetes.**

***Keywords*: Molecular docking, repurposing, Ketoconazole, Domperidone, alpha-amylase, mol dock score.**

1. INTRODUCTION

Drug Repurposing can be compared to recycling. It is the application of known drugs for treating. Conditions other than their original use. Drug repurposing offers drugs at a much cheaper, faster and accessible way to the patient population .The drug studied for repurposing are the shelved drugs which either could not made to the late phases of clinical trials or have failed in the market. Since the efficacy, safety and toxicity of the drug is already known, the initial phases of the clinical trials can be skipped which brings down the co stand duration of the clinical trials. It takes about 15years to bring a new drug to the market where are purposed drug is cuts down both the duration (3 to 12 years) and costs. The right the challenges are to identify the right compound for the new purpose, to use the resources judiciously and to not fail again after bringing the failed drugs back into clinical trials.(1)
Docking is a technique which predict the favored orientation of one molecule to a second when certain to each one-of-a -kind to structure a stable complex. docking plays an important role in the rational drug design.(2)

TARGET + LIGAND → DOCKING → TARGET LIGAND COMPLEX

Diabetes is a chronic condition where the body is unable to automatically regulate blood glucose levels, resulting in too much glucose (a sugar) in the blood. The blood glucose level is regulated with the help of insulin, a hormone (or chemical messenger) made in the pancreases. Insulin is the key that glucose needs to enter the body's cells so that it can be used as fuel. Diabetes develops when the pancreas stop producing insulin (Type 1 diabetes) or when the body does not respond properly to insulin (Type 2 diabetes). Insulin injections are necessary to treat type 1 diabetes. Type 2 diabetes can usually be controlled in the first instance by regular exercise and diet. The normal blood glucose level range is between 3.5-7.8 mmol/l.(3)
Alpha-Amylases are the prominent enzymes found in all over various organisms, showing diverse substrate specificities. It catalyzes the breakdown of internal α-1,4-glucan linkages in polysaccharides containing three or more α-1,4-linked D-glucose units, and gives up a combination of maltose and glucose. These enzymes are nothing but the glycoproteins basically with a single polypeptide chain of about 475 residues which consists of two free thiol groups, four disulfide bridges, and a tightly bound Ca2+Along with the diverse distribution in the nature, these enzymes are abundantly used in various industries because of their applications in food, brewing, textile, and detergents etc . In contrast, Inhibition of insect α-amylase is a proposed method of crop protection. On the other hand, inhibition of mammalian α-amylases is a proven therapeutic approach in diabetes and related disorders including obesity.(4)

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Chemicals

All the reagents and solvents which was used in study are of analytical and microbiogical grade and was purchased from Renchem, SDfine, Loba Chem, Merk and Himedia.

DMSO (Dimethyl sulfoxide)
DNS reagent (Di-nitro salicylic acid)

Sodium carbonate
Starch
Acarbose

2.1.2 Equipment and Glaswares

Major and Minor equipments like weighing balance, Hot air oven, Water-bath, Sonicator and UV-Visible Spectrophotometer (Schimadzu 1800) were used. All glassware of Borosil & Asgi was used.

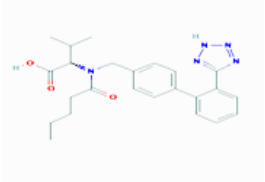
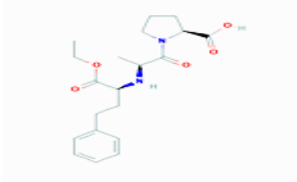
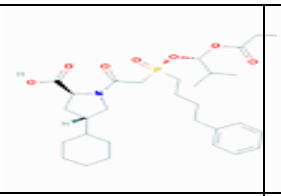
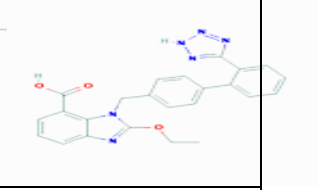
2.1.3 Softwares

- Molegro Virtual Docker 6.0
- Chem Draw

2.2 In-Silico Studies

2.2.1 Ligand Preperation

The initial structure of ligands were drawn on chem Draw Ultra and then converted to 3D orientation using Chem 3D Ultra. After that energy minimized by using MM2 force field, than converted to mole file for docking study. The energy minimized ligand were imported on the surface cavity created protein. The ligand and protein were selected in one worksheet for docking. [5]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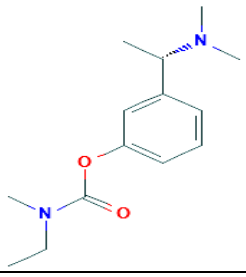	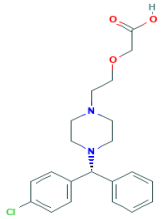	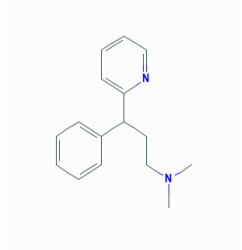	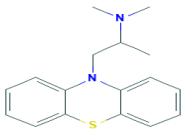
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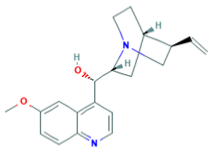	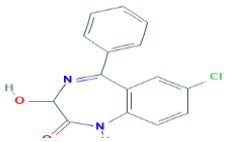	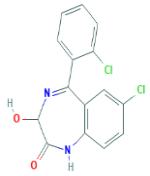	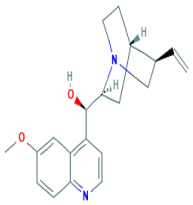
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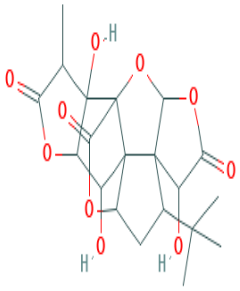
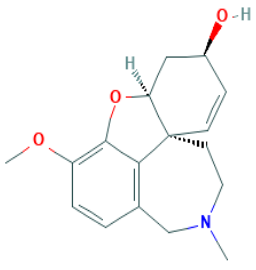
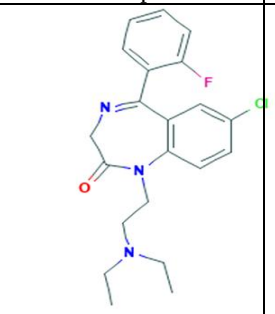
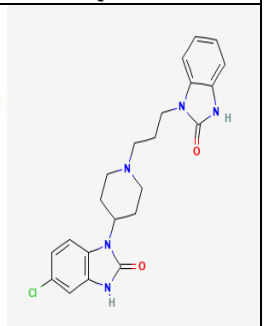
			
Ginkgolide B	Galantamine	Flurazepam	Domperidone

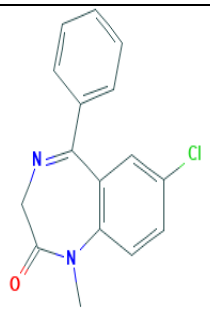
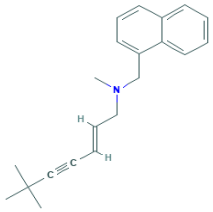
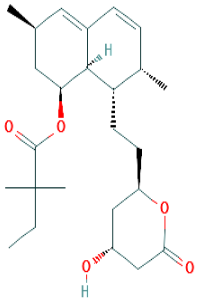
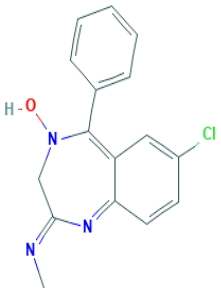
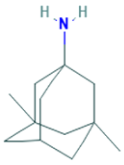
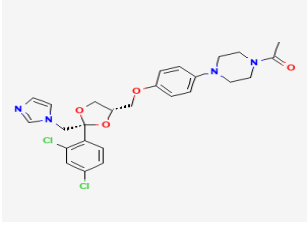
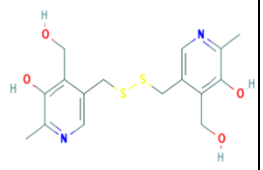
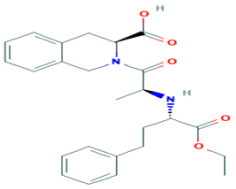
			
Diazepam	Miconazole	Simvastatine	Chlordiazepoxide
			
Memantine	Ketoconazole	Pyritinol	Quinapril

Table 2.1 Structure of Ligand

2.2.2 Enzyme or Protein Preparation

❖ From detail literature review potent enzyme alpha-amylase was selected for molecular docking studies. The enzyme was retrieved from Protein Data Bank. The identical 4GQQ alpha-amylase was selected. The selected enzyme will be used for molecular docking studies with synthetic ligands.

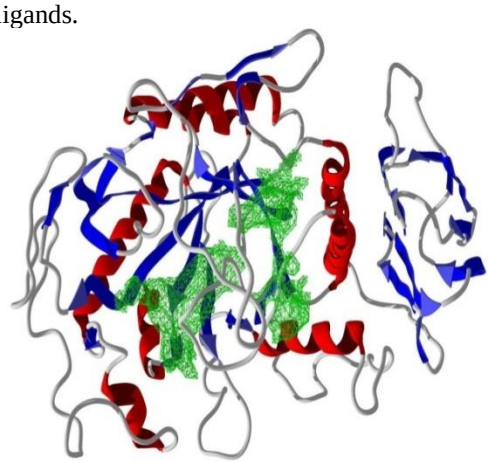


Figure 2.1. The Structures of 4GQQ alpha-amylase Enzyme

❖ The enzymes were then further processed in molegro software for creation of the protein surface. For docking studies electrostatic surface was created as showed in figure 2.2. [6]

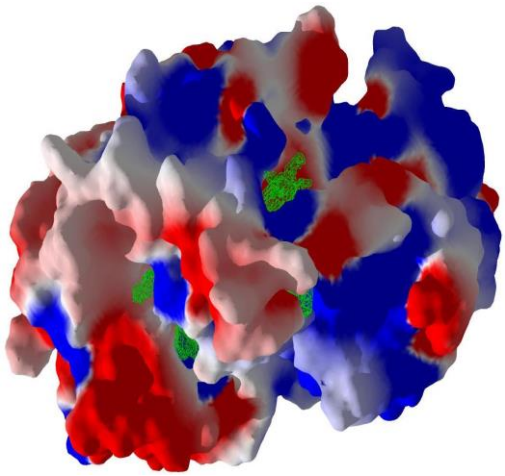


Figure 2.2. Protein Surface of 4GQQ alpha-amylase Enzyme

From the docking the interaction energies were studied to identified the potent ligand bind with the enzymes. The Interaction Energies of ligands are showed in table 2.2.

S.NO.	DRUG	MOL DOCK SCORE	RERANK SCORE	H BOND
1	Enalapril	-36.4261	-42.0937	-0.913304
2	Captopril	-71.2798	-63.5723	-4.55162
3	Fosinopril	-189.194	-146.567	-6.95184
4	Lasinopril	-128.556	-96.8062	-6.92208
5	Quinapril	-124.638	-41.9212	-2.57052
6	Galantamine	-107.635	-33.4983	0
7	Ginkgolide B	926.21	-24.873	-11.7716
8	Memantine	-51.2587	-49.2858	-3.78613
9	Pyritinol	-63.1614	20.4484	-8.43393
10	Rivastigmine	-88.0259	-75.9671	-3.27944
11	Simvastatine	-126.753	-59.6092	-3.24527
12	Levocetrizine	-97.0904	-82.2444	-0.242801
13	Pheniramine	-90.768	-74.1755	0
14	Promethazine	-90.2201	-63.7851	0
15	Quindine	-97.2415	-79.1584	-3.71152
16	Quinine	-97.2415	-79.1584	-3.71152
17	Chlordiazepoxide	-92.1223	-70.6817	-6.09183
18	Diazepam	-72.9474	-63.5867	0
19	Flurazepam	923.016	-41.3666	0
20	Lorazepam	-82.8568	-67.2289	0
21	Oxazepam	-82.624	-65.7814	0
22	Domperidone	-93.4013	-74.4395	-4.60736
23	Ketoconazole	-154.802	-101.91	-1.50888
24	Miconazole nitrate	-128.22	-85.8321	-0.852039

Table 2.2 Moldock Score of docked compounds Alpha-amylase

2.3. Biological Evaluation of Ligands

2.3.1. Alpha-Amylase Inhibitory Activity

- Different concentration of samples was prepared in DMSO.
- About 0.5 ml of different concentration of sample was treated with 0.5 ml of alpha amylase (0.5 mg/ml) in test tube.
- The solution was incubated at 250ºC for 10 minutes. About 0.5 ml of 1% starch solution in 0.02 M sodium.
- The reaction was stop by adding 1.0 ml of DNS reagent and the reaction mixture was kept in boiling water bath for 5 minutes and cool to room temperature.
- The solution was made up to 10 ml with distille water and the absorbance was read in the UV- Visible Spectrophotometer at 540 nm against phosphate buffer as blank solution.
- Acarbose is used as positive control. phosphate buffer of pH 6.9 was add to all the tubes and was incubate at 250ºC for 10 minutes.
- Absorbance was calculate by following formula:
- α-Amylase Inhibition Activity = (Ac+) – (Ac-) – (As-Ab) / (Ac+) – (Ac-) × 100 [7-10]

S.No.	Isolates	Absorbance				
		20	40	60	80	100
1	**Ketoconazole**	0.482±0.012	0.364±0.009	0.244±0.018	0.125±0.015	0.013±0.006
2	**Domperidone**	0.494±0.012	0.381±0.027	0.273±0.003	0.154±0.038	0.038±0.026
3	**Acarbose**	0.561 ±0.072	0.475 ±0.037	0.336 ±0.054	0.202±0.019	0.0814±0.025
4	**Control**	0.992±0.068				

Table 2.3 Absorbance of different concentration of selected drugs for Alpha- amylase

3. RESULTS

3.1 Docking Results

Total 24 drugs have been docked from which 2 compounds were selected which are ketoconazole and domperidone ligands for insilico studies. The 3D structures were downloaded from the Pub Chem / Drawn using the chemdraw office and were saved in mole file. All the structure are energy minimized using Chem Draw. The energy minization was succesfuly done by MM2 method using Chem 3D.

Molegro virtual docker 6.0 software was used for the raw enzymes optimization by removing the water molecules, Co factors and binded ligands and the protein surface of the enzyme was created and cavities were detected.

The minimized structures of ligands and alpha amylase were subsequently used in docking model. The conformation flexibility of ligands were considered by exhaustive conformational search that removed conformations which were not suitable for alpha amylase binding or had long range hydrogen bonds, whereas aldose reductase conformation remained fixed. The shape and properties were represented on grid by different fields, which provided more accurate position and orientation of ligands (termed pose) in the alpha amylase. Some poses were calculated per ligand molecule, which were docked to Alpha amylase. The resulting dock conformations were analyzed using molegero software. The conformation or poses that prepared the maximum number of interaction were measured to further analysis. Moldock score was considered for selection of the compounds which is shown in table 6.1.

The Molecular docking score of the interaction studies, as reported in table 3.1 were used to identify two best interactions. The two best selected ligands are ketoconazole and domperidone as shown in structures from figure 3.1 – 3.4.

Name of drug	Moldock Score	Rerank Score	H Bond
Ketoconazole	-159.594	-108.567	0.95898
Domperidone	-159.254	119.751	-7.76246
Acarbose	-148.656	-128.109	-24.8009

Table 3.1 Mol Dock Score of compounds

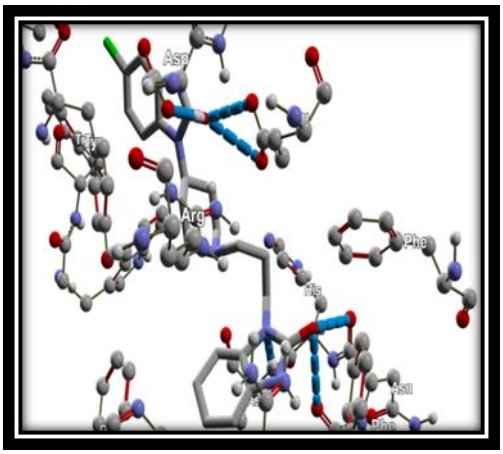


Fig. 3.1 Compact Structure of Domperidone

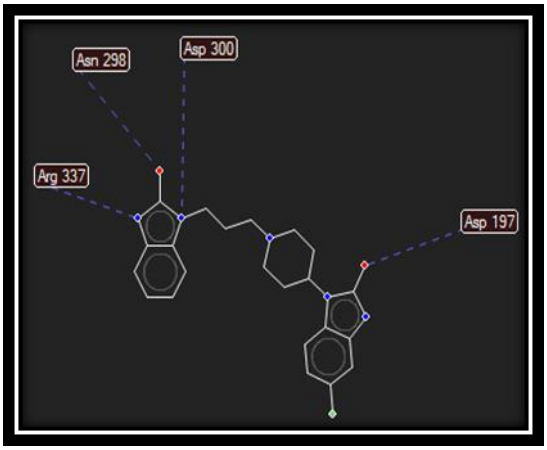
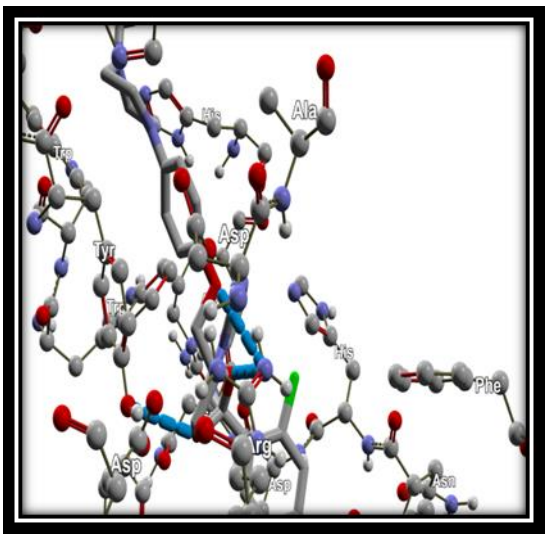


Fig. 3.2 Bond-line Structure of Domperidone

It is clear from the figure 3.1 & 3.2 that alpha-amylase form a potent interaction with all the ligand. It was observed that Domperidone in amylase complex forms 4 hydrogen bonds with amino acid Arginine 337, Asparagine 298, Aspartic Acid 300, Aspartic Acid 197.



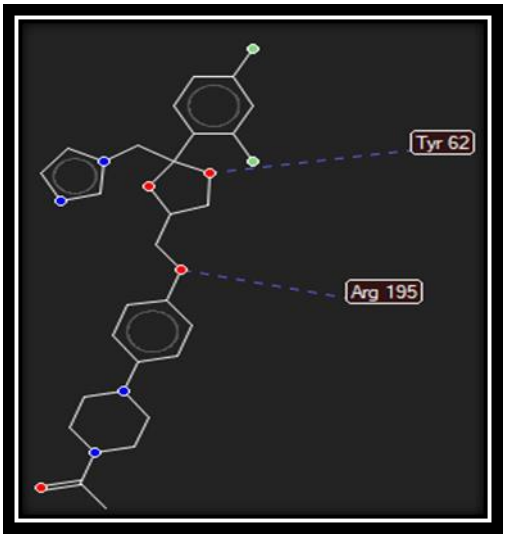


Fig. 3.3 Compact Structure of Ketoconazole **Fig. 3.4 Bond-line Structure of Ketoconazole**

It is clear from the figure 3.3 & 3.4 that alpha-amylase form a potent interaction with all the ligand. It was observed that Ketoconazole in amylase complex forms 2 hydrogen bonds with amino acid Tyrosine 62 and Arginine 195.

3.2 In-Vitro Evaluation Result

Sr. No.	DRUG	IC50 VALUE
1	Acarbose	35.5
2	Ketoconazole	25.4
3	Domperidone	20.84

Table 3.2 IC50 value of selected drugs

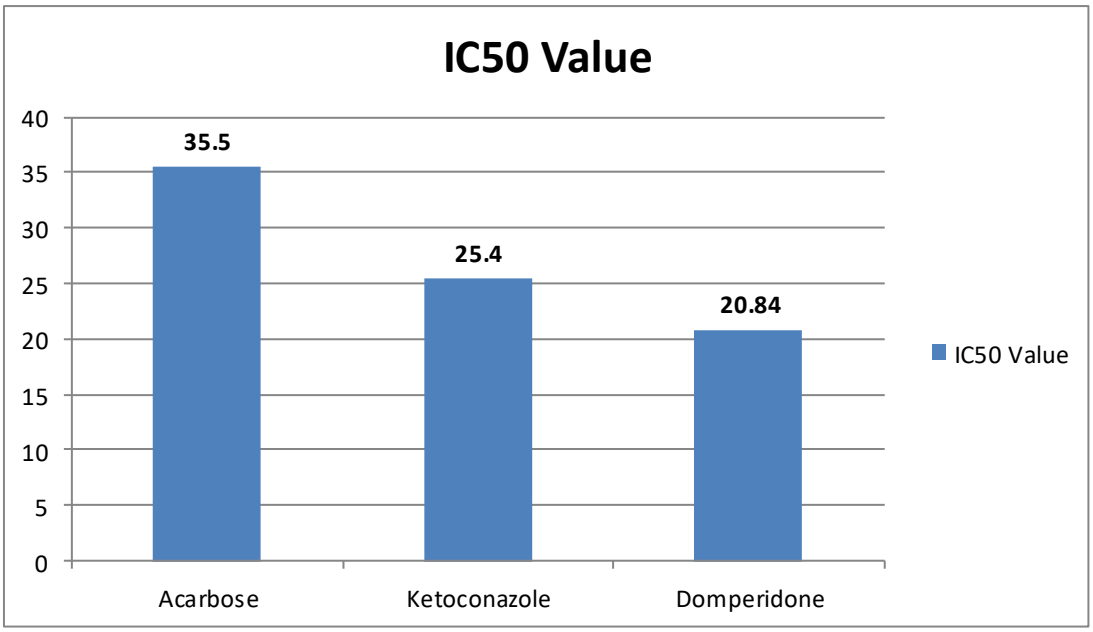


Fig. 3.5 Graphical Representation of IC50 value of selected drugs

4. DISCUSSION

Diabetes is one of the largest global public health concerns, imposing a heavy global burden on public health as well as socio-economic development. Diabetes is one of the top 10 causes of death globally. Individuals with diabetes have 2-3 fold risks of all-cause mortality. The global burden of diabetes has increased significantly in recent decades and will continue to soar in the next few decades.

Patients suffering from diabetes has to take medications in the form of oral tablets or insulin injection. In case, if a patient suffers from more than one disease such as hypertension, cardiovascular disease, etc. along with diabetes, then the patient has to take multiple drugs for living a normal life. The consumption of multiple drugs on a daily basis causes non-compliance in the patients. To overcome this problems repurposing of drugs is helpful for the management of diabetes mellitus.Many drugs which are used for the treatment of some other disease or disorder, can show its usefulness in the management of diabetes mellitus.

In this research work, around 300 drugs have been docked with alpha-amylase to form a ligand-protein complex. The docking has been performed to obtain mol dock score which is compared with standard acarbose drug. 2 drugs namely Ketoconazole and Domperidone have been found to be compatible for the purpose of management of diabetes.

The notable results of this research work has been concluded below:-

1. To identify new therapeutic opportunities for existing drugs for the management of diabetes and its complications.
2. To identify new uses for approved or investigational drugs.
3. Reducing the discovery and development timeline.
4. This decreases the overall cost of bringing the drug to market because the safety and pharmacokinetic profile of the repositioned candidates are already established.
5. Reduction in risk of failure.
6. High success rate.

5. CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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