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DISCOVERY OF NOVEL GLUCOKINASE ACTIVATORS THROUGH STRUCTURE-BASED PHARMACOPHORE MODELING, VIRTUAL SCREENING AND MOLECULAR DOCKING APPROACHES

CHHAJED PRIYANKA N* AND PATIL RAVINDRA B

DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, KBC NMU, Jalgaon, Maharashtra, India

***Corresponding Author: E Mail: chhajedpriyanka123@gmail.com**

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ABSTRACT

The prevalence of Diabetes mellitus has been increasing day-by-day. Available drug therapies are associated with some side-effects. Glucokinase is an emerging target to design newer anti-diabetic drugs by activating the enzyme. Glucokinase activators convert glucose into glucose-6-phosphate. In this study, structure-based Pharmacophore model was generated; virtual screening was performed using Asinex and Zinc database library compounds. Molecular docking was performed followed by ADMET studies. ZINC212072098, ZINC212076713, ZINC212080377, ZINC94222677 and ZINC95394375 have shown good binding energies, interactions with ideal amino acid residues and better ADMET results. These compounds will be further used to design novel Glucokinase activators.

Keywords: Glucokinase, 1V4S, Ligand Scout, PyRx, Structure-based Pharmacophore, Swiss-ADMET

ABBREVIATIONS: T2DM: Type-2 Diabetes Mellitus; GK: Glucokinase; GKA: Glucokinase Activator; PDB: Protein Data Bank; ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity

1. INTRODUCTION

Glucokinase is also known as Hexokinase IV. It involves in metabolism of Glucose into Glucose6-phosphate. It is present in Pancreas and Liver. It is an allosteric enzyme, which is having one active site for binding with glucose and other allosteric binding site for Glucokinase activator. Glucokinase activators are small molecules, bind to allosteric site of Glucokinase enzyme **[1-3]**. GK activators bind to GK enzyme in pancreas and increase glucose-sensitive insulin secretion. In liver, GK activators convert glucose in glycogen, thereby reducing blood glucose level. This role of Glucokinase activators makes them a better choice as an anti-diabetic treatment **[1, 2]**. The designing of GKAs have been increasing day-by-day. Among many designed and synthesized GKAs, Phenyl acetamides and substituted benzamides represent a major structural scaffold **[1-3]**. Thus, in this study, we attempted to design novel Glucokinase activators by taking 1V4S as a crystal structure of human Glucokinase enzyme. 1V4S contains 2-amino benzamide as a co-crystalized ligand.In this study, we tried to design a structure-based Pharmacophore model, screen it against different databases to find the hits matching the pharmacophoric features. **Figure 1** shows the flowchart of computational approaches we performed throughout the study.

2. MATERIALS AND METHODS

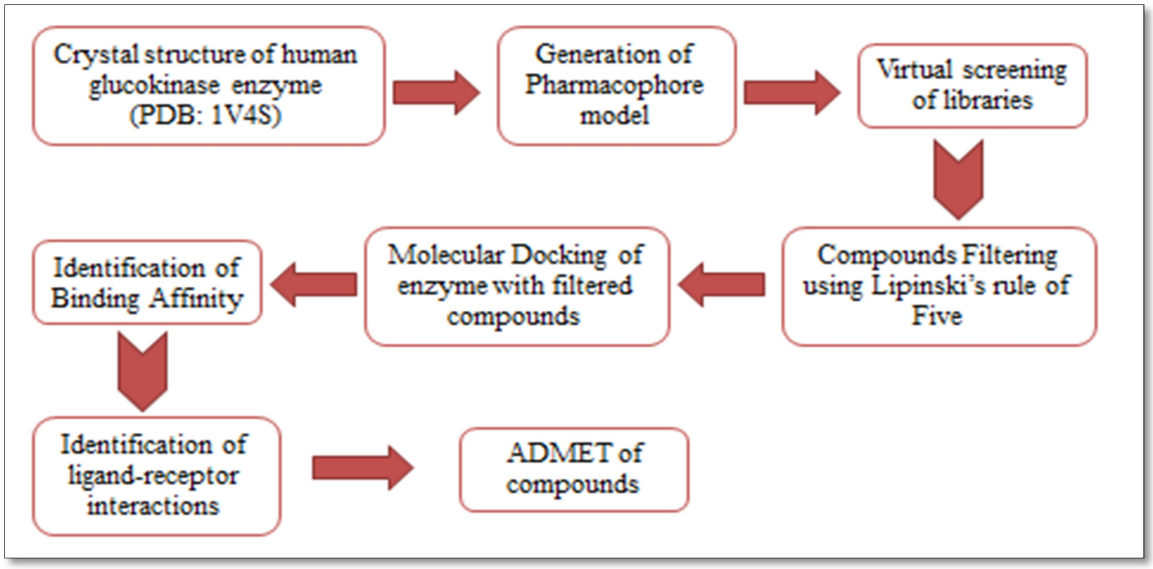

Figure 1: Flowchart of computational approaches used in the study

2.1. Enzyme/ target Selection

The Crystal Structure of human Glucokinase enzyme with PDB ID: 1V4S with resolution of 2.30 Å was downloaded from protein data bank [https://www.rcsb.org/structure/1V4S]. 1V4S is complexed with MRK as a ligandin allosteric site, GLC (Glucose) in

glucose-binding site and contains sodium ion **(Figure 2)**. The amino acid residues involved in Glucokinase and MRK binding are Tyr61, Val62, Arg63, Ser64, Thr65, Pro66, Gln98, Ile159, Met210, Ile211, Tyr214, Tyr215, Cys220, Glu221, Val452, Val455, Ala456 and HOH729.

2.2. Enzyme/ target Preparation

The downloaded 1V4S enzyme was prepared using Biovia Discovery studio software 2020 and its structure was validated by observing Ramachandran plot **(Figure 3)**. The Heteroatoms, MRK ligand and Water molecules were removed and Polar hydrogens were added.

2.3. Generation of Pharmacophore model

Structure-based Pharmacophore model was generated using Ligand Scout software v3.In macromolecule view of structure-based perspective of Ligand Scout software, the enzyme 1V4S was typed and clicked on 'download'. The 1V4S enzyme was then retrieved from Protein data bank directly **(Figure 4)**. Then, the MRK ligand was discovered and active site view was generated. The MRK and GK bonding interactions were observed. Then Structure-based Pharmacophore was generated **[5, 6]**. The Pharmacophore model showed 3 Hydrogen-bond acceptors, 2 Hydrogen-bond donors and 2 hydrophobic groups are necessary for binding to Glucokinase enzyme **(Figure 5)**.

2.4. Virtual Screening of Asinex and ZincPharmer databases

The generated Pharmacophore model was then copied to screening perspective of Ligand Scout software. The Asinex compounds library was downloaded in the software. The library was screened against the Pharmacophore model to search newer compounds matching the Pharmacophore points **[10, 11]**. Total 68 hits were found after screening of Asinex library of 6000 compounds. Similarly, Zinc Pharmer database was also screened for Pharmacophore model and 92 hits were found **[7]**.

2.5. Applying Lipinski's Rule of Five

The 68 hits from Asinex library and 92 hits from ZincPharmer database were then filtered by applying Lipinski's rule of five. Finally, 15 hits from Asinex and 22 hits from ZincPharmer database, total 37 hits were found.

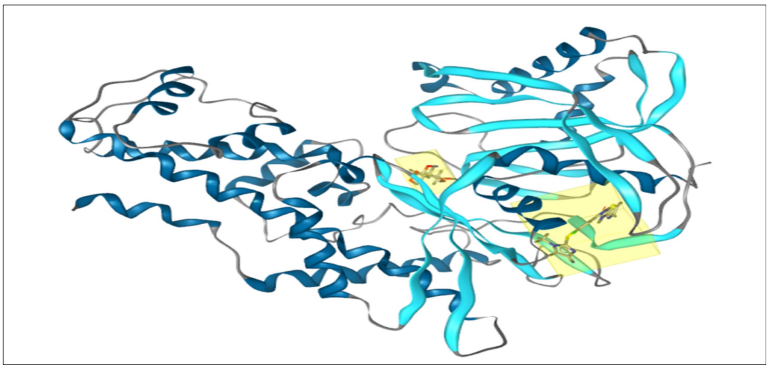

Figure 2: Crystal structure of Human Glucokinase enzyme with glucose and MRK

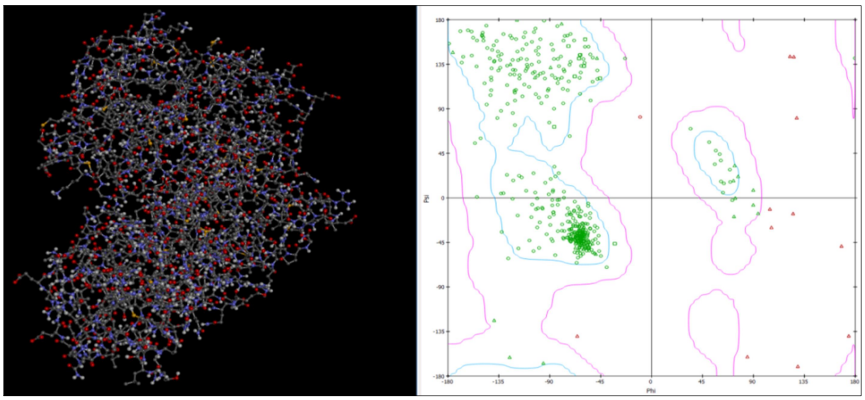

Figure 3: Crystal structure of Human Glucokinase enzyme with Ramachandran plot

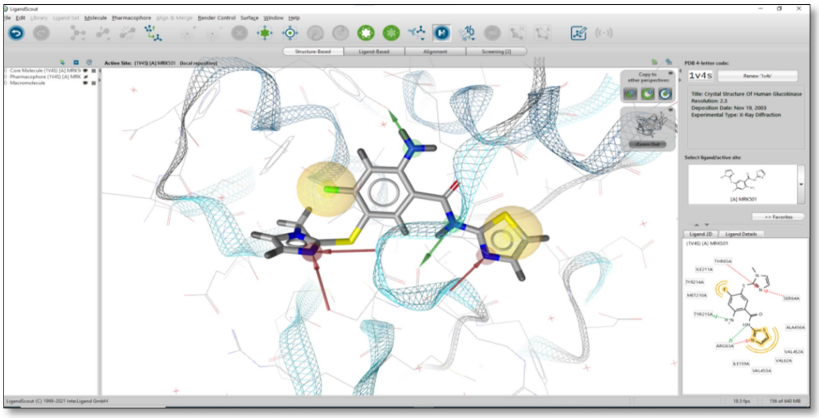

Figure 4: MRK ligand in the active site of human Glucokinase enzyme

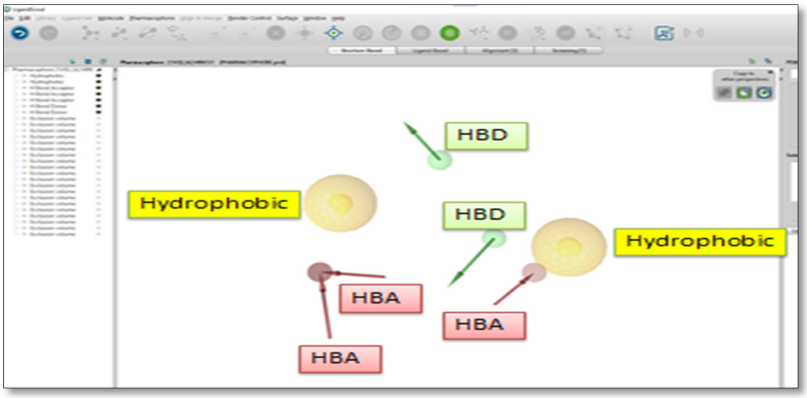

Figure 5: Generated Structure-based Pharmacophore model

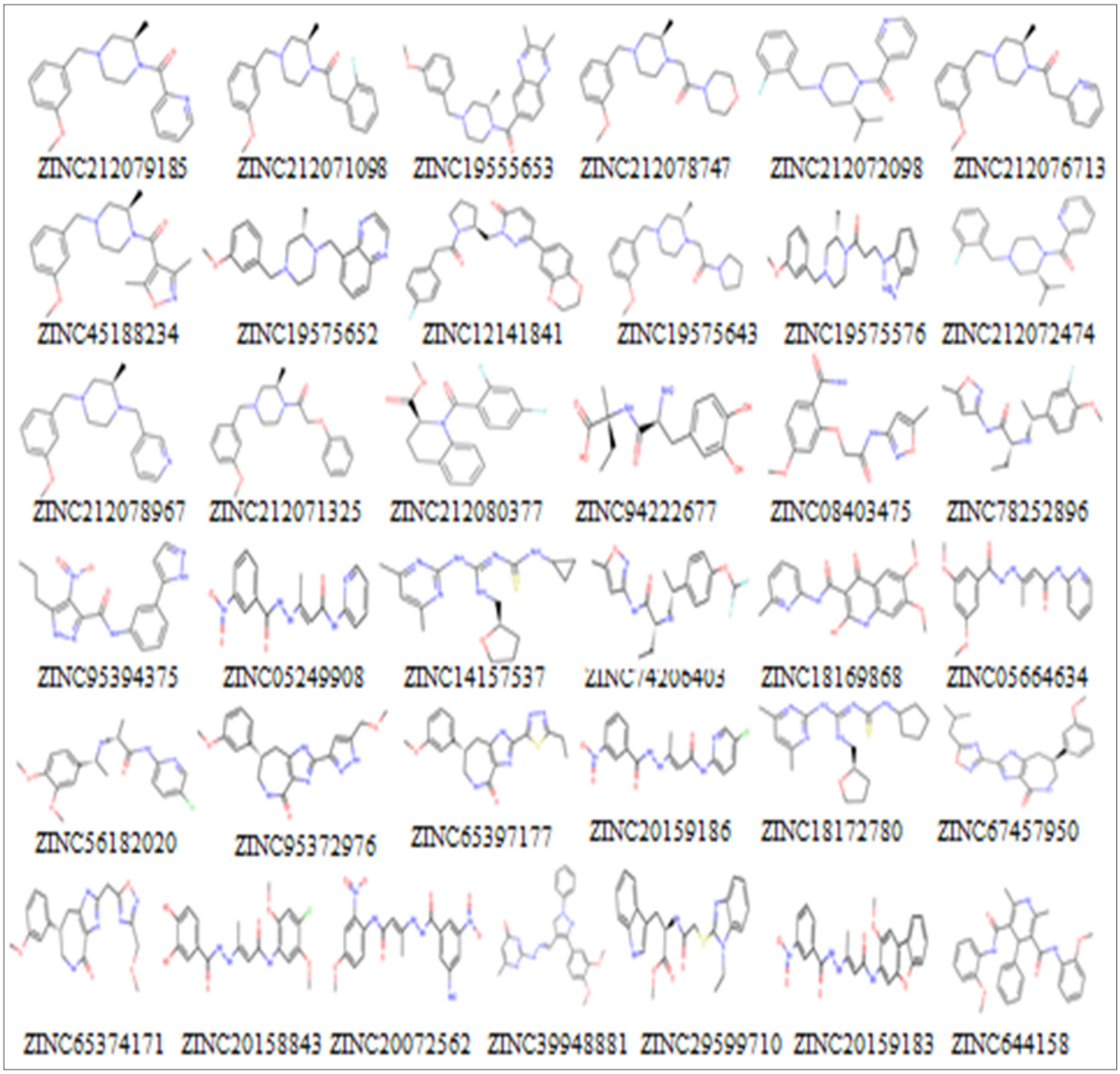

Figure 6: Screened compounds with their structures and ZINC IDs

2.6. Molecular Docking Studies [10,11]

2.7. The total 37 hits after virtual screening and Lipinski's rule of five were docked with prepared 1V4S enzyme using PyRx- Virtual screening software v 0.8, 2008-2010 **[8]**. Before docking, The Enzyme (macromolecule-1V4S) was prepared and converted into pdbqt format. Similarly, ligands were minimized and converted into pdbqt format. The grid box was generated at the active binding site of 1V4S. Then, docking was performed followed by binding affinity and RMSD observations.

2.8. Identification of ADMET properties and synthetic accessibility

The hits which shown good binding affinities and docking scores were identified for their Absorption, Distribution, Metabolism, Excretion, Toxicities and synthetic accessibilities using Swiss-ADME webserver **[9]**.

3. RESULTS AND DISCUSSION

From the results of docking **(Table 1)**, it was observed that ZINC20159183, ZINC67457950, ZINC20159186, ZINC20158843, ZINC20159186, ZINC65397177 compounds had docking score of -9.4, -8.9, -8.7, -8.6,-8.7 and -8.5 respectively. But, though ZINC20159183 had highest docking score, it has not showed any hydrogen bond interaction **(Table 2)**. We performed docking of 37 compounds, observed their binding energy and amino acid residues involved in interactions with Glucokinase enzyme. Out of 37 compounds, 21 compounds showed better interactions. Among 21 compounds, 18 compounds showed ideal hydrogen bond interactions **(Figure 7-12)**. Those 18 compounds were then studied for their Absorption, Distribution, metabolism, excretion, Lipinski's rule, Ghose rule, Toxicity, lead-likeness and synthetic accessibilities **[9]**. ZINC212072098, ZINC212076713, ZINC212080377, ZINC94222677 and ZINC95394375 have showed better results in ADMET and synthetic accessibility studies, though they were having less docking score **(Table No. 3 & 4)**.

Table 1: Binding energies of 37 screened compounds after Molecular docking

Sr. No.	Compound ZINC database ID	Binding Energy (kcal/mol)
1.	ZINC212079185	-7.3
2.	ZINC212071098	-7.2
3.	ZINC19555653	-8.2
4.	ZINC212078747	-6.6
5.	ZINC212072098	-7.3
6.	ZINC212076713	-7.6
7.	ZINC45188234	-7.1
8.	ZINC19575652	-7.9
9.	ZINC12141841	-8.1
10.	ZINC19575643	-6.9
11.	ZINC19575576	-7.3
12.	ZINC212072474	-7.4
13.	ZINC212078967	-7
14.	ZINC212071325	-6.7
15.	ZINC212080377	-7.4
16.	ZINC94222677	-7.1
17.	ZINC08403475	-7.1
18.	ZINC78252896	-7.5
19.	ZINC95394375	-8.3
20.	ZINC05249908	-8.1
21.	ZINC14157537	-7.3
22.	ZINC74206403	-8.3
23.	ZINC18169868	-8.3
24.	ZINC05664634	-8.2
25.	ZINC56182020	-7.9
26.	ZINC95372976	-8.4

27.	**ZINC65397177**	**-8.5**
28.	**ZINC20159186**	**-8.7**
29.	**ZINC18172780**	**-7.1**
30.	**ZINC67457950**	**-8.9**
31.	**ZINC65374171**	**-8.2**
32.	**ZINC20158843**	**-8.6**
33.	**ZINC20072562**	**-8.3**
34.	**ZINC39948881**	**-7.6**
35.	**ZINC29599710**	**-7.2**
36.	**ZINC20159183**	**-9.4**
37.	**ZINC644158**	**-7.2**

Table 2: Amino acid residues involved in interactions between enzyme and compounds (amino acid residues involved in Hydrogen bond interactions shown in Bold)

Sr. No.	Compound ZINC database ID	Amino acid residues involved in Hydrogen bond, Hydrophobic, vanderwaals interactions
1.	ZINC19555653	Tyr61, Val62, Pro66, Ile211, Ala456, Lys459, Lys459
2.	ZINC212072098	**Asp160,** Tyr61, Val62, Pro66, Asp58, Ile159, Val452, Val455, Ala456, Lys459, Ala460
3.	ZINC212076713	**Arg63,** Val62, Pro66, Val452, Val455, Ile211, Asp158, Lys459, Ala460
4.	ZINC19575652	Tyr61,Asp158, Ile159,Ile211,Tyr214, Val45, Ala456
5.	ZINC12141841	**Arg85, Lys169, Ser445,** Asp78, Gly80, Gly81, Ser151, Ile225, Thr228, Gly229, Asp409, Asp409, Gly410, Ser411, Ser414, Glu500
6.	ZINC212080377	**Thr82, Asn83, Arg85, Arg85,** Gly80, Asp409
7.	ZINC94222677	**Arg63, Arg63, Tyr215, Tyr215, Val452, Leu451,** Tyr61, Val62, Thr65, Pro66, Ile159, Ile211, Tyr214, Val455, Ala456, Lys459
8.	ZINC78252896	**Thr228, Thr228, Glu443, Glu444,** Gly227, Gly80, Gly81,
9.	ZINC95394375	**His218,** Val62, Thr65, Ile211, Met235, Val452, Val455
10.	ZINC5249908	**Arg250, Tyr214,** Val62, Arg63, Ser64, Thr65, Pro66, Met210, Ile211, His218, Cys220, Glu221, Met235, Leu451, Val452, Val455
11.	ZINC18169868	**Arg63**, Arg63, Val62, Pro66, Val452, Val455, Ile211, Lys459, Ala456, Asp158, Lys459
12.	ZINC5664634	**His218, Tyr214, Cys220,** Val62,Arg63, Ser64, Pro66, Ile211, Glu221, Met210, Met235, Val452, Val455
13.	ZINC56182020	**Arg63,**Tyr61, Val62, Thr65, Pro66, Asp158, Ile211, Tyr214, Tyr215, Ile159, Val452, Val455, Ala456, Lys459, Ala460
14.	ZINC95372976	**Arg63,Arg63, Arg63,** Asp158, Val62,Pro66, Ile159, Leu451, Val452, Val455, Ala456, Lys459, Leu451, Leu459
15.	ZINC65397177	**His218,** Tyr214, Tyr215, Met210, Ile211, Met235, Glu221, Arg250
16.	ZINC67457950	**Arg63, Arg63, Asp158,** Val62, Pro66, Ile159, Ile211, Val452, Val455, Ala456, Lys459, Ala460
17.	ZINC65374171	**Arg63, Asp158,** Tyr61, Val62, Pro66, Asp160, Ile211, Tyr215, Leu451, Ile159, Lys459, Ala456, Val452
18.	ZINC20158843	**Arg63,** Thr65, Pro66, Ile159, Met235, Arg250, Val452, Val455, Ala456
19.	ZINC20072562	**Tyr61, Arg63, Thr65,** Val62, Pro66, Ile211, Tyr215, Val452, Val455, Ala456, Lys459
20.	ZINC39948881	**Thr228, Ser411,** Asp78, Arg333, Asp409, Ser411, Lys414
21.	ZINC20159183	Ser64, Tyr214, Tyr215, Ile211, Val452, Ala456, Lys459

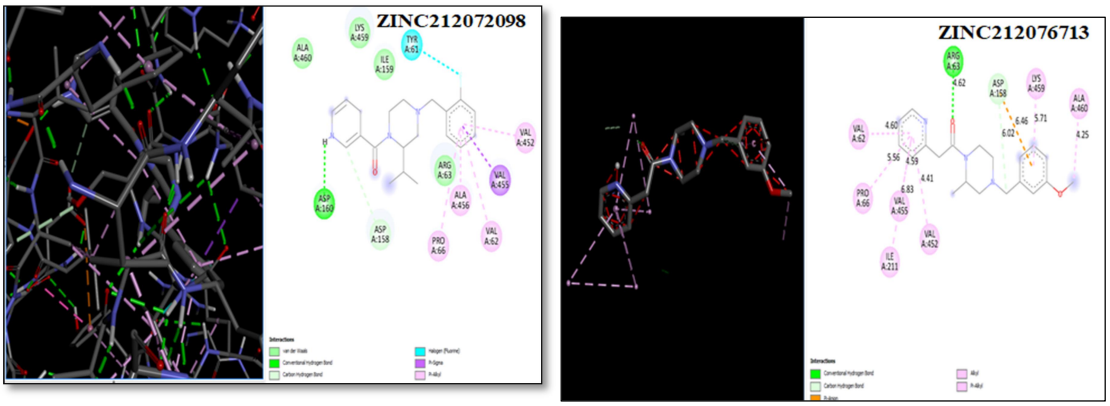

Fig 7, 8: Interactions of ZINC212072098 and ZINC212076713 with Glucokinase enzyme

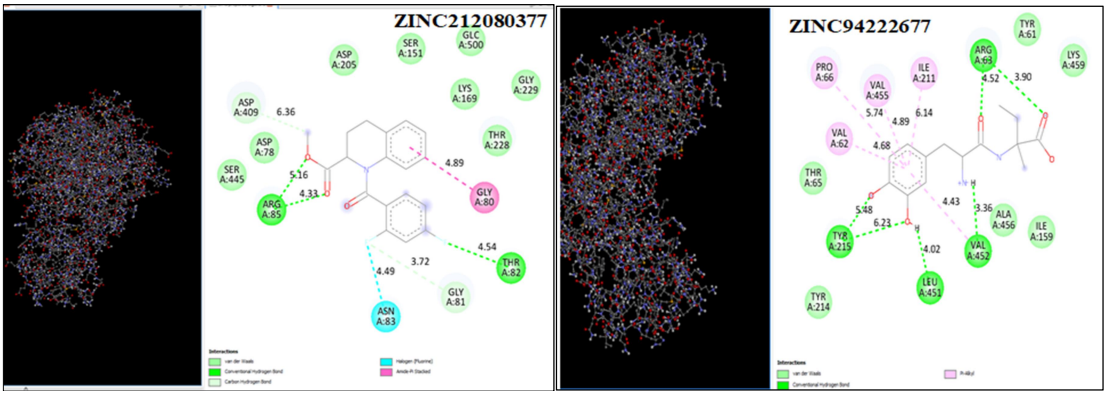

Fig 9, 10: Interactions of ZINC212080377 and ZINC94222677 with Glucokinase enzyme

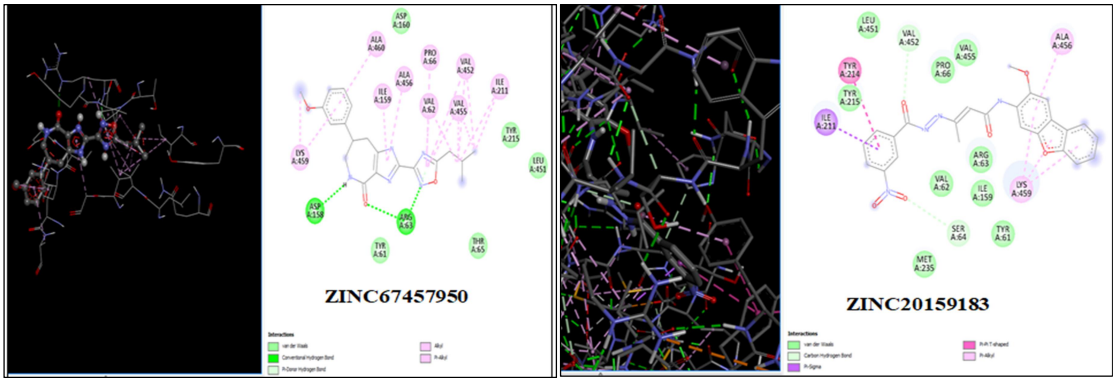

Fig 11, 12: Interactions of ZINC67457950 and ZINC20159183 with Glucokinase enzyme

Table 3: Physicochemical Properties of best docking score compounds from Screened compounds:

Sr. No.	ZINC ID of compounds	Mol. Formula	Mol. Wt. (g/mol)	No. rot. bonds	No. HBA	No. HBD	Molar Refractivity
1.	ZINC212072098	C20H24FN3O	341.42	5	4	0	104.03
2.	ZINC212076713	C20H25N3O2	339.43	6	4	0	105.54
3.	ZINC12141841	C25H24FN3O4	449.47	6	6	0	124.25
4.	ZINC212080377	C18H15F2NO3	331.31	4	5	0	86.97
5.	ZINC94222677	C14H20N2O5	296.32	7	6	5	76.62
6.	ZINC78252896	C17H22FN3O3	335.37	8	6	2	88.75
7.	ZINC95394375	C16H16N6O3	340.34	7	5	3	93.79
8.	ZINC5249908	C16H15N5O4	341.32	8	5	3	91.83
9.	ZINC18169868	C18H17N3O5	355.34	5	6	3	96.55
10.	ZINC5664634	C18H20N4O4	356.38	9	5	3	95.99
11.	ZINC56182020	C18H22ClN3O3	363.84	8	5	2	98.25
12.	ZINC95372976	C19H21N5O3	367.4	5	5	3	102.58
13.	ZINC65397177	C18H19N5O2S	369.44	4	5	2	102.81
14.	ZINC67457950	C20H23N5O3	381.43	5	6	2	106.82
15.	ZINC65374171	C19H21N5O4	383.4	6	7	2	102.3
16.	ZINC20158843	C19H20ClN3O6	421.83	9	6	5	107.25
17.	ZINC20072562	C18H18N6O7	430.37	10	7	4	113.75
18.	ZINC39948881	C23H24N6O3	432.48	7	5	3	131.48

Table 4: Results of lead-likeness, Bioavailability score, and synthetic accessibility of compounds showing best docking score and interactions from Screened compounds

Sr. No.	Compound	Lipinski's rule; violation	Ghose rule; violation	Bioavailability score	Leadlikeness; violation	Synthetic accessibility
1.	ZINC212072098	0	0	0.55	0	2.81
2.	ZINC212076713	0	0	0.55	0	2.95
3.	ZINC12141841	0	0	0.55	1	4.03
4.	ZINC212080377	0	0	0.55	0	2.92
5.	ZINC94222677	0	0	0.55	0	2.76

6.	**ZINC78252896**	**0**	**0**	**0.55**	**1**	**3.58**
7.	**ZINC95394375**	**0**	**0**	**0.55**	**0**	**2.96**
8.	**ZINC5249908**	**0**	**0**	**0.55**	**1**	**3.04**
9.	**ZINC18169868**	**0**	**0**	**0.55**	**1**	**2.74**
10.	**ZINC5664634**	**0**	**0**	**0.55**	**2**	**3.1**
11.	**ZINC56182020**	**0**	**0**	**0.55**	**2**	**3.32**
12.	**ZINC95372976**	**0**	**0**	**0.55**	**1**	**3.82**
13.	**ZINC65397177**	**0**	**0**	**0.55**	**1**	**3.88**
14.	**ZINC67457950**	**0**	**0**	**0.55**	**1**	**4**
15.	**ZINC65374171**	**0**	**0**	**0.55**	**1**	**3.92**
16.	**ZINC20158843**	**0**	**0**	**0.55**	**2**	**3.26**
17.	**ZINC20072562**	**1**	**0**	**0.55**	**2**	**3.55**
18.	**ZINC39948881**	**0**	**1**	**0.55**	**1**	**4.08**

4. CONCLUSION

In this in-silico work, we generated structure-based Pharmacophore model using crystal structure of 1V4S Glucokinase enzyme co-crystalized with substituted benzamide, MRK as a ligand. The Pharmacophore model showed that 3 Hydrogen-bond acceptors, 2 Hydrogen-bond donors and 2 hydrophobic groups are important pharmacophoric features to activate Glucokinase enzyme. Then we performed virtual screening of Asinex and ZincPharmer database library compounds to find out the compounds matching to Pharmacophoric features. We found 37 compounds after screening and applying Lipinski's rule of five. The 37 compounds were then docked into allosteric site of glucokinase enzyme and binding energies were observed. It was observed that all the compounds with good docking score did not show good hydrogen bond interactions. The 18 compounds out of 37 showed good interactions were then studied for their ADMET profile. ZINC212072098, ZINC212076713, ZINC212080377, ZINC94222677 and ZINC95394375 were found to be showing good docking score, interactions and ADMET profile. Thus, these compounds can be used further to design derivatives and used as Glucokinase activators.

5. CONFLICTS OF INTEREST

There are no conflicts of interest on the part of the authors with respect to the publication of this paper.

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