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ORIGINAL ARTICLE **OPEN ACCESS**

Ligand Based Pharmacophore Modelling, Virtual Screening And Molecular Docking Of Novel Compounds Against Diabetes

Fariha Maryam,Hana Mukhtar*, Iqra Bibi,Muhammad Rizwan, Sajid Khan, Azhar Mehmood, Anum Munir
Department of Bioinformatics, Government Post Graduate College Mandian Abbottabad
E-mail: hanamukhtar06@gmail.com

ABSTRACT
Diabetes Mellitus is also called Type 2 diabetes, a disorder related to metabolism considered by insulin resistance, high sugar level in blood and lack of insulin production in body. It primarily occurs as a result of obesity, sedentary lifestyle, stress, nutrition and toxins.CAPN10, which encodes the cysteine protease calpain 10, was the first type 2 diabetes mellitus (T2DM) susceptibility gene identified through a genome-wide scan followed by positional cloning. A haplotype combination comprising three intronic CAPN10 single-nucleotide polymorphisms (UCSNP-43, -19, and -63) was associated with increased risk of T2DM in the population in which linkage was first found. In this study an attempt was madeto design a novel antidiabetic compound. A total of 21 existing compounds were taken from which 6 compounds were set as test and remaining 15 as training. Pharmacophore models were generated and their shared pharmacophore feature was identified. Virtual screening was performed and hit compounds were selected as inhibitor compounds. The generated shared feature pharmacophore showed 2 main features as 2 hydrogen bond acceptors and 1 aromatic ring. After the complete analysis, pharmacophores of all compounds were matched and novel pharmacophore was identified. Virtual screening was performed against the shared feature pharmacophoreto identify hit compounds 3 hit compounds were retrieved and docked.
Keywords: *Diabetes Mellitus, CAPN10, Pharmacophore Modelling, Ligand, Molecular Docking, Virtual Screening.*

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INTRODUCTION

Diabetes mellitus (DM) is one of the first diseases occurring in human. It had been first identified in Egyptians3000 years back,when interaction between hereditary components and environmental factors[1]. DM was first described as metabolic syndrome component in 1988. It is also known as non-insulin dependent characterized by the lack of insulin, insulin conflict and hyperglycemia. Environmental, hereditary and behavioral factors are also responsible for the development of disease. The people having diabetes mellitus are more in danger to different kinds of complications that frequently causes their early death. It is a long-lasting metabolic disease increasing day by day around the world[2],[3].

There are two types of diabetes established by Hinsworth in 1935. Both forms of diabetes are described by constant increase of sugar level in plasma[1], type 1 diabetes has been known as insulin dependent and is an autoimmune disease caused when the B-cells in pancreatic islets are completely lost resulting in insulin deficiency, this type can be treated with insulin injections[4], on the other hand T2D is caused when the insulin secreted by islets cannot circulate properly in the tissues where it is required e.g. in liver, muscles and fats. It is also called Insulin non-dependent and is most common in adults. It can be treated by hypoglycemic drugs and diet control[5].

There are number of genes contributing to diabetes mellitus, calpain-10 gene (CAPN10) is one of the proteases which serves as intracellular calcium-dependent cysteine proteases. Secretion of insulin and its metabolism is regulated by CAPN10 protein. It is the first gene to be recognized through a genome scan and positional cloning involved in DM, in which the expression of CAPN10 is changed with polymorphisms. The mRNA of CAPN10 is mostly expressed in the heart, kidney, liver,pancreas and brain. It is present on chromosome 2q37.3 and it is the first candidate gene for diabetes mellitus. Different tests

are required to diagnose Diabetes Mellitus i-e glycated hemoglobin (A1C) test, random blood sugar test, fasting blood sugar test and oral glucose tolerance test[6],[1].

Two therapeutic approaches have been developed to this problem; (GLP-1 analoguesimproving half-life and DPP IV inhibitor stops the breakdown of endogenous GLP-1) these agents are used to control the fasting and postprandial glucose level and in improved functioning of beta cells[7]. For the designing of Ligand based drugs for DM molecular docking methods has been commonly used. [7],[12].

Pharmacophore modelling is greatly being used in drug discovery. In 1967 the conception of Pharmacophore modelling has been given by Kier and according to him Pharmacophore modelling "is the arrangement of functional feature that a compound or drug must have for desired expression" and the term Pharmacophore was coined by Ehlrich[13].. They are the collections of atoms in 3D with functional groups which shows interaction with receptors. Pharmacophore modelling is divided into two types: structure based pharmacophore modelling and ligand based pharmacophore modelling[14],[15].

This study intends to identify novel compounds for DM using Ligand based pharmacophore modelling approach.

MATERIAL AND METHODS

Figure 1 show the methods which were used to conduct this research.

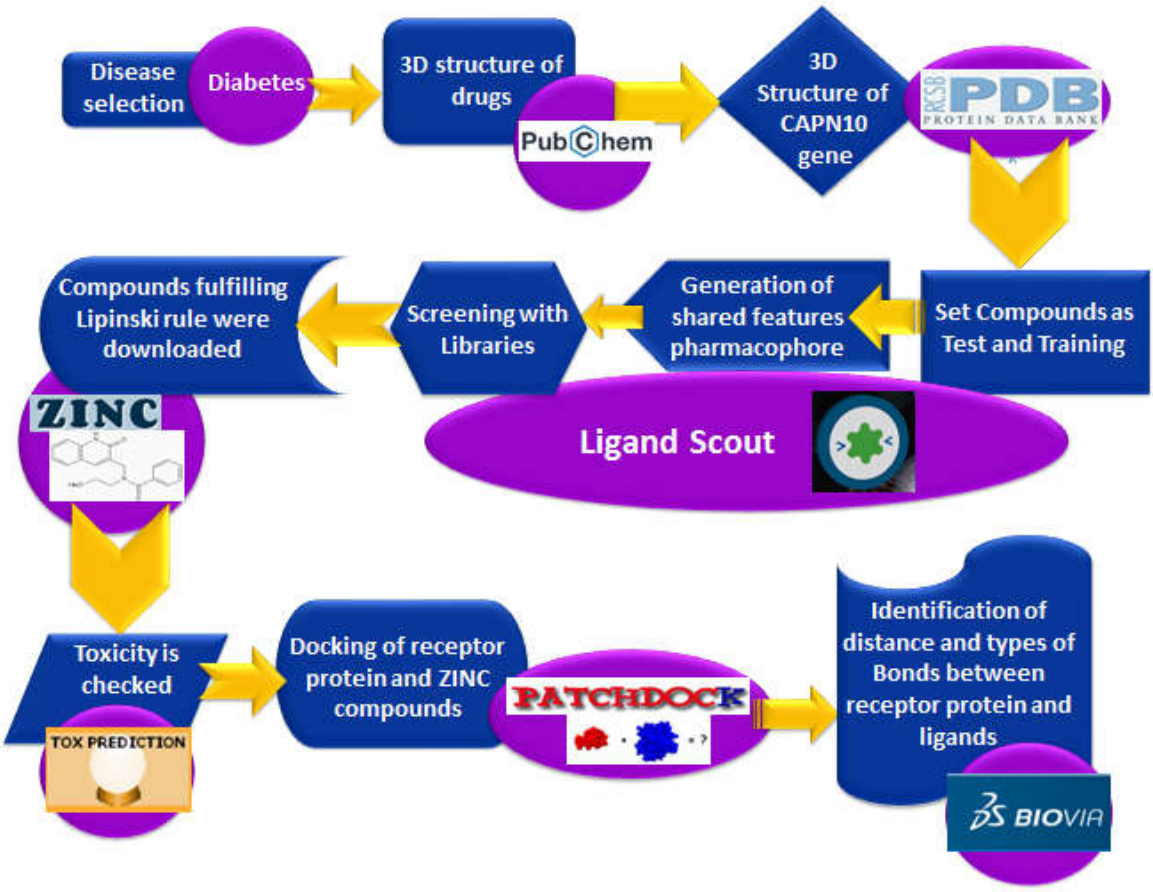


Figure 1: Flowchart of methodology applied for the pharmacophore modelling procedure

Selection of CAPN10 Protein and existing Drugs:

From literature review CAPN10 gene was selected[1] after screening and the mutated structure with 1 mutation was downloaded with protein ID 3BOW from RCSB PDB after applying some filters. PDB(PDB; http://www.rcsb.org/pdb/) a useful database that contains the 3D structures of nucleic acids or proteins basically by using techniques like NMR and X-Ray Crystallography[16]. The existing compounds for Diabetes were also screened and the 3D structures of these compounds were downloaded from PubChem. Pubchem (https://**pubchem**.ncbi.nlm.nih.gov) a freely accessible chemistry database that contain chemical molecules along with their activities against biological assays[17].

Protein Preparation:

The protein with Id name 3BOW is the 3D crystal structure of CAPN10 gene in complex with Calpastatin.The structures were then imported to Ligand Scout(www.inteligand.com/ligandscout) a software that allows creating 3D pharmacophore models[18]. After importing the structure the energy of these compounds is minimized and are then further used for pharmacophore generation.

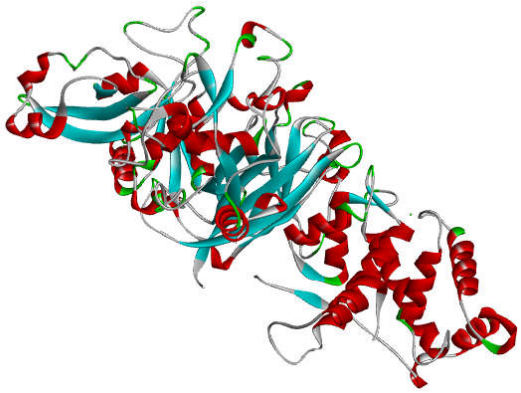


Figure 2: 3D structure of 3BOW

Generation of Pharmacophore:

For every compound the pharmacophore is generated because it is the first important step to understand the relationship between ligand and receptor. From all the pharmacophores generated a shared feature pharmacophore was generated which shows and gives all the common and shared features of all the generated pharmacophores

Virtual Screening of hit compounds against shared feature pharmacophore:

Then performed screening of shared feature pharmacophore with the zincdb.ldb library and 76 compounds were obtained by performing screening that had 90% similarity with these compounds. By using the known pharmacophore validation set of antidiabetic drugs which are available in the market the models of pharmacophore were tested.

Use of Lipinski's rule for Validation of Hit compounds:

In order to estimate the drugs likeliness property, Lipinski's rule (rule of five) is used, it is an important and standard rule to evaluate the properties of drugs and define the toxicity of these compounds by the Protox server and only three compounds were obtained after screening that were fulfilling Lipinski rule[19]. These three compounds were downloaded. The rules of five are:The hydrogen bond donors should not be more than 5, M. Weight should not exceed 500Da, Log P should be less than 5 (or MLogP is over 4.15) and the hydrogen bond acceptor should not be more than 10.

Docking of CAPN10 Protein with Hit compounds:

The Hit compounds which were fulfilling the Lipinski rule of 5 were downloaded and docked with mutated CAPN10 protein for validation by using PatchDock server. And these were compared and analyzed by using Discovery Studio.

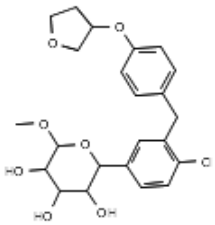
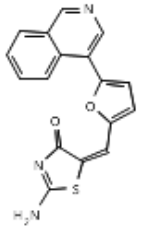
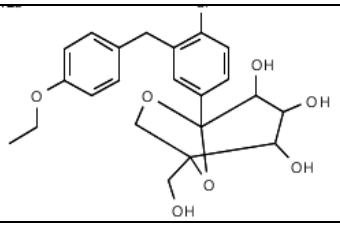
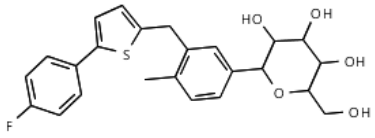
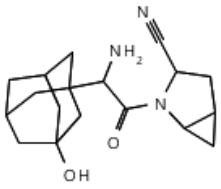
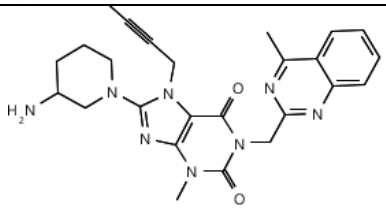
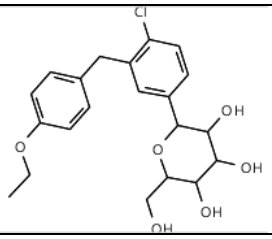
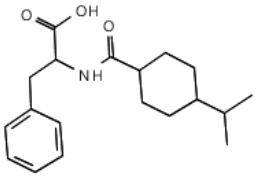
RESULTS:

The protein sequence of 3BOW has 993 residues of amino acid and a molecular weight 114049.26 Daltons.It is vital in Pharmacophore modelling to select the test set and training set compounds[15].For the discovery of novels compounds two types of pharmacophore modelling is used. First one is ligand based and the second one is structure based. Here we used the ligand based pharmacophores for the discovery of novel antidiabetic compounds. Ligand based pharmacophore models are significant for those proteins whose 3D structures are still not predicted[20].

The activity and the properties of not only test compounds but also training compounds can be identified through a good pharmacophore model.The 21 existing antidiabetic compounds were used as the test ant training set. The compounds for test set were selected on the fact that these compounds are active in many models of animals and are mostly used in the treatment of Diabetes. Among these 21 compounds, six were set as test and 15 were set as training.

Table 1 shows the structure and ID's of the training set compounds along with their names which were not being used actively for the treatment or these compounds were used in combinations with other drugs.

Table 1: Training set and their chemical structures

S.no	ID NO	COMPOUND NAME	CHEMICAL STRUCTURE
1	56843247	SYNJARDY	
2	54592203	FURAN THIAZOLIDINEDIONES,A47	
3	44814423	ERTUGLIFLOZIN	
4	24812958	CANAGLIFLOZIN	
5	11243969	SAXAGLIPTIN	
6	10096344	LINAGLIPTIN	
7	9887712	DAPAGLIFLOZIN	
8	5311309	NATEGLINIDE	

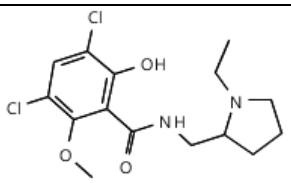
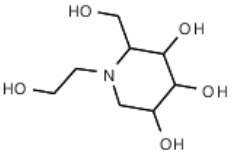
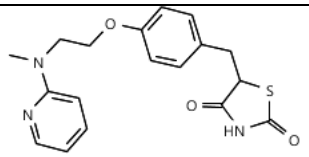
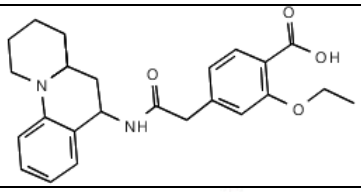
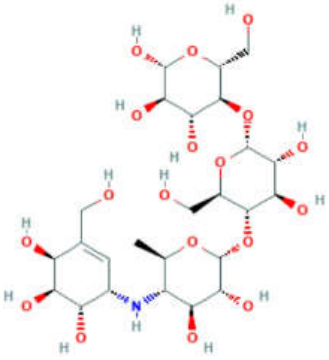
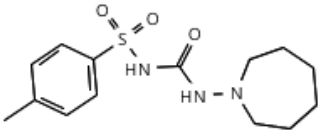
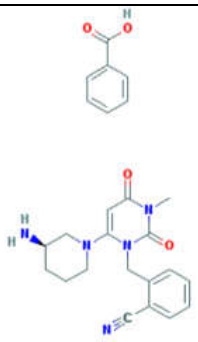
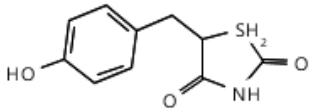
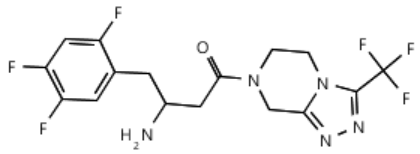
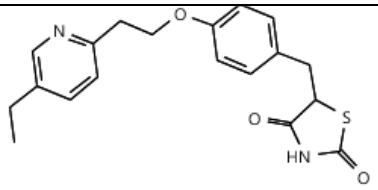
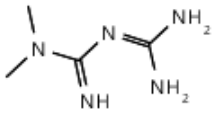
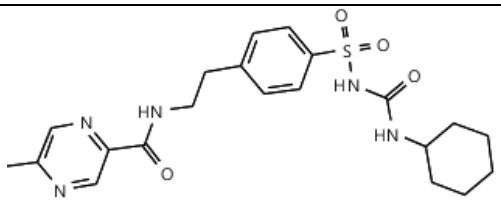
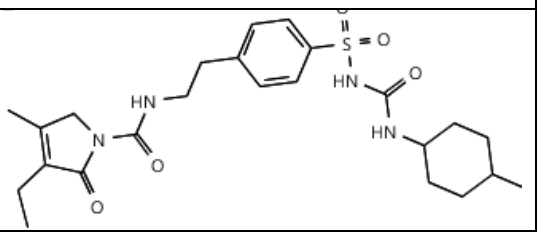
9	3033769	RACLOPRIDE	
10	441314	MIGLITOL	
11	77999	ROSIGLITAZONE	
12	65981	REPAGLINIDE	
13	41774	ACARBOSE	
14	5503	TALAZAMIDE	
15	11450633	ALOGLIPTIN BENZOATE	

Table 2 shows the structure and ID's of the test set compounds along with their names which were being actively used for the treatment.

Table 2: Test set and their chemical structures

S.no	ID NO	COMPOUND NAME	CHEMICAL STRUCTURES
1	10198397	2,4 Thiazolidinedione	
2	4369359	SITAGLIPTIN	
3	4829	PIOGLITAZONE	
4	4091	METFORMIN	
5	3478	GLIPIZIDE	
6	3476	GLIMEPIRIDE	

Here we used the ligand based pharmacophores for the discovery of novel antidiabetic compounds.For this study Ligand Scout (www.inteligand.com/ligandscout) was used to generate the pharmacophore models for the test and training set. Before pharmacophore generation minimization of energy of ligand is important. The pharmacophore for each compound has been generated using "default settings" by clicking on "create pharmacophore" command in menu.

Figure 3 shows the five main Pharmacophoric features for 25 ligands: Hydrogen bond acceptor (HBA), Hydrogen bond donor (HBD), Hydrophobic region (H), Aromatic rings (AR) and ionizable positive regions (PI). In each pharmacophore model of the compounds the red arrows represent HBA, green arrow symbolizes HBD, a yellow sphere donates AR.

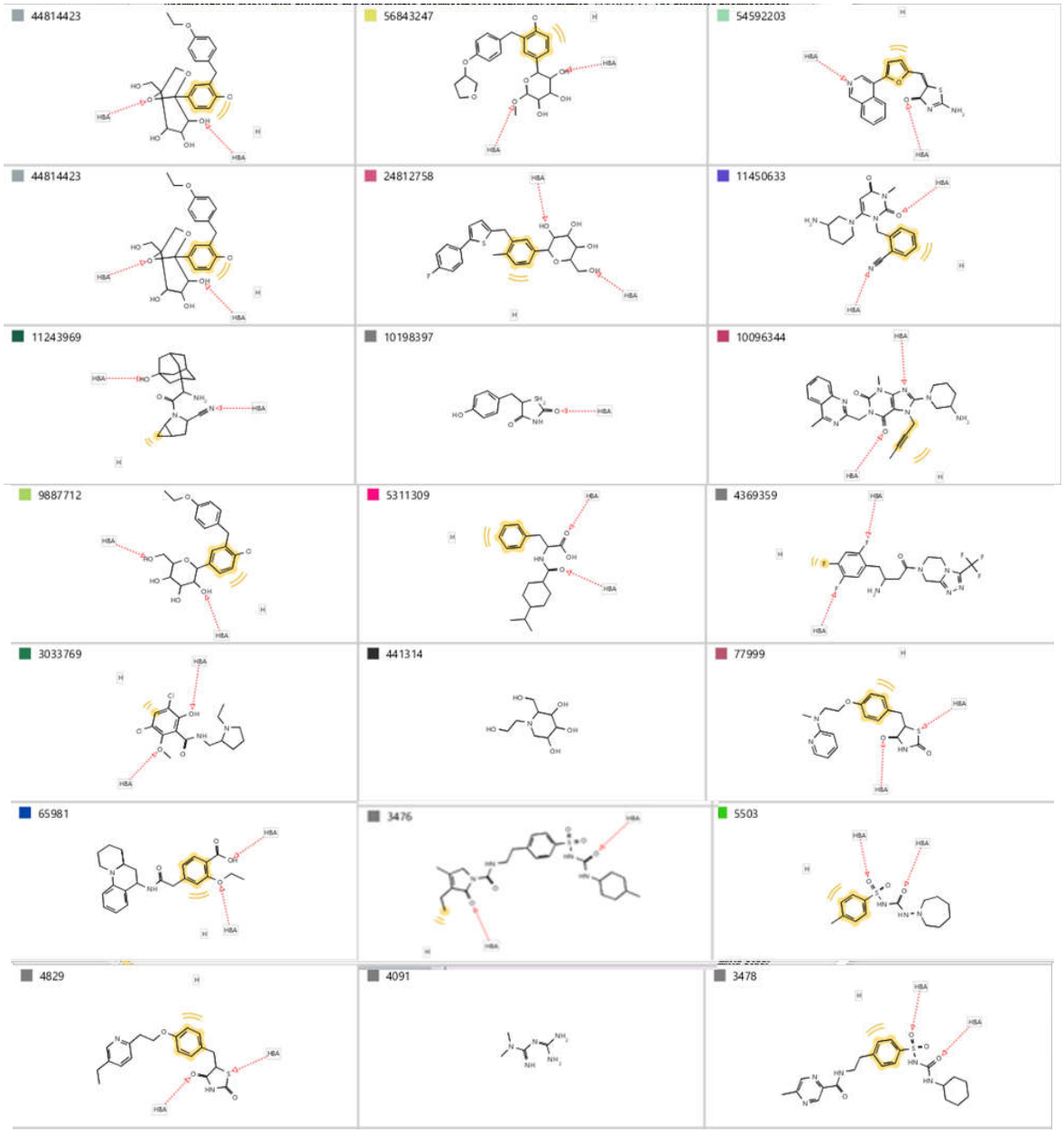


Figure 3: Pharmacophores of all the compounds

A shared feature pharmacophore model was developed on the basis of common Pharmacophoric features present in all pharmacophore models and comprises two HBD's and one AR. The shared pharmacophore model is shown in Figure 4.

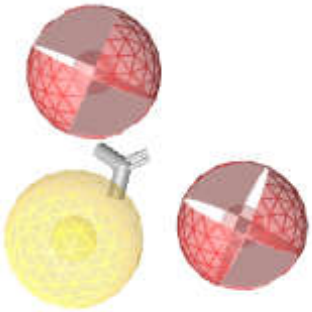
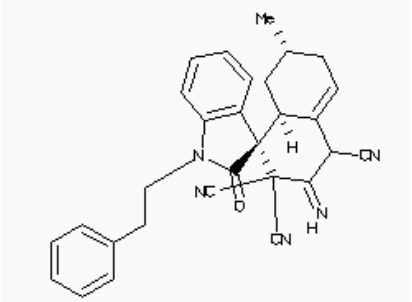
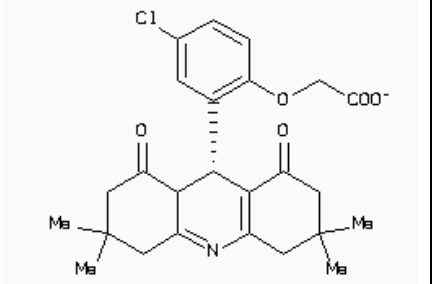
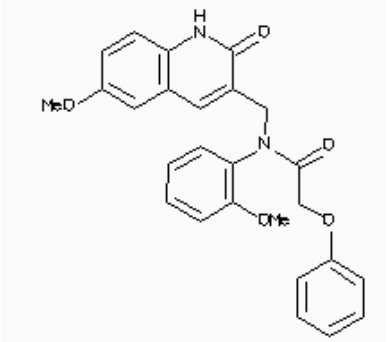


Figure 4: Shared feature Pharmacophore showing Hydrogen bond Acceptors (Red spheres) and Aromatic ring (Yellow Sphere)

The screening was performed against shared pharmacophore model and total 76 Hit compounds were obtained that shown 90% similarity. Lipinski rule was applied on these 76 hit compounds for validation purpose. Only three compounds were fulfilling the Lipinski rule and their toxicity values and LD50 values were also checked shown in Table 3.

Table 3: Toxicity class, LD50 value, structure and Zinc ID's for Hit compounds fulfilling Lipinski's rule.

S.no	Hit compound Zinc ID	Toxicity Class	LD50 Value	Structure
1	Zinc_8442109	4	325mg/kg	
2	Zinc_8442186	4	730mg/kg	
3	Zinc_8442268	4	650mg/kg	

The ligands were docked in the active pocket of the mutated CAPN10 protein for further validation. Docking is a powerful tool and the main purpose of docking is to bind the ligand with 3D structure of protein. The docking score with the types and distance of bonds were measured. The interaction of amino acids residues and high docking score show that this attempt of designing a novel compound against DM is successful. The docking results of ligands and CAPN10 protein are shown in Figure 5, 6 and 7.

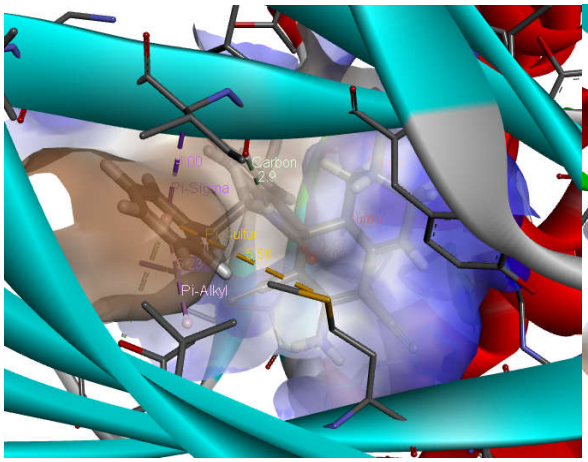


Figure 5: Actively docked conformation of First Zinc compound into CAPN10's cavity

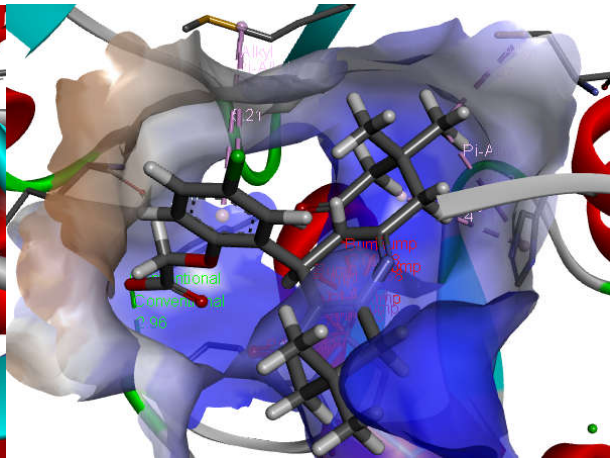


Figure 6: Actively docked conformation of second Zinc compound into CAPN10's cavity

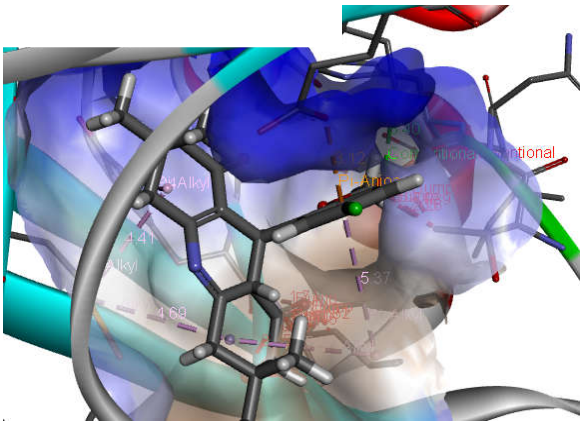


Figure 7: Actively docked conformation of third Zinc compound into CAPN10's cavity

Figure 5 shows the docking result of Zinc_8442109 with mutated CAPN10 protein, with binding score of 5340 and binding energy of -190.33 in this docked complex the common interactive amino acids identified were MET, ILE, LEU and VAL. Figure 6 shows the docking result of Zinc_8442186 with mutated CAPN10 protein, with binding score of 5268 and binding energy of -132.85 in this docked complex the common interactive amino acids identified were TYR, LYS, MET, HIS, GLN and CYS. And Figure 7 shows the docking result of Zinc_8442268 with mutated CAPN10 protein, with binding score of 5450 and binding energy of -260.27 in this docked complex the common interactive amino acids identified were GLU, MET, LEU, LYS, and PRO

DISCUSSION

Pharmacophore models are useful for designing lead structure and identified also for Binding explaining site. Ligand-based methods use only ligand information for predicting activity depending on its similarity/dissimilarity to previously known active ligands. It relies on knowledge of other molecules that bind to the biological target of interest, which may be used to derive a pharmacophore model that will define the minimum necessary structural characteristics a molecule must possess to bind to the target. It relies on knowledge of other molecules that bind to the biological target of interest, which may be used to derive a pharmacophore model that will define the minimum necessary structural characteristics a molecule must possess to bind to the target.[20],[21]

2,4 Thiazolidinedione, Acarbose, Actoplus, Albiglutide, Alogliptin Benzoate and Metformin are currently being used as treatment regimens. Several side effects headache. digestive discomfort, fatigue, hypoglycemia, death and liver cell injury have been reported[8],[9]. In curing diabetes, the main task is to normalize the sugar level of blood. For maximum control of glycaemia these drugs or therapies are used

individually or in combination with other drugs, but these drugs have some limitations as they are expensive with some side effects, their pharmacokinetics properties and also their success rate is very low[10],[11]. So the search for new drugs or class of compounds is on-going which would have less side effects and more success rate than existing drugs.

In this present work, the pharmacophores were generated from the 21 antidiabetic compounds and same technique of identification and pharmacophore generation has been reported in many researches. The selection of compounds for dataset is the first and the most crucial step in pharmacophore model generation. The pharmacophore was generate by using Ligand Scout which showed two main features as Aromatic rings which are identified in Yellow color and Hydrogen bond Acceptors which are identified in Red color. All the ligands showed uniformity in these two features. The pharmacophores of all compounds are shown in Figure 2.

The similar features of all compounds were identified by generating their Shared feature Pharmacophore. Screening of shared featured pharmacophore was done with zinc libraries that helped to discover hit compounds and then on these hit compounds Lipinski rule of 5 was applied. The compounds for further validation were docked with the receptor protein. The actively docked conformation of zinc compounds into the binding cavity of CAPN10 gene and the strong binding interaction of ligand andCAPN10 showed the validation of pharmacophore model shown in Figure 3, 4 and 5.

CONCLUSION

The present work was done to determine the novel compounds against Diabetes Mellitus and an inhibitor for CAPN10 gene which successfully binds to its active site and helps to resist this disease. These novel compounds were obtained by using the strategies like ligand based pharmacophore modelling, Validation by docking and Virtual screening. The newly generated pharmacophore had shown two main common features i-e Hydrogen bond acceptors and Aromatic rings.Compounds show least variations from each other structurally but have the similar mode of action. Treatment choices for Diabetes Mellitus are limited so these novel compounds can aid for the better treatment for DM who are taking a good diet and maintaining a healthy lifestyle and who do not respond to any treatment.The predicted Pharmacophore for Diabetes Mellitus will help in the identification of novel and more effective drugs with less side effects and more success rates. This study can be further studied for developing more antidiabetic compounds.

REFERENCES

1. O. Ali, (2013).“Genetics of type 2 diabetes,” *World J. Diabetes*, vol. 4, no. 4, p. 114.
2. K. Sriram and P. A. Insel,(2018). “G Protein-Coupled Receptors as Targets for Approved Drugs: How Many Targets and How Many Drugs?,” *Mol. Pharmacol.*, vol. 93, no. 4, pp. 251–258.
3. D. Patel, R. Kumar, S. Prasad, K. Sairam, and S. Hemalatha, (2011).“Antidiabetic and in vitro antioxidant potential of Hybanthus enneaspermus (Linn) F. Muell in streptozotocin–induced diabetic rats,” *Asian Pac. J. Trop. Biomed.*, vol. 1, no. 4, pp. 316–322.
4. A. L. Notkins, (2002).“Immunologic and Genetic Factors in Type 1 Diabetes,” *J. Biol. Chem.*, vol. 277, no. 46, pp. 43545–43548.
5. L. W. Singh,(2011).“Traditional medicinal plants of Manipur as anti-diabetics,” *J. Med. Plants Res.*, vol. 5, no. 5, pp. 677–687.
6. K. N. Mistry, A. Solanki, and A. Thakkar, (2013). “Calpain 10 Gene Polymorphism and Its Association in Cardiomyopathy and Type 2 Diabetes,” *Int. J. Adv. Diabetes Res.*, vol. 1, no. 1, p. pp–16.
7. T. Hansotia and D. J. Drucker,(2005). “GIP and GLP-1 as incretin hormones: lessons from single and double incretin receptor knockout mice,” *Regul. Pept.*, vol. 128, no. 2, pp. 125–134.
8. H. Hui, G. Tang, and V. Go, (2009).“Hypoglycemic herbs and their action mechanisms,” *Chin. Med.*, vol. 4, no. 1, p. 11.
9. J. Neustadt and S. R. Pieczenik,(2008). “Medication-induced mitochondrial damage and disease,” *Mol. Nutr. Food Res.*, vol. 52, no. 7, pp. 780–788.
10. M. Jung, M. Park, H. C. Lee, Y.-H. Kang, E. S. Kang, and S. K. Kim, (2006). “Antidiabetic agents from medicinal plants,” *Curr. Med. Chem.*, vol. 13, no. 10, pp. 1203–1218.
11. M. Y. Donath and J. A. Ehses, (2006). “Type 1, type 1.5, and type 2 diabetes: NOD the diabetes we thought it was,” *Proc. Natl. Acad. Sci.*, vol. 103, no. 33, pp. 12217–12218.
12. S. vani Guttula, A. A. Rao, G. R. Sridhar, and M. S. Chakravarthy, (2011). “Protein ligand interaction analysis an insilico potential drug target identification in diabetes mellitus and nephropathy,” *J. Bioinforma. Seq. Anal.*, vol. 3, no. 5, pp. 95–99.
13. N. Brooijmans and I. D. Kuntz, (2003). “Molecular Recognition and Docking Algorithms,” *Annu. Rev. Biophys. Biomol. Struct.*, vol. 32, no. 1, pp. 335–373.
14. G. Wolber and T. Langer, (2005).“LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters,” *J. Chem. Inf. Model.*, vol. 45, no. 1, pp. 160–169.

15. S. Bibi, S. Kalsoom, and H. Rashid, (2003). "Ligand based approach for pharmacophore generation for identification of novel compounds having anti-diabetic activity," *Int. J. Pharm. Pharm. Sci.*, vol. 5, no. 4, pp. 303–314.
16. H. M. Berman *et al.*,(2000). "The Protein Data Bank," *Nucleic Acids Res.*, vol. 28, no. 1, pp. 235–242.
17. S. Kim *et al.*, (2016). "Literature information in PubChem: associations between PubChem records and scientific articles," *J. Cheminformatics*, vol. 8, no. 1, p. 32.
18. G. Wolber and T. Langer, "LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and Their Use as Virtual Screening Filters," *J. Chem. Inf. Model.*, vol. 45, no. 1, pp. 160–169, 2005.
19. M. N. Drwal, P. Banerjee, M. Dunkel, M. R. Wettig, and R. Preissner, "ProTox: a web server for the in silico prediction of rodent oral toxicity," *Nucleic Acids Res.*, vol. 42, no. W1, pp. W53–W58, 2014.
20. N. Kandakatla and G. Ramakrishnan, (2014)."Ligand Based Pharmacophore Modeling and Virtual Screening Studies to Design Novel HDAC2 Inhibitors," *Adv. Bioinforma.*, vol. 2014, pp. 1–11.
21. T. Kaserer, K. Beck, M. Akram, A. Odermatt, and D. Schuster, (2015)."Pharmacophore Models and Pharmacophore-Based Virtual Screening: Concepts and Applications Exemplified on Hydroxysteroid Dehydrogenases," *Molecules*, vol. 20, no. 12, pp. 22799–22832.

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