Molecular Docking Studies to explore Potential Drug for Diabetic Kidney Disease

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Abstract
The kidney is the organ most susceptible to microvascular damage caused by diabetes. Many individuals with diabetes are susceptible to developing kidney disease due to their medical condition or additional co-morbidities such as hypertension and age-related nephron loss. The computational analysis of protein-ligand interactions has gained significant importance in elucidating the functional properties of both ligands and proteins. This work conducts molecular docking and molecular dynamic simulation studies on some pharmacological compounds used to treat diabetic kidney disease (DKD). The analyses focus on the interactions between these drug molecules and essential functional proteins, namely ABCA1, LXR α, ACE, GLP1, PKC and VEGF.

The binding energy of nintedanib with all substances exhibits the lowest binding energy and a strong affinity towards all protein molecules. The present study's findings indicate that the medicinal molecule nintedanib exhibits substantial affinity for the functional protein associated with diabetic kidney disease (DKD), suggesting its potential utility as a therapeutic intervention for this condition.

Keywords: Diabetic kidney disease, Nintedanib, ABCA1, LXR α, ACE, GLP1, PKC, VEGF, Molecular docking.

Introduction

Diabetes mellitus is a widespread medical condition distinct from gout, characterized by elevated blood glucose levels. Numerous studies indicate that individuals with diabetes often have higher blood uric acid levels compared to healthy individuals. This elevation is frequently associated with severe albuminuria and increased serum creatinine levels. Recent clinical investigations have shown that using uric acid-lowering medications such as allopurinol, can potentially reduce urinary albumin excretion rates (UAER) and serum creatinine (Scr) levels, while simultaneously improving the estimated glomerular filtration rate (eGFR).

Consequently, this therapeutic approach may effectively mitigate renal impairment in individuals diagnosed with diabetes[9]. Diabetic kidney disease (DKD) is a type of chronic kidney disease (CKD) linked to diabetes mellitus (DM). The pathophysiology of DKD is complex and is marked by increased persistent albuminuria and a gradual decline in the glomerular filtration rate (GFR), which can ultimately lead to end-stage renal disease (ESRD)[19]. DKD is a primary cause of ESRD and also increases the risk of cardiovascular and cerebrovascular diseases[18]. According to a global survey conducted by the International Diabetes Federation (IDF), 2% of individuals with type 2 diabetes have cardiovascular risk factors or have experienced cardiovascular events[20].

The onset and progression of diabetic kidney disease present a significant clinical challenge, leading to higher rates of morbidity and mortality and severely affecting individuals' quality of life. Identifying effective treatments and pharmaceutical interventions for DKD is therefore of utmost importance. Implementing fundamental clinical interventions such as managing hyperglycemia, hypertension, hyperlipidemia, smoking cessation and dietary modifications, has the potential to decrease the incidence of end-stage renal disease (ESRD) among individuals with diabetes[10]. The characteristic clinical presentations of diabetic kidney disease (DKD) commonly involve the presence of albuminuria, which may advance to macroalbuminuria or overt proteinuria as the condition develops. Additionally, a small subset of patients may exhibit microscopic hematuria whereas the rate of decline in renal function tends to be relatively slow.

Traditionally, diabetic kidney disease (DKD) is categorized into five distinct stages. Stages 1 and 2 represent the preclinical phases of the condition, wherein there is an elevation in glomerular filtration rate (GFR), the presence of normoalbuminuria (stage 1), or intermittent microalbuminuria (MA; stage 2) and normal blood pressure. Stage 3 represents the commencement of the clinical phase, distinguished by the presence of persistent microalbuminuria (MA), mild hypertension and a GFR that either remains within the normal range or experiences a modest decrease. Stage 4 of the disease is marked by the presence of macroalbuminuria, hypertension and a progressive decrease in GFR. Stage 5 is the final stage of renal illness.

However, current epidemiological research indicates that the above classification may not apply to all patients with diabetic kidney disease (DKD), especially those with type 2 diabetes mellitus (DM). An increasing body of evidence suggests that proteinuria does not consistently precede the

decline in renal function in individuals with diabetes. This finding challenges the conventional understanding of the course of diabetic kidney disease (DKD)[4,13,17.]

In the past, insulin has been regarded as the preferred therapeutic option for managing diabetic individuals with renal impairment. Recently, promising oral pharmacological alternatives in diabetes therapy has emerged, prompting a re-evaluation of the prescription and dosing protocols associated with conventional drugs. Typically, metformin is the primary pharmaceutical intervention employed in the management of type 2 diabetes. The primary mechanism of action of this medication involves reducing hepatic glucose production, enhancing peripheral glucose absorption, improving glucose tolerance and reducing fasting and postprandial plasma glucose levels. However, the administration of metformin is contraindicated in individuals with diabetic kidney disease (DKD) due to its renal excretion process[1,15].

The recent efficacy of sodium-glucose cotransporter-2 (SGLT2) inhibitors as a novel therapeutic approach for diabetic kidney disease (DKD) is a source of enthusiasm and motivation for nephrologists. The sodium-glucose cotransporter 2 (SGLT2) typically contributes to around 90% of glucose re-absorption in the kidney's proximal tubule[18].

Bioinformatics has experienced significant advancements, leading to the emergence of network pharmacology as an effective method that utilizes large datasets to elucidate the intricate mechanisms behind pharmacological systems comprehensively. This approach enables the detailed investigation of drug actions, ranging from the molecular to the pathway level. Simultaneously, this methodology has arisen as a potential strategy to expedite the process of drug investigation and advancement[6,14,17]. The utilization of molecular docking (MD) techniques has the potential to improve the molecular pharmacological impact and to elucidate the fundamental cause of drug action at a molecular level[3].

Material and Methods

Selection of Target Protein: The protein structures were retrieved from the RCSB PDB database. Proteins such as ABCA1, ACE, GLP-1, LXR, PKC and VEGF were identified in the literature. The 3D structures of these target proteins were obtained from the PDB database (https://www.rcsb.org). The PDB IDs for these structures are *7ROQ, 2XY9, 6LN2, 7UHL, 1XJD and 2C7W*. For the protein structures, structural waters and ligands were removed using Discovery Studio software.

Ligand Preparation: Drug molecules were downloaded from PubChem. The 3D structure of all compounds was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and utilized in this study. All compounds underwent minimization, with hydrogen atoms added, followed by a minimization step using Avogadro software. The ligands were constructed and energy-minimized using the MMFF94 force field.

ADMET analysis: ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) studies are crucial in drug screening for determining pharmacokinetic parameters. The SWISS ADME tool is essential for analyzing structural analogs and predicting key physical and pharmacological features. Principal descriptors and physicochemical qualities are significant, with an emphasis on metrics such as log P (Octanol/Water), log S, molecular weight and so forth. The program also assesses analogs using Lipinski's Rule of 5, an important criterion for rational medication design.

Docking studies: Docking studies for the target protein with PDB IDs: *7ROQ, 2XY9, 6LN2, 7UHL, 1XJD and 2C7W* and drug molecules were performed using the HDOCK server. The protein and the drug molecules were submitted or uploaded to the HDOCK server for computational analysis and molecular docking studies. This platform was utilized to predict and to assess potential interactions and binding affinities between the drug molecules and the protein of interest, aiding in exploring their binding modes and potential therapeutic applications. The lowest-binding energy conformations that replicated key interactions were chosen. The results were also analyzed using Discovery Studio 2021.

Results

Preparation of target protein: Table 1 presents the crystal structure details of various proteins retrieved from the Protein Data Bank. ABCA1 (PDB ID 7ROQ) comprises 13971 amino acid residues with a molecular weight of 263.05 kDa, at a resolution of 4.01 Å. ACE (PDB ID 2XY9) consists of 5355 amino acid residues with a molecular weight of 70.44 kDa, resolved at 1.97 Å. GLP1 (PDB ID 6LN2) contains 6524 amino acid residues with a molecular weight of 103.21 kDa, resolved at 3.20 Å. LXRα (PDB ID 1UHL) is composed of 3707 amino acid residues with a molecular weight of 57.62 kDa, resolved at 2.90 Å.

PKC (PDB ID 1XJD) includes 2502 amino acid residues with a molecular weight of 41.29 kDa, resolved at 2.00 Å. VEGF (PDB ID 2C7W) comprises 1474 amino acid residues with a molecular weight of 22.43 kDa, resolved at 2.48 Å. Using Discovery Studio, non-receptor molecules including water were removed from these protein structures and the data were saved in PDB format.

Ligand preparation: Table 2 shows that our study is based on a literature review, the structures of ligands i.e. all 15 drug compounds were obtained in SDF format from the PubChem database and converted to PDB format.

ADMET analysis: *In silico* pharmacokinetic predictions play a crucial role in drug development as they examine Absorption, Distribution, Metabolism and Excretion

(ADME). These computational techniques have become increasingly important in the drug selection process, aiding in the assessment of a compound's potential for human medicinal application.

Docking Studies: The objective of ligand-protein docking is to elucidate the interaction between a ligand and a protein with a known three-dimensional structure, specifically focusing on the binding mechanism within the molecule's active site[8].

Table 1
Protein PDB ID

S.N.	Protein name	PDB ID	Molecular weight
1	ABCA1	7ROQ	254 kDa
2	LXRα	1UHL	57.62kDa
3	GLP1	6ln2	103.21kDa
4	VEGF	2c7w	22.43kDa
5	PKC	1xjd	41.29kDa
6	ACE	2xy9	70.44kDa

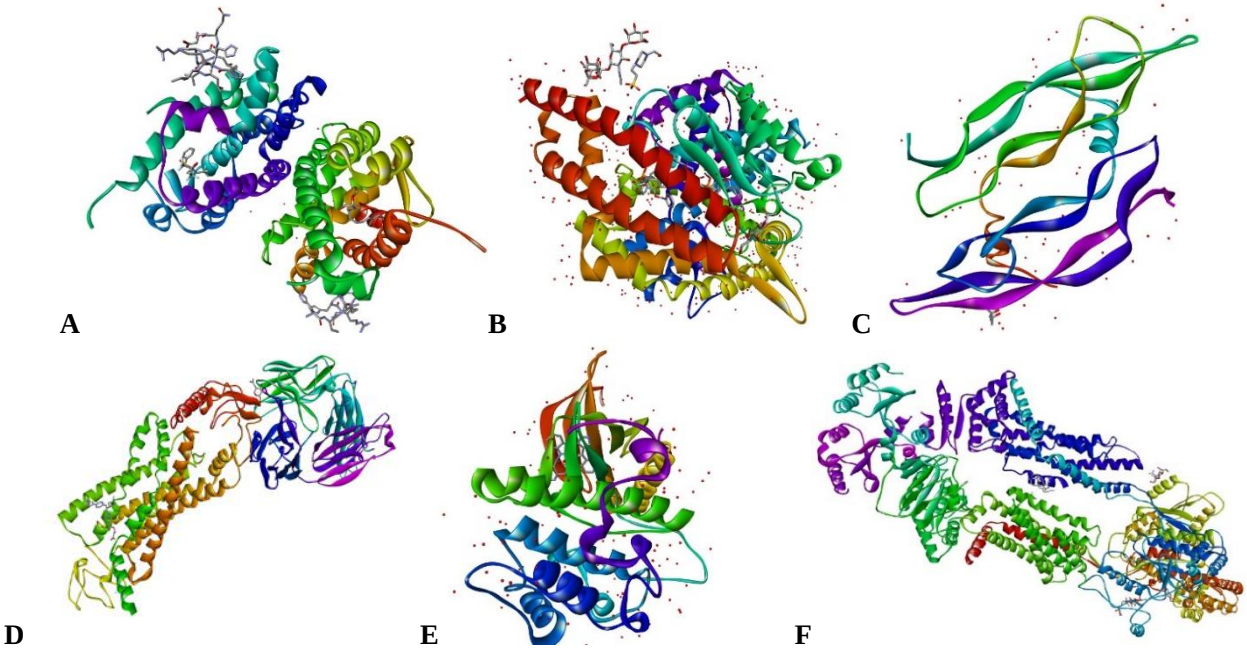

Fig. 1: Protein structure (A) LXR α (B) ACE (C) VEGF (D) GLP1 (E) PKC, (F) ABCA1

Table 2
Drug molecules

S.N.	Compound Name	PubChem ID	Molecular Weight
1	ALLOPURINOL	135401907	136.11 g/mol
2	ATRASENTAN	159594	510.6g/mol
3	CANAGLIFLOZIN	24812758	444.5g/mol
4	DOXYCYCLINE	54671203	444.4g/mol
5	EMPAGLIFLOZIN	11949646	450.9g/mol
6	NINTEDANIB	135423438	539.6g/mol
7	PENTOXIFYLLINE	4740	278.31g/mol
8	PIRFENIDONE	40632	185.22 g/mol
9	PYRIDOXAMINE	1052	168.19 g/mol
10	STREPTOZOTOCIN	29327	265.22 g/mol
11	CILASTAZOL	2754	369.5 g/mol
12	DAPAGLIFLOZIN	9887712	408.9 g/mol
13	PARICALCITOL	5281104	416.6 g/mol
14	RUBOXISTAURINE	153999	468.5 g/mol
15	TRAMETINIB	11707110	615.4 g/mol

The docking analysis targeted specific pharmacological targets, namely ABCA1, ACE, GLP-1, LXR, PKC and VEGF. Evaluation of the binding energies of all compounds revealed that Nintedanib exhibits the lowest critical point among all the target drug molecules (Table 3 and fig. 2).

Discussion

Diabetes mellitus (DM) is a persistent metabolic condition characterized by increased levels of glucose in the bloodstream, resulting from inadequate insulin production or resistance to insulin[2]. Disease progression invariably involves the emergence of pathological changes such as nephropathy, retinopathy and cardiovascular complications[16]. Individuals with diabetes are at a risk of experiencing severe macrovascular complications, specifically cardiovascular disease and microvascular complications. These issues have significant consequences including increased death rates, blindness, kidney failure and a general reduction in quality of life[21]. Diabetic kidney disease manifests in approximately 40% of individuals diagnosed with diabetes, representing the primary etiology of chronic kidney disease (CKD) globally. While end-stage renal disease (ESRD) is commonly associated with diabetic kidney disease, it is essential to note that a significant proportion of patients succumb to cardiovascular illnesses and infections before requiring kidney replacement therapy.

Table 3
Pharmacokinetics properties of selected drug molecules.

S.N.	Compound Name	Mol.Wt(g/mol)	Rotatable bonds	Hydrogen bond acceptor	Hydrogen bond donor	Lipinski Rule	Violation
1	ALLOPURINOL	136.11 g/mol	0	3	2	Yes	0
2	ATRASENTAN	510.6g/mol	0	3	2	Yes	0
3	CANAGLIFLOZIN	444.5g/mol	2	9	6	Yes	1
4	DOXYCYCLINE	444.4g/mol	6	7	4	Yes	0
5	EMPAGLIFLOZIN	450.9g/mol	9	7	2	Yes	1
6	NINTEDANIB	539.6g/mol	5	4	0	Yes	0
7	PENTOXIFYLLINE	278.31g/mol	2	4	3	Yes	0
8	PIRFENIDONE	185.22 g/mol	5	8	5	Yes	0
9	PYRIDOXAMINE	168.19 g/mol	2	4	3	Yes	0
10	STREPTOZOTOCIN	265.22 g/mol	5	8	5	Yes	0
11	CILASTAZOL	369.5 g/mol	7	5	1	Yes	0
12	DAPAGLIFLOZIN	408.9 g/mol	6	6	4	Yes	0
13	PARICALCITOL	416.6 g/mol	5	3	3	Yes	1
14	RUBOXISTAURINE	468.5 g/mol	2	4	1	Yes	0
15	TRAMETINIB	615.4 g/mol	6	5	2	No	1

Table 4
Docking score (Kcal/mol) of the selected drugs with Target proteins.

S.N.	NAME OF DRUG	Binding energy with Target proteins					
		ACE	GLP1	LXR	PKC	ABCA1	VEGF
1	ALLOPURINOL	-97.10	-89.25	-97.23	-82.34	-100.04	-80.97
2	ATRASENTAN	-230.97	-159.59	-184.88	-170.63	-176.94	-124.80
3	CANAGLIFLOZIN	-242.08	-151.27	-191.20	-152.23	-156.59	-119.16
4	CILASTAZOL	-190.29	-140.19	-170.55	-152.95	-170.66	-116.41
5	DOPAGLIFLOZIN	-204.26	-142.75	-188.03	-153.38	-159.95	-119.78
6	DOXYCYCLINE	-236.67	-167.83	-204.24	-161.34	-189.59	-140.45
7	EMPAGLIFLOZIN	-227.19	-173.05	-186.54	-158.30	-166.15	-121.37
8	NINTEDANIB	**-261.88**	**-171.93**	**-185.75**	**-180.00**	**-190.76**	**-131.47**
9	PARICALCITROL	-190.48	-154.52	-166.00	-131.20	-136.22	-106.68
10	PENTOXIFYLLINE	-149.51	-115.43	-135.73	-121.54	-165.07	-106.83
11	PIRFENIDONE	-124.35	-94.76	-101.14	-85.16	-101.64	-74.26
12	PYRIDOXAMINE	-96.08	-97.91	-87.68	-84.07	-108.46	-74.85
13	RUBOXISTAURINE	-222.21	-165.36	-160.52	-165.56	-164.07	-120.90
14	STREPTOZOTOCIN	-171.99	-137.21	-135.54	-115.72	-161.56	-104.41
15	TRAMETINIB	-235.43	-162.62	-169.95	-169.69	-167.97	-126.36

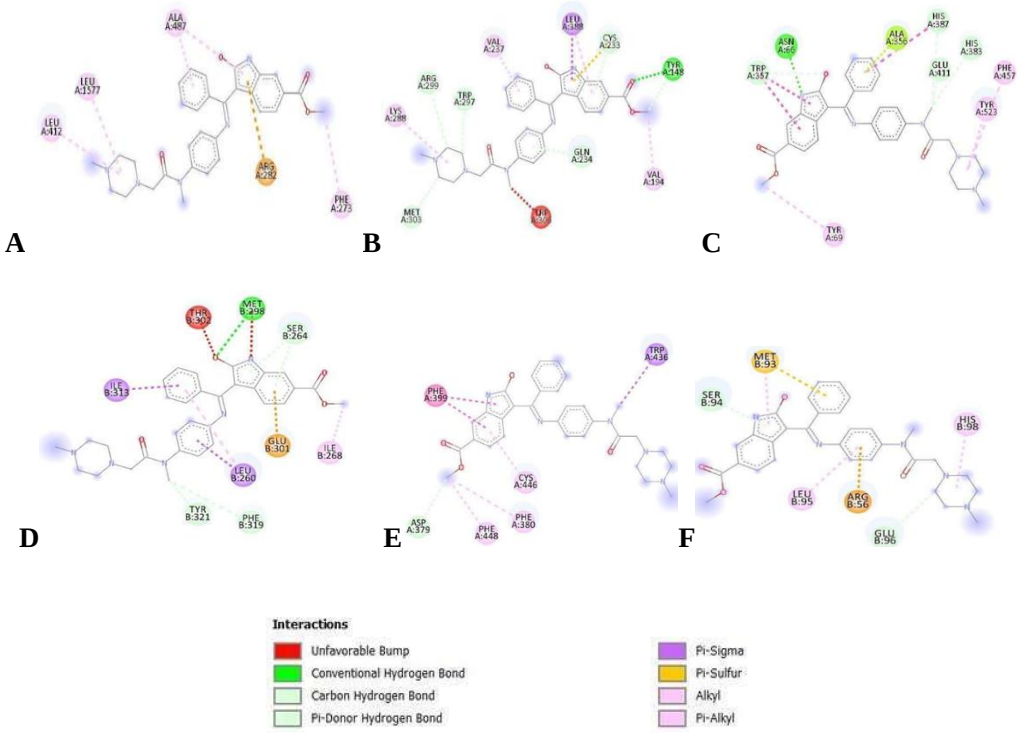

Fig. 2: (A) Nintedanib with ABCA1, (B) Nintedanib with ACE, (C) Nintedanib with GLP1, (D) Nintedanib with LXR *α*, (E) Nintedanib with PKC, (F) Nintedanib with VEGF.

The progression of diabetic kidney disease includes glomerular hyperfiltration, a gradual increase in albuminuria, decline in glomerular filtration rate (GFR) and eventually, end-stage renal disease (ESRD). Diabetes is characterized by metabolic alterations that give rise to glomerular hypertrophy, glomerulosclerosis, tubulointerstitial inflammation and fibrosis. Despite existing therapeutic interventions, a significant residual risk remains for developing and advancing diabetic kidney disease[12].

Diabetic kidney disease (DKD) frequently progresses to end-stage renal disease or precipitates cardiovascular events, resulting in mortality for approximately 50% of afflicted individuals. Hence, it is imperative to prioritize early awareness, detection and intervention to enhance clinical outcomes[11].

Molecular docking is a highly significant technology utilized in structural molecular biology, computer-aided drug design and the analysis of phytoconstituent interactions with target molecules. The primary objective of ligand-protein docking is to elucidate how a ligand, along with a protein with a well-established three-dimensional structure, will interact with one another, specifically in terms of its binding mechanism within the active site of the molecule[8].

The results of the docking analysis showed that selected drugs had significant binding modes and exhibited beneficial binding energy scores while interacting with specific target proteins, namely ABCA1, ACE, GLP-1, LXR, PKC and VEGF. This conclusion was substantiated by the observation that the chosen pharmaceutical compounds could establish enduring complexes with the targeted proteins. Nintedanib, one of the drugs, demonstrated the highest binding affinity with a score of -261.88 kcal/mol.

In an identical study, researchers explored the protective mechanism of dehydromethionine in the context of diabetic kidney disease[5]. Their study employed network pharmacology techniques and experimental validation to elucidate this process. They performed molecular docking and molecular dynamic simulations to investigate the interaction between dehydromethionine (DHT) and PIK3CA. The results of the docking analysis showed that a binding energy threshold of $\leq$ -5 kJ/mol was utilized as the screening criterion. The top three active components based on degree (DHT, Sclareol and Aethiopinone) were selected for molecular docking. During the molecular docking analysis, it was observed that the binding energy between DHT and PIK3CA was the lowest, measuring -8.6 kJ/mol.

Conclusion

The selected drugs show potential in influencing diabetic kidney disease (DKD) by mitigating oxidative stress, reducing inflammation, combating fibrosis, lowering levels of albuminuria and uric acid, protecting podocytes from damage and altering the extracellular matrix (ECM) remodeling process. However, it should be noted that the precise mechanisms of action for some of these drugs remain unclear and there is limited clinical trial data available. Further experiments will be necessary to validate the effectiveness and safety of these drugs as viable treatments for DKD in future research.

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